EISSN 2148-2373

BEZMIALEM VAKIF UNIVERSITY
1845

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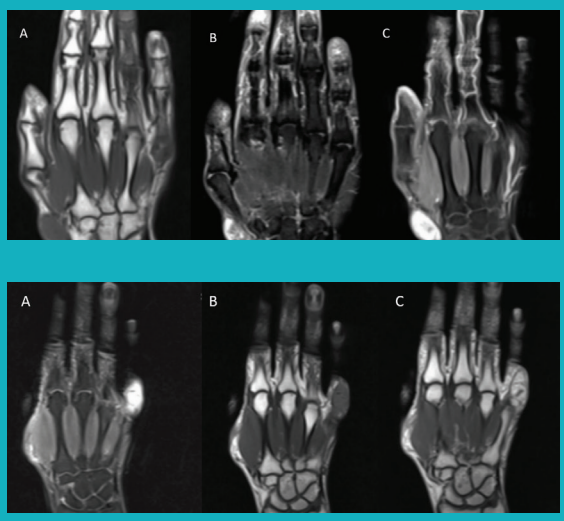

Volume 10 • Issue 2 • March-April 2022

bezmialemscience.org

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Web: www.galenos.com.tr
Publisher Certificate Number: 14521
Publication Date: April 2022
E-ISSN: 2148-2373
International scientific journal published quarterly.

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The target population of this journal includes medical academicians, specialists, assistants, and medical students. The aim of the journal is to publish high-ranking original reseaches in basic and clinical sciences, reviews covering contemporary literature about medical education and practice, reports of rare cases, and manuscripts that would contribute to continuous medical education.

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Title: Bezmiâlem Science

Official abbreviation: Bezmialem Science

E-ISSN: 2148-2373

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to Medical Subject Headings (MeSH) terms prepared by the National Library of Medicine (NLM) and contain minimum 3 and maximum 6 items; keywords should be written in Turkish and English just below the abstract. The main text should contain Introduction, Methods, Results, Discussion, Limitations of the Study, Conclusion, References, Tables, Figures and Images, and should be limited to 5000 words excluding references. References not exceeding 50 would be acceptable.

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In addition to the pictures in Case Reports and Original Images, video/ motion images and extra images / static images can be published on the web page of our journal if submitted with the following technical specifications.

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2. Presentations in image / static image format: JPG, GIF, TIFF, BMP

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4. The file size should be a maximum of 100 MB.

5. The file size should be no more than 10 minutes.

6. Main text should not exceed 500 words.

7. References are welcomed and should not be more than 5.

8. Video and images must be cited within main text.

9. There should be no identification of patient / physician / institution / city / country in pictures and especially video images.

The tables, figures and illustrations included in your article should be declared as original. When received from sources other than the original,the source must be cited, and hardcopy format or electronic versions of the source should be submitted to the Editor-in-Chief office with permission from the copyright holder (publisher, journal or author). The rules for references, figures and tables apply to all article types.

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signed by no more than 3 authors. Figure legends are limited to 200 words and the number of figures are limited to 3. Video submissions will not be considered.

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All tables, figures, graphs and other visual media must be numbered in order of citation within the text and must not disclose the names of the patients, doctors or institutions. Tables must be prepared in a Microsoft Office Word document using the "Insert Table" command and be placed at the end of the references section in the main document. Tables should not be submitted in JPEG, TIFF or other visual formats. In microscopic images, magnification and staining techniques must be specified in addition to figure captions. All images should be in high resolution with minimum 300 dpi. Lines in the graphs must be in adequate thickness. Therefore, loss of details would be minimal if a reduction is needed during press. The width must be 9 cm or 18 cm. It would be more appropriate if the drawings are prepared by professionals. Gray color should be avoided. Abbreviations must be explained in alphabetical order at the bottom. Roman numerals should be avoided while numbering the Tables and Figures, or while citing the tables in the text. Decimal points in the text, tables and figures should be separated by a comma in Turkish sections and by dots in English sections. Particularly, tables should be explanatory for the text and should not duplicate the data given in the text.

Pharmaceuticals should be specified with their generic names, and medical products and devices should be identified with brand name and company name, city and country.

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Book Section: Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine.St Louis: Mosby; 1974.p.273-85.

Books with Single Author: Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Marcel Dekker; 1993.

Editor(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992.p.1561-5.

Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

Thesis: Kaplan SI. Post-hospital home health care: the elderly access and utilization (dissertation). St. Louis (MO): Washington Univ. 1995.

Manuscripts accepted for publication, not published yet: Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med In press 1997.

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EDITORIAL

Dear Readers,

As you know, we have increased the number of publications to six with the new year. I would like to state that we have been working very hard on incoming articles and evaluations, and the burden of our assistant editors has also increased. With the increase in the number of our publications, we have observed that the biggest problem of our assistant editors is the reviewer evaluations. This prolongs the evaluation process of the submitted articles and causes delay in publication of your articles that is beyond our control. My request from you, our esteemed readers, is that you accept to evaluate the manuscripts you have received for reviewing, spare time for this and support us. I know that there are problems with reviewing in many foreign journals. Although some journals have found different solutions, we have no other way than your voluntary support. Peer-reviews are one of the most important factors that determine the quality of a journal and its contribution to the scientific community. We add the authors to the list of reviewers who have submitted articles to our journal. We try to send incoming articles according to your field of interest. Thank you in advance for your support.

We published the first special issue of this year. I would like to thank my friends who supported and contributed to this issue, which consisted of papers traditionally presented at our student congress.

I am happy to share many good articles with you again in this issue. We chose the cover art of this issue from the article of Yılmaz et al. Thank you for this beautiful picture. In addition, in the comentary section, I wrote the “Current Status of Approach to Thyroid Nodules”, which concerned many branches. I think it will contribute to our colleagues who are interested in this field.

Our eye-catching articles are; Yılmaz et al. Radiological Appearences of Benign Soft-tissue Tumors of the Hand and Wrist With Special Emphasis on MRI, Yabacı Tak et al. Statistical Errors in Medical Residency Theses, Salık et al. Anatomical Evaluation of The Major Vessels with Ultrasound in Children Undergoing Cardiac Surgery, Aslan et al. Comparisons of the Radiolucent Lines Between Cemented and Cementless Oxford Unicondylar Knee Arthroplasty: A Non-designer Group Report, Süleyman et al. Safety of Cefazolin in Prophylaxis in B-Lactam Allergic Children.

Dear readers,

It is pleasing that scientific meetings are starting to be held face-to-face again these days when the pandemic slows down the pace and the number of cases decreases. We are waiting for you to send your original articles to our journal, which will be created with increasing scientific activities. Also, as a small reminder, I would like you to cite our valuable published articles in your articles in order to increase the number of citations of our journal.

All the best, see you in our next issue.

Kind regards
Prof. Dr. Adem AKÇAKAYA
Chief Editor

Comentary

DOI: 10.14235/bas.galenos.2022.30075
Bezmialem Science 2022;10(2):131-4

Current Status of Approach to Thyroid Nodules

Tiroid Nodüllerine Yaklaşımda Güncel Durum

Adem AKÇAKAYA

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The prevalence of thyroid nodules (TNs) in the community is high, and the rate of detection of TNs by using high-resolution ultrasonography (US) is approximately 60%. Less than 5% of TNs are malignant (1). TNs are seen approximately 4 times more frequently in women than in men. The prevalence of palpable TNs in areas without iodine defficiency is approximately 5% in women and 1% in men. The rate of thyroid cancer is two times higher in men than in women (4% vs. 8%) (2). In a screening study conducted with randomly selected individuals in the population, TNs were detected more frequently in women and the elderly (3). It is very important to distinguish between benign and malignant nodules. Clinical evaluation and US should be performed in palpable TNs (4). Physical examination alone may reveal 5% to 7% of TN. Ultrasound shows a prevalence of 20% to 76% in the same population, and this is in line with autopsy findings (2).

People with TN are usually euthyroid. Most patients are asymptomatic, but the absence of symptoms does not rule out malignancy. Less than 1 percent of TNs cause hyperthyroidism or thyrotoxicosis. If spontaneous bleeding into the nodule has occurred, patients may complain of a feeling of pressure or pain in the neck. When any nodule is detected; history of goiter, family history of thyroid carcinoma and autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease, family history of polyposis (Gardner syndrome), hypothyroidism or hyperthyroidism should be questioned.

TNs can be solitary, multinodular, or cystic (5). TNs can also be classified as adenoma, carcinoma, cyst, colloidal nodule, and inflammatory nodules. The most common TN type with a low risk of malignancy is colloids. Most follicular adenomas are benign, but some may have features of follicular carcinoma. About 5% of microfollicular adenomas are proven to be follicular cancer. Thyroiditis may also present as a nodule. Thyroid carcinoma usually presents as a solitary palpable TN. The most common type of malignant TN is papillary carcinoma (6).

A TN is a lesion within the thyroid gland that is radiologically different from the surrounding thyroid parenchyma. Some palpable lesions may not have obvious radiological features. TNs can be solid or liquid. TNs that are detected in imaging studies performed for other reasons and that are not-palpable are called incidental nodules or "incidentalomas". There is no difference in malignancy risk between non-palpable nodules and sonographically confirmed palpable nodules of the same size.

Generally, nodules larger than 1 cm are considered clinically significant and are evaluated because they have malignant potential. Although rare, lymphadenopathy and nodules smaller than 1 cm with clinical symptoms may also cause morbidity and mortality, therefore they should be evaluated with advanced imaging techniques. The widely accepted view is that TNs smaller than 1 cm do not need to be investigated invasively for malignancy.

The clinical significance of TNs is due to the risk of development of thyroid cancer, which occurs in 7-15% of patients, depending on age, gender, radiation exposure, family history, and other factors. The generally accepted view is that most nodules are at low risk for malignancy, and even if malignancy is diagnosed, it can be successfully treated to a large extent (3).

High-resolution US, sensitive thyroid-stimulating hormone (TSH) assays, and fine needle aspiration biopsy (FNAB) are used together with clinical findings to detect the features of the TN. Thyroid scintigraphy is not required for diagnosis in most of the

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Received: 07.04.2022
Accepted: 14.04.2022

Cite this article as: Akçakaya A. Current Status of Approach to Thyroid Nodules. Bezmialem Science 2022;10(2):131-4

patients, but can be used to detect functional autonomy in iodine deficient soils in patients with low serum TSH or multinodular thyroid gland. Serum TSH measurement is the first laboratory test to detect thyroid function. Afterwards, measurement of free thyroxine (FT4) and free triiodothyronine (FT3) levels, and thyroid peroxidase antibodies (TPOAbs) may be requested. Measurement of unstimulated serum calcitonin should only be performed when medullary thyroid cancer (MTC) is suspected based on FNAB results or the patient's history (4).

1- **Measurement of serum thyrotropin level:** During the initial evaluation of a patient with a TN, serum thyrotropin level should be measured. If the serum TSH level is below normal, a radionuclide thyroid scan should be performed. If serum TSH level is normal or elevated, radionuclide scanning should not be performed as the initial imaging evaluation.

2- **Serum thyroglobulin level:** Routine measurement of serum thyroglobulin (Tg) level is not recommended for the initial evaluation of TNs. Serum Tg levels may be elevated in most thyroid diseases and measuring its level is a low-sensitivity and non-specific test for thyroid cancer.

3- **Serum calcitonin level:** Routine measurement of serum calcitonin level is not recommended for the initial evaluation of TNs. If the unstimulated serum calcitonin level is higher than 50-100 µpg/mL, it is significant for the diagnosis of MTC. There are studies showing that calcitonin measurement from the FNAB material of TN may be helpful in the preoperative evaluation of patients with moderately elevated basal serum calcitonin level (20-100 µpg/mL).

4- **18F-Fluorodeoxyglucose positron emission tomography scan (18FDG-PET**): Focal 18FDG-PET uptake in a sonographically confirmed TN is significant for thyroid cancer risk, and FNAB is recommended for nodules larger than 1 cm. Extensive 18FDG-PET uptake with sonographic and clinical signs of chronic lymphocytic thyroiditis does not require further imaging or FNAB.

5- **Thyroid sonography:** Thyroid sonography, including examination of cervical lymph nodes, should be performed in all patients with known or suspected TN. Thyroid US is widely used to assess the risk of malignancy in TN and to help decide whether FNAB is indicated. AACE/ACE/AME guidelines recommend that the position, shape, size, border, content, echogenicity and vascular status information of TN be included in US reports (3,7).

When evaluating the thyroid parenchyma with ultrasound, homogeneity or heterogeneity of the thyroid gland, size of the gland, size, location and sonographic characteristics of any nodule, presence or absence of suspicious cervical lymph nodes in the central or lateral compartments are evaluated. The report should note the size of the nodule in three dimensions and its location (for example, the right upper lobe) and all of the sonographic characteristics, including the structure of the nodule (solid, cystic ratio or spongy), echogenicity, borders, vascularity, presence and type of calcifications. The pattern of sonographic features and the size of the nodule provide important information in the decision of FNAB.

Thyroid ultrasound is an important imaging modality used to evaluate TNs. Thyroid sonography, including examination of cervical lymph nodes, should be offered in all patients with known or suspected TNs. US can provide information about dimensions, vascularity, structure and parenchymal changes and detect lesions as small as 2 mm. It is widely used to avoid unnecessary use of invasive procedures and to differentiate benign and malignant lesions (2).

The FNAB can be performed with palpation if diagnostic US confirms the presence of a solid nodule corresponding to the palpable nodule. When clinically indicated, it is preferred to perform FNAB under ultrasound guidance in the evaluation of TNs. FNAB is the most accurate and cost-effective method for evaluating TNs. Retrospective studies have shown that US-guided FNAB procedure has lower rates of both non-diagnostic and false-negative cytology compared to palpation. US-guided FNAB should be preferred, especially in deeply located, difficult to palpate or posteriorly located nodules that are not larger than 1 cm, are not palpable, have non-diagnostic cytology in the previous biopsy, have mixed or cystic components. Cystic or spongy lesions are considered to pose a low risk for malignancy, and if they are larger than 2 centimeters, they are either monitored or biopsied (2,3).

If a cyst is large and symptomatic, aspiration and ethanol ablation may be considered as therapeutic interventions. If aspiration is performed, it should be evaluated cytologically. When TNs are detected, sonographic evaluation of the anterior cervical lymph node compartments (central and lateral) is required. If US detects cervical lymph nodes sonographically suspicious for thyroid cancer, FNAB of the suspected lymph node should also be performed. Microcalcifications, cystic appearance, peripheral vascularity, hyperechogenicity and round shape are features suggestive of malignancy in lymph nodes. Lymph nodes with these features usually require histopathological evaluation (7).

The ATA guideline provides the following recommendations for the US findings of TNs, the estimated risk of malignancy, and the decision whether to perform FNAB (3).

A. High suspicion (**risk of malignancy >70%-90%**), FNAB recommended

A solid hypoechoic nodule or a solid hypoechoic component of a partial cystic nodule together with microlobulated, infiltrative irregular borders, microcalcifications, the length of the nodule being longer than the width, marginal calcifications, and the presence of one or more of the signs of extrathyroidal extension are risk factors for malignancy. A nodule with these sonographic features is likely to be papillary thyroid cancer (PTC). Nodules with a pattern of high suspicion and greater than 1 cm in size should undergo diagnostic fine-needle biopsy to rule out or confirm malignancy. However, in the absence of extrathyroidal spread, metastatic cervical lymph nodes, or evidence of distant metastases, micropapillary thyroid cancers (<1 cm) usually have a slow course,

although this may depend on the age of the patient. The patient's age and preference can alter the decision-making process.

B. Moderate suspicion **(risk of malignancy 10-20%)**, FNAB is recommended.

Hypoechoic solid nodule with regular borders without microcalcification, longer nodule length than width, and extrathyroidal extension finding constitute this group. This appearance has the highest sensitivity (60-80%) for PTC but a lower specificity than the previous high suspicion pattern, and FNAB should be considered for these nodules ≥1 cm to rule out malignancy.

C. Low suspicion **(risk of malignancy 5%-10%)**, FNAB is recommend if >1.5 cm

There are isoechoic and hypoechoic solid nodules which do not have microcalcification, irregular border, a shape where the neck is longer than the width and evidence of extrathyroidal extension, or there are cystic nodules with eccentric solid areas. Only about 15-20% of thyroid cancers on ultrasound are iso- or hyperechoic, and these are usually PTC or the follicular variant of follicular thyroid cancers (FTC). Less than 20% of these nodules are partially cystic. Therefore, these appearances have a lower probability of malignancy and those less than 1.5 cm in size can be followed up.

D. Very low suspicion **(≤3),** consider FNAB if it is >2 cm, follow-up without FNAB is possible.

Spongy or partial cystic nodules that do not have sonographic characteristics described in low, moderate, or high suspicion samples have a low malignancy risk (<3%). If FNAB is to be performed, the nodule should be at least 2 cm in size. For nodules smaller than 2 cm, observation without FNAB may also be considered.

E. Benign **(≤1%)**, FNAB is not recommended

Completely cystic nodules are unlikely to be malignant and diagnostic fine needle biopsy is not indicated. If a cyst is large and symptomatic, aspiration is performed and ethanol ablation may be added in appropriate cases. If aspiration is performed, it should be done in cytological examination (3).

In the cytological evaluation of the material obtained by using FNAB, the **Bethesda classification** is used, which classifies the lesions into 6 main categories with different characteristics. The Bethesda system defines six diagnostic categories, and cancer risk estimation is made in each category based on literature review and expert opinion. These categories are (1) non-diagnostic/unsatisfactory, (2) benign, (3) atypia/follicular lesion of undetermined significance (AUS/FLUS), (4) follicular neoplasm/suspicious follicular neoplasm (FN/SFN) category including Hurthle cell neoplasm/suspicious Hurthle cell neoplasm, (5) suspected malignancy (SUSP), and (6) malignancy (3).

In the Bethesta classification;

1- Non-diagnostic category represents an inadequate sample with an insufficient number of follicular cells. 2- Benign, normal thyroid tissue showing adenomatous or multinodular goiter nodules, common conditions include adenomatous nodules, Hashimoto's thyroiditis, and subacute granulomatous thyroiditis. 3- Follicular lesion of undetermined significance (FLUS) or atypia of undetermined significance (AUS): the lesions that cannot be determined to be benign are in this category. AUS includes lesions with nuclear atypia and lesions with extensive oncocytic changes, although not sufficient to be classified as a Hurthle cell neoplasm. FLUS includes a combined microfollicular and macrofollicular pattern. 4-Follicular neoplasm or suspicious follicular neoplasm includes microfollicular or cellular adenomas. Because the FNAB samples only a portion of the nodule, surgical excision is required to determine whether the microfollicular lesion is benign or malignant. Microfollicles are defined, colloid is absent or insufficient, and cells are more numerous than in macrofollicular nodules. 5- Suspicion of malignancy: It includes lesions with uncertain malignant features for thyroid cancer. 6-Malignancy: The cytology will differ according to the different types of possible thyroid malignancies. In papillary cancers, microscopy shows large cells with ground glass cytoplasm, prominent nucleoli, and intranuclear cytoplasmic inclusions. In medullary cancer microscopy shows scattered cells with eccentrically displaced nuclei and slightly granular cytoplasm often structured as a teardrop. Anaplastic cancer shows marked pleomorphism, peculiar giant cells, and spindle cells.

How should a surgeon who sees the cytological evaluation in the Bethesda classification interpret these results and what should he do? Non-diagnostic biopsies (Bethesda 1) are considered cytologically inconclusive. If insufficient follicular tissue is obtained, the absence of malignant cells should not be interpreted as a negative biopsy. The FNAB is usually repeated in 4 to 6 weeks. Patients with benign nodules (Bethesda 2) such as macrofollicular and colloid adenomas, nodular goiter, and Hashimoto's thyroiditis are usually followed up without surgery. Initially, periodic US monitoring at increasing intervals of 12 to 24 months is preferred. If US shows highly suspicious findings, FNAB should be repeated within 12 months despite a benign initial biopsy. For nodules with indeterminate cytology (Bethesda 3 and 4), the approach varies with institutional practice. Some institutions take an additional FNAB sample for molecular testing, while other centers repeat the FNAB 6 to 12 weeks later. A radionuclide scan may also be obtained if repeated aspirations show only structural atypia. For nodules in the Bethesda V category with suspected malignancy, treatment should include surgery. Molecular markers should not be used. Bethesda 6 includes papillary cancer, medullary thyroid cancer (MTC), thyroid lymphoma, anaplastic cancer, and metastatic cancer to the thyroid. In these patients, surgery is the appropriate method. [2]

Patients with a solitary nodule larger than 1 cm in multiple nodules should be evaluated in the same way. Each nodule larger than 1 cm carries an independent risk of malignancy and therefore requires a separate FNAB from each nodule.

Sonographic patterns should be considered for FNAB. The guideline recommends annual US and FNAB follow-up in patients with benign FNAB and nodules with high suspicion on US. In this group, patients with nodules with low or moderate suspicion on US can be followed up at 12-24 month intervals. If an increase in size (more than 20% increase in two different diameters or 50% increase in volume) or new suspicious nodule formation is detected during follow-up, FNAB should be applied again. US follow-up is not required in patients with nodules who have undergone two FNABs and have benign cytological results.[7]

As a result, most of the TNs seen quite frequently in the community are benign nodules. Close follow-up is important to detect cancerous nodules. The follow-up protocol should be made in accordance with the guidelines and this should be determined according to the risk status of each patient.

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Original Article

DOI: 10.14235/bas.galenos.2021.6349
Bezmialem Science 2022;10(2):135-8

The Role of Platelet Indices, Red Cell Distribution Width and the Ratios of Neutrophil to Lymphocyte, Platelet to Lymphocyte and Lymphocyte to C-reactive Protein as Markers for the Diagnosis of Acute Appendicitis in Children

Çocuklardaki Akut Apandisit Tanısında Trombosit İndeksleri ve Kırmızı Hücre Dağılım Genişliği ile Nötrofil/Lenfosit, Trombosit/Lenfosit ve Lenfosit/ C-reaktif Protein Oranlarının Rolü

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ABSTRACT

Objective: To determine the role of mean platelet volume (MPV), platelet distribution width (PDW), red cell distribution width (RDW), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and lymphocyte to C-reactive protein ratio (LCR) for the diagnosis of complicated and uncomplicated acute appendicitis (AA) in children admitted to the hospital with right lower quadrant pain.

Methods: The patients presented with right lower quadrant abdominal pain were grouped as acute uncomplicated appendicitis (Group 1), acute complicated appendicitis (Group 2) and right lower quadrant abdominal pain-other than appendicitis (Group 3) groups.

Results: In this retrospective study, 1,050 patients were included. There was not a statistically significant difference regarding age and gender between groups (p=0.555 and p=0.641, respectively). No significant difference was observed in the comparison of all groups and between-groups in terms of all laboratory parameters (MPV, p=0.528; PDW, p=0.181; RDW, p=0.479; NLR, p=0.953; PLR, p=0.848; LCR, p=0.535)

Conclusion: It was shown that MPV, PDW, RDW, NLR, PLR and LCR aided neither in the differential diagnosis of patients with

ÖZ

Amaç: Sağ alt kadran ağrısı ile hastaneye başvuran hastalarda komplike olan ve olmayan akut apandisit (AA) tanısını koymada ortalama trombosit hacmi (MPV), trombosit dağılım genişliği (PDW), kırmızı hücre dağılım genişliği (RDW), nötrofil/lenfosit oranı (NLR), trombosit/lenfosit oranı (PLR) ve lenfosit/C-reaktif protein (LCR) oranının rolünü belirlemek.

Yöntemler: Sağ alt kadran ağrısı ile başvuran hastalar akut komplike olmayan apandisit (Grup 1), akut komplike apandisit (Grup 2) ve apandisit dışında sağ alt kadran karın ağrısı olanlar (Grup 3) şeklinde gruplandırıldı.

Bulgular: Bu retrospektif çalışmaya sağ alt kadran karın ağrısı nedeniyle başvuran ardışık 1.050 hasta dahil edildi. Gruplar arasında yaş ve cinsiyet açısından istatistiksel olarak anlamlı bir fark yoktu (sırasıyla; p=0,555 ve p=0,641). Tüm laboratuvar parametreleri açısından tüm gruplar arasında ve gruplar ikişerli karşılaştırıldığında anlamlı farklılık gözlenmedi (MPV, p=0,528; PDW, p=0,181; RDW, p=0,479; NLR, p=0,953; PLR, p=0,848; LCR, p=0,535).

Sonuç: Bu çalışmada; MPV, PDW, RDW, NLR, PLR ve LCR'nin hem sağ alt kadran ağrısı olan olgularının ayırıcı tanısına hem de pediyatrik akut komplike ve komplike olmayan apandisit olgularının ayırt edilmesine yardımcı olmadığı gösterilmiştir. Bunun nedeni,

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Cite this article as: Süzen A. The Role of Platelet Indices, Red Cell Distribution Width and the Ratios of Neutrophil to Lymphocyte, Platelet to Lymphocyte and Lymphocyte to C-reactive Protein as Markers for the Diagnosis of Acute Appendicitis in Children. Bezmialem Science 2022;10(2):135-8

Received: 26.04.2021
Accepted: 17.06.2021

right lower quadrant pain nor in the discrimination of pediatric acute complicated and uncomplicated appendicitispatients. The explanation for this might be that complete blood cell count could be affected by an inflammatory process other than AA and other characteristics such as anemia, age and sex. Clinical observation and diagnostic imaging modalities should still be the most reliable diagnostic methods in acute appendicitis. The roles of these parameters in the diagnosis of AA can be understood more clearly with multi-center prospective studies with larger sample sizes.

Keywords: Mean platelet volume, platelet distribution width, red cell distribution width, acute appendicitis, children, right lower quadrant pain

tam kan sayımının AA dışındaki bir enflamatuar süreçten veya anemi, yaş ve cinsiyet gibi diğer bozukluk ve özelliklerden etkilenmiş olması olabilir. AA'da klinik gözlem ve tanısal görüntüleme yöntemleri hala en güvenilir tanı yöntemleri olmalıdır. AA tanısında bu parametrelerin rolleri, daha geniş örneklem büyüklüğüne sahip çok merkezli prospektif çalışmalarla daha net anlaşılabilir.

Anahtar Sözcükler: Ortalama trombosit hacmi, trombosit dağılım genişliği, kırmızı hücre dağılım genişliği, akut apandisit, çocuklar, sağ alt kadran ağrısı

Introduction

Acute appendicitis (AA) is one of the most common causes of acute abdomen. Mostly, the diagnosis is easy due to well-known typical symptoms and clinical findings of AA. However, sometimes these symptoms and clinical findings are not clear. A number of diseases mimic AA and misdiagnosis can lead to negative appendectomy at a rate of 5-42% (1). On the other hand, it is important to diagnose AA rapidly and accurately because diagnostic delay is associated with perforation and increased complication rate (2,3).

In recent years, radiological imaging methods such as ultrasound, computerized tomography and magnetic resonance imaging have been used frequently in diagnosis to improve diagnostic accuracy in patients with suspected AA, but they are not completely sufficient (2,3). Moreover, these methods can be expensive and sometimes not available in emergency departments. Also, the patients are exposed to radiation and contrast allergy may occur in computed tomography (3).

Increased white blood cell and neutrophil counts are the earliest indicators of inflammation in AA with low sensitivity and specificity (2,3). Therefore, new biomarkers are needed for diagnosis. Mean platelet volume (MPV), platelet distribution width (PDW) and red cell distribution width (RDW) are the parameters of complete blood cell count (CBC) calculated automatically. MPV, PDW and RDW have been shown as markers of many inflammatory conditions such as inflammatory bowel disease, celiac disease, acute pancreatitis, rheumatoid arthritis, ankylosing spondylitis, and familial Mediterranean fever (4-7). Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and lymphocyte to C-reactive protein (CRP) ratio (LCR) are parameters that have been studied as the predictive factors of AA in several articles and can be determined simply by using CBC and CRP (8-10).

In this study, it was aimed to determine the roles of all these laboratory parameters for the diagnosis of complicated and uncomplicated AA in children admitted to the hospital with right lower quadrant pain.

Methods

This retrospective study was conducted on children who were admitted to Muğla Sıtkı Koçman University Training and Research Hospital, Muğla, Turkey with right lower quadrant pain between January 2018 and December 2019. The study was approved by the local ethics committee on 23.07.2020 with the decision number 160.

The participants were divided into three groups as:

Group 1: Patients with acute uncomplicated appendicitis

Group 2: Patients with acute complicated appendicitis (patients with perforated or gangrenous appendix)

Group 3: Patients with right lower quadrant pain- other than AA

The patients with a history of peripheral vascular disease, hematological disease, acute or chronic infection, and anticoagulant or steroid usage were excluded from the study.

Venous blood samples of the children were collected during admission using the Standard venipuncture technique to analyze CBC and CRP. The Cell-Dyn 3700 Haematology analyzer (Abbott Diagnostics, USA) was used to assess CBC measurements. The reference intervals of MPV, PDW and RDW levels were accepted as 9.4-12.3 fL, 9.9-15.4 fL and 36.4-46.3 fL, respectively. CRP concentrations were quantified with a dual-radius enhanced latex techonology (Cobas C 501 Roche Diagnostics, Rotkreuz, Switzerland).

Statistical Analysis

The SPSS (SPSS Inc, version 22.0 software, Chicago, Illionis, USA) was used for statistical analysis. The results were expressed as mean ± standard deviation. One-Way analysis of variance (ANOVA) was performed to compare age, levels and ratios of parameters which were normally distributed among groups. Kruskal-Wallis test was used to compare levels and ratios of parameters which were not normally distributed among groups. $\chi 2$ test was performed to compare gender. For comparison of groups with each other, post-hoc tests were performed. $p<0.05$ was considered statistically significant.

Results

Records of 1,050 patients were evaluated. Of these, 110 (10.48%) had uncomplicated AA, 37 (3.52%) had complicated AA, and 903 (86%) did not have AA. The demographic features

of the groups are presented in Table 1 and the comparisons of laboratory parameters of all groups are shown in Table 2. There was not a statistically significant difference regarding age and gender between groups. No significant difference was observed in the comparison of all groups and between-groups in terms of all laboratory parameters.

Discussion

Although AA is one of the most common causes of abdominal pain that requires surgical interventions in childhood, the diagnosis still remains a challenge despite clinical examination, laboratory tests and radiological studies (1-3). The need for a novel, widely-available and inexpensive laboratory marker is obvious to decrease morbidity rates due to either negative appendectomies or complications such as perforation, wound infection, bowel obstruction, abdominal abscess and sepsis. Inexpensive, readily available and convenient inflammatory markers presented in CBC have good potential for the differential diagnosis of AA with these properties. On the other hand, numerous conflicting results have been reported about various inflammatory markers and ratios determined simply by using CBC and CRP in the diagnosis of AA. This is the first study comparing all these parameters in the diagnosis of AA.

In this study investigating the diagnostic value of MPV, PDW, RDW, NLR, PLR and LCR in complicated and uncomplicated AA, we did not determine the role of these parameters in diagnosis. Although MPV, PDW and RDW levels were higher in complicated and uncomplicated appendicitis groups, the difference between groups was not statistically significant. Furthermore, in patients with right lower quadrant pain but not having AA, LCR was higher than complicated and uncomplicated AA groups. This was not statistically significant either.

The MPV is a measurement of the average size of platelets in the blood, whereas the PDW is a measurement of platelet anisocytosis. Numerous contradictory results have been observed about the diagnostic roles of MPV and PDW in patients with right lower quadrant pain. Some studies reported that MPV increased in AA, while others found that MPV was lower (11-16). Meanwhile, Uyanik et al. (3) suggested that MPV had no diagnostic value in children with AA. Also, Yigit et al. (2) and Ceylan et al. (15) found no difference in terms of PDW between patients with and without AA. Similarly, in the present study, there were no statistically significant differences between the groups in terms of MPV and PDW.

The RDW is an automated measure of the heterogeneity of red blood cell size. Alteration of RDW level has been found in some inflammatory disorders and infections (5,6). Daldal and Dagmura (17) reported that RDW level was significantly lower in patients with AA. On the other hand, Boshnak et al. (8) and Tanrikulu et al. (13) did not findany significant diagnostic value of RDW in AA, similiar to our result.

The role of NLR in AA was examined in a few studies. Daldal and Dagmura (17) found that NLR was significantly higher in patients with AA than patients without AA. Khan et al. (18) suggested NLR was a predictor of severity of AA. On the other hand, there was no statistically significant difference between the studied groups with respect to NLR in the current study.

Table 1. Summary of patients' characteristics

	Uncomplicated appendicitis (n=110)	Complicated appendicitis (n=37)	Non-appendicitis (n=903)	p
Age (mean ± SD)	12.03±4.92 years	11.73±5.06 years	11.49±5.03 years	0.555
Gender (M/F) (%)	46/64 (41.8/58.2)	15/22 (40.5/59.5)	412/491 (45.6/54.4)	0.641

F: Female, M: Male, SD: Standard deviation

Table 2. Comparison of groups regarding the parameters (mean ± SD)

Parameter	Uncomplicated appendicitis n=110	Complicated appendicitis n=37	Non-appendicitis n=903	p	p1	p2	p3
MPV (fL)	10.03±0.99	10.22±1.22	10.02±1.06	0.528	1	0.778	1
PDW (fL)	11.71±3.20	12.22±3.13	11.51±2.32	0.181	1	0.262	0.845
RDW (fL)	38.07±6.61	39.4±7.31	37.72±3.73	0.479	1	0.058	0.305
NLR	4.03±7.25	3.82±4.02	3.85±5.67	0.953	1	1	1
PLR	148.03±68.31	157.54±99.23	151.71±92.9	0.848	1	1	1
LCR	1.82±2.77	1.91±4.22	2.46±6.45	0.535	0.914	1	1

p= Comparision of all groups
p1= Comparison between acute appendicitis and non appandicitis
p2= Comparison between perforated appendicitis and non-appendicitis
p3= Comparison between acute appendicitis and perfotated appendicitis
MPV: Mean platelet volume, PDW: Platelet distribution width, RDW: Red cell distribution width, NLR: Neutrophile-to-lympocyte ratio, PLR: Platelet to lymphocyte ratio, LCR: lymphocyte to C-reactive protein ratio, SD: Standard deviation

The findings of studies examining the association between the PLR and AA have been controversial. Liu et al. (9) systematically reviewed the literature and found a significant increase in PLR levels in adults with AA with age ≥30 years compared to those in adults with AA with age <30 years or in children with AA. This is consistent with our result.

The ratio of lymphocyte to CRP has recently been introduced as a new parameter in differentiating AA (10). We didn't find a significant diagnostic value of LCR, similiar to the result of Daldal and Dagmura (17).

Study Limitations

This was a retrospective study. The roles of these parameters in the diagnosis of AA could be understood more clearly with multi-center prospective studies with larger sample sizes.

Conclusion

The MPV, PDW, RDW, NLR, PLR and LCR are far from being significant predictors in the differential diagnosis of AA. The explanation for this might be that CBC could be affected by an inflammatory process other than AA and other disorders and characteristics such as anemia, diet, age and sex. Clinical observation and diagnostic imaging modalities should still be the most reliable diagnostic methods in AA.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee on 23.07.2020 with the decision number 160.

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Financial Disclosure: The author declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5279
Bezmialem Science 2022;10(2):139-43

Could Vitamin K1 Deficiency be the Problem in Iron Deficiency and/or Anemia in Premenopausal Women?

Premenopozal Kadınlarda, Demir Eksikliği ve/veya Anemisinde Sorun Vitamin K1 Yetersizliği Olabilir Mi?

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ABSTRACT

Objective: The etiology of iron deficiency anemia, which develops as a result of menstrual bleeding in the premenopausal period, is unknown. Vitamin K1 has an important role in the coagulation cascade and is not a well known vitamin. The aim of this study was to investigate whether or not Vitamin K1 had a role in anemia developing in the premenopausal period, for which no additional reason could be found.

Methods: This study included a patient group of women aged 18-50 years, who had a regular menstrual cycle. Patients who were found to have iron deficiency, who were evaluated hematologically, gastrointestinally and gynecologically, and who did not have a pathology that would lead to iron deficiency were included in the study group.The control group comprised volunteers with regular menstrual cycles who had not been previously determined with iron deficiency. In the study, Vitamin K1, Hemogram, ferritin, iron, total iron binding capacity were examined. The Vitamin K1 level was measured by two different methods both using ELISA and liquid chromatography-mass spectrometry (LC-MS/MS) methods. In addition, a record was made for all participants including demographic characteristics, lifestyle habits, and number of menstruating days. The obtained data were then compared between the groups.

Results: A total of 88 voluntary participants were included in the study as 45 patients with iron deficiency anemia (IDA) and

ÖZ

Amaç: Premenopozal dönemdeki menstrüasyon kanamaları sonucu gelişen demir eksikliği anemisinin önemli bir kısmında etiyoloji bilinmemektedir. Vitamin K1 ise koagülasyon kaskatında önemli görevleri olan ve az bilinen bir vitamindir. Amacımız premenapozal dönemde anemi gelişen ve araştırmalara rağmen ek bir neden saptanmayan gönüllülerde Vitamin K1'in rolünün olup olmadığını araştırmaktır.

Yöntemler: Çalışmaya menapoza girmemiş, düzenli adet gören, 18-50 yaş arası kadınlar alınmıştır. Çalışma grubuna demir eksikliği saptanan, hematolojik, gastrointestinal ve jinekolojik olarak değerlendirilmiş ve demir eksikliğine yol açacak bir patoloji saptanmamış hastalar alınmıştır. Kontrol grubuna ise daha önce demir eksikliği saptanmamış ve düzenli adet gören, gönüllü kadınlar alınmıştır. Çalışmada Vitamin K1, hemogram, ferritin, demir, total demir bağlama kapasitesi bakılmıştır. Vitamin K1 düzeyi ELISA ve sıvı kromatografisi-kütle spektrometresi (LC-MS/MS) kitleri kullanılarak iki farklı yöntemle çalışılmıştır. Ayrıca hastaların demografik özellikleri, alışkanlıkları ve menstrüasyon gün sayısı sorgulanarak kaydedilmiştir. Toplanan veriler karşılaştırılmıştır.

Bulgular: Çalışmaya 45'i demir eksikliği anemisi (DEA) olan, 43'ü de kontrol olmak üzere toplam 88 katılımcı alındı. Çalışmaya katılan her iki grupta yaş, beden kitle indeksi, protrombin zamanı, Uluslararası düzeltme oranı, aktif parsiyel tromboplastin zamanı, folik asit ve Vitamin B12 ortalamaları benzer bulundu. Çalışmada

**This study was presented at 19th National Internal Diseases Congress.*

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Cite this article as: Karatoprak C, Şekerci A, Karaaslan T, Olgaç A, Özer ÖF, Selek Ş, Köktaşoğlu F, Ekinci İ. Could Vitamin K1 Deficiency be the Problem in Iron Deficiency and/or Anemia in Premenopausal Women? Bezmialem Science 2022;10(2):139-43

Received: 30.08.2020
Accepted: 21.04.2021

a control group of 43 subjects. The age, body mass index, partial thromboplastin, International normalized ratio, active partial thromboplastin time, folic acid, and Vitamin B12 values were similar in both groups. In both methods, no significant difference was determined between the groups in respect of the Vitamin K1 level (p=0.9 in ELISA method and p=0.3 in LC-MS/MS method). The number of menstruation days was determined to be significantly higher in the anemic group than in the control group (p=0.002).

Conclusion: From the results of this study, it was considered that IDA developed in premenopausal women with a longer period of menstrual bleeding. However, Vitamin K1 deficiency was not considered to be one of the underlying reasons for longer menstrual bleeding.

Keywords: Vitamin K1, menstruation, iron deficiency anemia

Vitamin K1 düzeyi ELISA yöntemi ile değerlendirildi ve gruplar arasında anlamlı fark saptanmadı (p=0,9). LC-MS/MS yöntemi kullanılarak ölçülen Vitamin K1 sonuçları karşılaştırıldığında da gruplar arasında fark olmadığı görüldü (p=0,3). Çalışmada menstrüasyon gün sayısı anemik grupta anlamlı olarak daha uzun saptandı (p=0,002).

Sonuç: Premenapozal dönemdeki kadınların menstrüasyon kanamaları daha uzun sürdüğü için DEA geliştiğini düşünmekteyiz. Ancak daha uzun menstrüasyon kanaması nedenlerinden birinin Vitamin K1 yetersizliği veya eksikliği olduğu gösterilemedi.

Anahtar Sözcükler: Vitamin K1, menstrüasyon, demir eksikliği anemisi

Introduction

Iron deficiency anemia (IDA) is seen in one third of women in the menstrual period (1). Excessive menstrual bleeding and menstrual irregularities developing associated with gynaecological problems in the premenopausal period, absorption problems or losses originating from the gastrointestinal system, and hematological problems such as factor or vitamin deficiencies are thought to be the most common causes of iron deficiency or IDA. However, despite similar nutrition and lifestyles, it is not known why IDA develops in some menstruating women (2). A significant proportion of this anemia of unknown cause is seen to improve with menopause (3).

When the causes of excessive menstrual bleeding have been investigated, no organic pathology can be determined in at least 50% of patients (4). Although many studies have been conducted on the etiology in this subject, the effect of Vitamin K1 level on menstrual bleeding has not been researched. Vitamin K has an important role in the activation of Factors II, VII, IX and X, protein C and protein S (5). As the body does not synthesize this vitamin, it cannot be stored in large amounts, and acquired Vitamin K1 deficiency can develop in adults and excessive menstrual bleeding may be seen (5). When Vitamin K1 deficiency develops, first Factor VII decreases, prolonging the prothrombin time/international normalized ratio (PT/INR). When Vitamin K deficiency continues, the active partial thromboblastin time (aPTT) can prolong as other factors decrease. However, when Vitamin K1 deficiency develops and significant reductions in factor levels are not seen, it is not known whether or not bleeding increases without a longer PT/INR. In previous observations in our clinic, it was determined that in patients with iron deficiency continuously taking iron supplementation, when the Vitamin K level was at the lower limits, the PT/INR level was within the normal range.

The aim of this study was to investigate whether or not there was a difference in terms of Vitamin K1 level between a control group and patients with IDA, for which no additional reason could be determined, and which was thought to develop associated with menstrual bleeding.

Methods

This cross-sectional study included a patient group of women aged 18-50 years, who had not entered the menopause and had a regular menstrual cycle, and who had been determined with iron deficiency, for which no pathology could be determined from hematological, gastrointestinal and gynaecological evaluations. The control group comprised volunteers with regular menstrual cycles who had not entered the menopause and who had not been previously diagnosed with iron deficiency and had therefore never received any treatment. Patients or control group subjects were excluded from the study if they had any known disease or were taking any drugs, if they had prolonged PT/INR anda PTT tests, von Willebrand's disease, low thrombocyte count or function impairment, or had any abnormality in terms of Vitamin B12, folic acid, celiac antibody tests, stool occult blood test or full urine analysis. In addition, those using any method of birth control, who were pregnant or breastfeeding, were vegetarian or vegan, or who did not wish to participate in the study, were excluded. Vitamin K1, hemogram, ferritin, iron, and total iron-binding capacity were examined in all participants. To obtain more reliable results, Vitamin K1 levels were examined separately with two different methods. In addition, a record was made for all participants including demographic characteristics, lifestyle habits, and number of menstruating days. To determine the response to oral iron treatment, the hemoglobin and ferritin levels of the anemia group were evaluated after 3 months. The obtained data were then compared between the groups.

Procedure Technique: Vitamin K1: Venous blood samples were taken into 2 separate non-gel vacuum tubes (Becton Dickinson) wrapped in silver foil to prevent any effect from light, and the Vitamin K1 levels were examined with 2 different methods. Within 30 mins, the samples were centrifuged at 1,600 g for 15 mins at 4 ˚C. The serums were separated and stored at -80 ˚C until assay. Hemolytic and lipemic samples were not included in the study analyses. The Human Vitamin K1 levels were measured with an ELISA using commercial kits (Elabscience; Lot no:AK0018MAR21043; PRC) and an ELISA reader (Multiskan FC® Microplate Photometer; Thermo Scientific; USA). The results were expressed in 100-2,100 pg/mL.

The other sample taken from the same patients was examined with the liquid chromatography-mass spectrometry (LC-MS/MS) method to measure the Vitamin K1 level. The normal range was accepted as 0.1-2.1 ng/mL.

Approval for the study was granted by the Ethics Committee of Bezmialem Vakıf University (decision no: 71306642-050.01.04). The costs of the tests applied were met by the Scientific Research Program fund. Informed consent was obtained from all the study participants.

Statistical Analysis

Data obtained in the study were analysed statistically using SPSS for Windows vn 16.0 software (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL, USA). In addition to definitive statistical methods (mean, median, standard deviation), the Student's t-test was used to compare normally distributed parameters, and the Mann-Whitney U test for those not conforming to normal distribution. In the comparison of numerical data, the Pearson correlation coefficient test was used. A two sided p value of <0.05 was considered statistically significant.

Results

A total of 88 voluntary participants were included in the study as 45 patients with IDA and a control group of 43 subjects with a mean age of 34.3±6.7 years (range, 21-49 years). The age, body mass index, PT, INR, aPTT, folic acid, and Vitamin B12 values were similar in both groups, with no statistically significant difference determined (Table 1). The Vitamin K1 level was measured using both the ELISA and the LC-MS/MS methods. In both methods, no significant difference was determined between the groups in respect of the Vitamin K1 level (Table 2).

Using both methods, no correlation was observed between Vitamin K1 and hemoglobin, ferritin, transferrin saturation, INR, and PT (Table 3). The number of menstruation days was determined to be significantly higher in the anemic group than in the control group (p=0.002) (Table 2). A significant correlation was determined between the number of menstruation days and ferritin. As the number of menstruation days increased, the serum ferritin level was found to decrease (r=-0.34, p=0.003). The hemoglobin and ferritin levels of all the patients in the iron deficiency group were determined to increase with oral iron treatment.

Table 1. Demographic data and laboratory test results of the anemic group and the control group

	Anemic group (45)	Control grup (43)	p
Age (years)	35.6±6.5	32.9±6.8	0.07
BMI (kg/m²)	24.3±3.3	23.1±3.3	0.1
PT (sn) (10.8-15.3)	13.7±0.8	13.9±0.8	0.15
INR (0.8-1.2)	1.05±0.08	1.07±0.08	0.3
aPTT (sn) (24-42)	30.4±2.7	31.4±3	0.1
Folic acid (pg/mL)	6.9±2.6	5.9±1.6	0.06
Vitamin B12 (ng/mL)	321.2±110	324±146	0.9

BMI: Body mass index, PT: Prothrombin time, aPTT: Active partial prothrombin time, INR: International normal range

Discussion

Although seen in 30% of women of reproductive age, iron deficiency and/or anemia caused by menorrhagia is not fully understood. This means that the majority of these women have to use various iron preparates until menopause. These drugs have many unwanted effects, and a significant proportion of these women undergo hysterectomy before the age of 60 years (4). The basic underlying cause of this problem that affects a large section of society remains unknown.

Table 2. Comparisons of values between the anemic group and the control group

	Anemic group (n=45)	Control group (n=43)	p
Hemoglobin (g/dL)	9.5±1.3	13.3±0.7	0.001
MCV (fL)	70.3±6.5	86.9±3.7	0.001
Thrombocyte (10*³/uL)	288±102	246±49	0.005
Ferritin (ng/mL) (4.63-204)	4.4±3	31.9±19.1	0.001
Iron (ug/dL) (50-170)	19.9±6.2	92.3±30.8	0.001
TIBC (ug/dL) (250-425)	425.9±46.6	324.3±52.1	0.001
Transferrin saturation (%)	4.7±1.4	29.5±11.5	0.001
No of menstrual bleeding days	7.2±2.2	5.9±1.8	0.002
Vitamin K1 (ng/mL) (LC-MS/MS)	0.27±0.16	0.23±0.17	0.3
Vitamin K1 (pg/mL)/ELISA)	104±38	103±39	0.9

MCV: Mean corpusculer volume, TIBC: Totaliron binding capasity, LC-MS/MS: Liquid chromatography-mass spectrometry

Table 3. Correlation analyses related to Vitamin K1

Vitamin K1 ELISA	r	p
Hemoglobin (g/dL)	-0.16	0.17
Ferritin (g/dL)	-0.06	0.65
Transferrin saturation (%)	-0.04	0.73
INR (0.8-1.2)	-0.09	0.45
PT (sn)	0.004	0.97
Vitamin K1 (LC-MS/MS)	r	p
Hemoglobin (g/dL)	-0.08	0.46
Ferritin (g/dL)	0.016	0.9
Transferrin saturation (%)	-0.19	0.1
INR	-0.05	0.64
PT (sn)	-0.06	0.58

PT: Prothrombin time, INR: International normal range, LC-MS/MS: Liquid chromatography-mass spectrometry

Vitamin K is known to be a vitamin that cannot be directly synthesized in the body. Vitamin K1 is synthesized with nutrients and Vitamin K2 more by the bacterial flora in the intestinal system. Vitamin K provides activation of the coagulation cascade with clotting. When this vitamin is insufficient, firstly prolongation of PT (INR) is seen because of the shortened half-life of Factor 7, and in more severe deficiencies, prolongation of aPTT may be seen as Factors II, IX and X are affected (6). Various bleeding disorders may be seen in the neonatal period because of Vitamin K1 deficiency passing from mother to infant and insufficient synthesis as the intestinal flora has not developed (7).

However, these disorders are believed to be rarely seen in adults, as in addition to the wide availability of leafy green vegetables throughout the world, it can be synthesised in the intestines. Nevertheless, it may be seen in adults taking Vitamin K antagonists, those with chronic liver disease, fat absorption disorder, and those with long-term use of drugs similar to antibiotics which disrupt the intestinal flora (8,9). However, when there is subclinical Vitamin K deficiency without factor deficiencies reaching a severe level, the PT (INR) level remains within the normal range. In this situation, Vitamin K deficiency may cause a slight increase in bleeding or subcutaneous bleeding. Thus, more sensitive methods than INR should be used to determine subclinical Vitamin K deficiency (9). To this end, Vitamin K1 and K2 levels have been examined where there is a relationship between osteoporosis and atherosclerosis (8). It is thought that Vitamin K2 corrects bleeding diathesis by supporting Vitamin K1 (10). Deficiency of Vitamin K2 in particular is seen more often in some countries with different nutritional habits and causes more diseases such as osteoporosis (11). The prevalence in Turkey is unknown as to the best of our knowledge there have been no studies conducted on this subject.

To be able to obtain more reliable results in the current study, the Vitamin K levels were examined with the two most frequently used methods with the highest proven reliability. In both methods, when the Vitamin K level was determined below the accepted reference range of 0.1-2.1 ng/mL (100-2,100 pg/mL), the INR (PT) level was determined within the normal reference range. This suggested that subclinical Vitamin K deficiency was widespread and the study hypothesis could be correct. However, when compared with the control group of those with normal menstruation who had never developed iron deficiency, the mean Vitamin K1 values were found to be similar. These results could be explained by 3 different hypotheses.

First, not all Vitamin K was included in the current study as only the Vitamin K1 level was examined. Therefore, a sufficient Vitamin K2 level might be compensated for those with a low Vitamin K1 level. Or when Vitamin K1 was at the lower limit but within the reference range, Vitamin K2 might be insufficient. Due to these types of variations, the results might be misleading. If both Vitamin K1 and Vitamin K2 had been examined in this study, the results would have been more comprehensive, and results might support the hypothesis. Therefore, this study should be considered a preliminary study for further research, and as such, sheds light on future studies.

Second was that Vitamin K analyses were extremely complex and difficult tests, which might be affected by several factors. As there were no reference tests with proven international reliability, the tests were not well known and were performed in few centres, this could affect the results and made it difficult to reach accurate results (8,12). Although the utmost care was taken to obtain robust results and two different methods were used, the results were not statistically significant. In previous studies conducted on Vitamin K1, the results were found to be conflicting (8,13,14).

Finally, the Vitamin K1 level might not have an effect on longer duration of menstrual bleeding, as found in the current study, but as no similar studies could be found in literature, it was not possible to make comparisons.

In the patients followed up throughout the current study, the iron deficiency was observed to be easily corrected with oral iron treatment. Thus, it was determined that these women were not able to provide sufficient iron in the body as they lost more of the required nutritional iron intake in comparison to the control group. In each menstrual cycle, an extra 10 mg iron is lost, and in those with heavy bleeding this amount can increase to 42 mg (15). Therefore, every month, reserves are used up after a time and this can lead to the development of iron deficiency again. In the current study, the number of menstrual bleeding days was found to be higher in the iron deficiency group than in the control group. Moreover, as the number of menstrual days increased, so the level of serum ferritin was determined to decrease. However, Vitamin K deficiency could not be shown as the reason for an increasing number of menstrual bleeding days leading to iron deficiency. These results suggest that there could be one of two possibilities or the two together.

The first possibility is that when menstrual bleeding increases, the iron absorption capacity does not increase. Iron is absorbed in the duodenum and jejunum in the intestines. While iron absorption increases with ascorbic acid (Vitamin C) and meat, it decreases with the intake of calcium, fibre, tea and coffee (15). This type of effect was not expected in the current study as the patients and control group were of the same culture with the same nutritional conditions.

The other possibility is that as proposed in the current study, there is a disorder causing the longer duration of bleeding. Although no relationship could be shown between Vitamin K level and menstrual bleeding, it could be considered a disorder prolonging menstrual bleeding. This is supported by the fact that iron deficiency seen in the premenopausal period is corrected after menopause (16). It can also be that there is another factor like Vitamin K, which has not yet been determined, which affects the coagulation mechanism and contributes to the longer duration of bleeding.

Study Limitations

There were some limitations to this study, primarily that not all types of Vitamin K (K1, K2) were measured. Another limitation was that the amount of menstrual bleeding of the women in the study was not measured. Although Vitamin K levels were

detected too low to be measured, subjects with non-prolonged PT (INR) were determined in both groups. However, the reason that INR was not prolonged in this situation or why the bleeding of the control group was not prolonged, could not be explained.

Conclusion

In women developing iron deficiency and/or anemia because of menstrual bleeding, there is thought to be a greater loss of iron because of a longer duration of menstrual bleeding. However, no relationship could be determined between Vitamin K1 level and iron deficiency and/or anemia developing in pre-menopausal women. We believe that further research is needed about what is the factor that increases and prolongs menstrual bleeding without changing bleeding tests and Vitamin K1 levels.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Ethics Committee of Bezmialem Vakıf University (decision no: 71306642-050.01.04).

Informed Consent: Informed consent was obtained from all the study participants.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: C.K., A.Ş., T.K., A.O., Ö.F.Ö., Ş.S., F.K., İ.E., Design: C.K., A.Ş., T.K., A.O., Ö.F.Ö., Ş.S., F.K., İ.E., Data Collection or Processing: C.K., A.Ş., T.K., A.O., Ö.F.Ö., Ş.S., F.K., İ.E., Analysis or Interpretation: C.K., A.Ş., T.K., A.O., Ö.F.Ö., Ş.S., F.K., İ.E., Literature Search: C.K., A.Ş., T.K., A.O., Ö.F.Ö., Ş.S., F.K., İ.E., Writing: C.K., A.Ş., T.K., A.O., Ö.F.Ö., Ş.S., F.K., İ.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: Financial Disclosure: The authors declared that support was received from BAP Coordination Unit of Bezmialem Vakıf University and project code: 12.2017/23.

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Original Article

DOI: 10.14235/bas.galenos.2021.6113
Bezmialem Science 2022;10(2):144-9

The Effect of Exogenous Human Albumin Administration on Acute Kidney Injury Development in Hypoalbuminemic Patients in the Intensive Care Unit

Yoğun Bakım Ünitesindeki Hipoalbüminemik Hastalarda Eksojen Human Albümin Uygulamasının Akut Böbrek Hasarı Gelişimine Etkisi

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ABSTRACT

Objective: Hypoalbuminemia is an independent risk factor for acute kidney injury (AKI) and mortality. The primary aim of our study was to investigate the effect of exogenous human albumin (EHA) administration on hypoalbuminemic patients in the intensive care unit (ICU) regarding the development of AKI. Our secondary aim was to compare the ICU admission duration and mortality rates of these patients.

Methods: After receiving ethics committee approval, the researchers retrospectively screened database for 5,989 patients admitted to the adult ICU from 01.01.2014 to 01.06.2018. The demographic data, serum albumin and creatinine levels, ICU admission duration and mortality rates of patients were recorded. Stage 2-3 AKI was accepted based on the AKI network criteria, while hypoalbuminemia was accepted as serum albumin values below 3.5 g/dL. Patients not given EHA were assigned to group none human albumin (Group NHA), while patients given EHA were assigned to group human albumin (Group HA). The rate of AKI development, duration of stay in ICU and mortality rates were compared between the groups.

Results: The mean age, AKI development rate, mortality rate and ICU admission duration in Group HA were statistically significantly higher than in Group NHA (p=0.0001, p=0.0001, p=0.0001, p=0.0001). There was no difference in terms of the gender distribution in the groups. The mean albumin value in Group HA was statistically significantly lower than Group NHA (p=0.0001).

ÖZ

Amaç: Hipoalbüminemi, akut böbrek hasarı (ABH) ve mortalite için bağımsız bir risk faktörüdür. Çalışmamızın temel amacı, yoğun bakım ünitesinde (YBÜ) izlenen hipoalbüminemik hastalarda eksojen human albümin (EHA) uygulamasının ABH gelişimi üzerine etkisini araştırmaktı. İkincil amaçlarımız ise YBÜ'de kalış süresi ile mortalite oranlarını karşılaştırmaktı.

Yöntemler: Etik kurul onayı alındıktan sonra 01.01.2014-01.06.2018 tarihleri arasında erişkin YBÜ'de izlenen 5.989 hastayı retrospektif olarak inceledik. Hastaların demografik verileri, serum albümin ve kreatinin düzeyleri, YBÜ başvuru süreleri ve mortalite oranları kaydedildi. ABH ağı kriterlerine göre evre 2-3 ABH olarak kabul edilirken, 3,5 g/dL'nin altındaki serum albümin değeri hipoalbüminemi olarak kabul edildi. EHA verilmeyen hastalar Grup NHA, EHA verilen hastalar Grup HA olarak adlandırıldı. ABH gelişimi, YBÜ'de kalış süresi ve mortalite oranları gruplar arasında karşılaştırıldı.

Bulgular: Grup HA'daki ortalama yaş, ABH gelişimi, mortalite oranları ve YBÜ'de kalış süresi Grup NHA'ya göre istatistiksel olarak anlamlı derecede yüksekti (p=0,0001, p=0,0001, p=0,0001, p=0,0001). Gruplar arasında cinsiyet dağılımında herhangi bir fark yoktu. Grup HA'daki ortalama albümin değerleri Grup NHA'dan istatistiksel olarak anlamlı derecede düşüktü (p=0,0001).

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Cite this article as: Yeşiltaş S, Güzel C, Sümer İ, Uysal H, Daşkaya H, Türkay M, Karaaslan K. The Effect of Exogenous Human Albumin Administration on Acute Kidney Injury Development in Hypoalbuminemic Patients in the Intensive Care Unit. Bezmialem Science 2022;10(2):144-9

Received: 27.02.2021
Accepted: 01.05.2021

Conclusion: In conclusion, EHA administration in hypoalbuminemic patients prolong stay in ICU in addition to the increase in the development of AKI and mortality.

Keywords: Acute kidney injury, exogenous human albumin, hypoalbuminemia, intensive care units

Sonuç: Sonuç olarak, hipoalbüminemik hastalarda EHA uygulaması ABH ve mortalitenin artmasına ek olarak YBÜ'de kalış süresini uzatmaktadır.

Anahtar Sözcükler: Akut böbrek hasarı, eksojen human albümin, hipoalbüminemi, yoğun bakım üniteleri

Introduction

The global health problem of acute kidney injury (AKI) affects millions of people each year (1). AKI, a syndrome characterised by rapid disruption of renal functions within hours or days, has multifactorial pathogenesis. A variety of changeable (dehydration, intravascular volume loss, hypotension, anaemia, hypoxia and body mass index) or unchangeable (age, gender, invasive interventions, high-risk surgeries, cancer and comorbidities, such as lung, liver or gastrointestinal pathologies) risk factors are reported in the aetiology (2). AKI develops more often in critically ill patients compared to the general population due to increased risk factors. Early diagnosis of risk factors improves prognosis for critically ill patients (3). Serum albumin concentration of less than 3.5 g/dL, defined as hypoalbuminemia, is an independent risk factor for AKI development (3,4). Albumin comprises 60% of plasma proteins and is multifunctional (5). Albumin is the main determinant of colloid osmotic pressure and is an important extracellular non-enzymatic antioxidant that regulates capillary membrane permeability (5,6). Endogenous albumin has a protective effect on the kidney and is proposed to play a role in the integrity of proximal tubule cells and to maintain functions through a variety of signal transduction pathways (3,7). The protective effect in the kidney is thought to be due to scavenging reactive oxygen fragments, preventing oxidative injury via lysophosphatidic protective acid distribution and binding and reducing nephrotoxicity caused by interleukin-2 (3). Administration of exogenous human albumin (EHA) as colloids is not nephrotoxic, unlike some artificial colloids (3). We did not find any studies regarding the effect of administering EHA to patients developing hypoalbuminemia in the intensive care unit (ICU) on the development or prevention of AKI. The primary aim of our study was to investigate the effect of EHA administration on hypoalbuminemic patients in the ICU on the development of AKI. Our secondary aim was to compare the duration of stay in ICU and mortality rates of these patients.

Methods

This study was a single-centre retrospective study and was completed after receiving approval from Hospital Institutional Ethics Committee (date: 02/10/2018, no: 18/235). A total of 5,989 patients who were followed up at our hospital adult ICU between 01.01.2014-01.06.2018 were included in the study after obtaining the ethics committee approval. Only the first admission of patients with multiple admissions in this period was analysed. This study included all patients above the age of 18 who were monitored for more than 48 hours in the ICU, with initial serum creatinine and serum albumin values recorded. Patients who received EHA after developing AKI were excluded from the study. Additionally, patients with a kidney transplant, known AKI on admission to ICU and chronic renal failure diagnoses were excluded from the study.

Clinical characteristics, demographic data and laboratory data were obtained from the institutional electronic medical records system. The AKI was defined according to AKI network (AKIN) criteria, using only serum creatinine levels. The first serum creatinine level measured upon the admission of the patient to ICU or when an increase in serum creatinine began was accepted as basal creatinine. The second serum creatinine value measured within 48 hours was determined to be the highest creatinine value. If the basal creatinine value increased by more than 2 times, AKIN stage 2-3 patients were accepted as having AKI. Serum albumin was measured in the hospital laboratory using an Architect C 16000 (Abbot/USA) device. Serum albumin value lower than 3.5 g/dL was assessed as hypoalbuminemia (8). Patients who were not administered EHA (Human Albumin 20% CSL Behring, Gmbh, Marburg, Germany) were included in Group none human albumin (NHA), while patients administered EHA comprised Group HA. The development of AKI, duration of stay in ICU and mortality rates were compared between the groups.

Statistical Analysis

In this study, statistical analyses were completed by using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program.

Descriptive statistical methods (mean, standard deviation) were used in addition to Independent t-test for comparison of two groups and chi-square test for comparison of qualitative data. Logistic regression analysis was used to determine factors affecting mortality and AKI. Results were assessed at a significance level of $p<0.05$.

Results

A total of 5,989 patients were recorded. Of the 1,672 (27.91%) hypoalbuminemic patients, 1,206 who met our criteria were included in the study. Patients were divided into two groups based on whether EHA replacement was administered. There were 625 patients in group NHA (51.82%) and 581 patients in group HA (48.18%). AKI developed in 167 patients in group NHA and 275 patients in group HA (Figure 1).

The mean age in group HA was statistically significantly higher than group NHA ($p=0.0001$). There was no statistically significant difference observed between the groups regarding gender distribution ($p=0.074$). The AKI development and mortality rates

in group HA were statistically significantly higher than group NHA (p=0.0001). The mean albumin value in group HA was statistically significantly lower than group NHA (p=0.0001). The mean duration of stay in group HA was statistically significantly higher than group NHA (p=0.0001) (Table 1).

The mean age, AKI development rate, EHA administration and number of days in ICU were statistically significantly higher in patients with mortality compared to surviving patients (p=0.0001). The albumine value in mortal patients was statistically significantly lower than surviving patients (p=0.0001). There was no statistically significant difference observed in the gender distribution of surviving and mortal patients (p=0.326) (Table 2).

Logistic regression analysis was performed to determine factors affecting mortality among age, AKI presence, number of days in ICU and EHA use. Advanced age (p=0.0001), AKI development (p=0.0001) and EHA administration (p=0.0001) were determined as factors affecting mortality. The number of days in ICU was not determined to affect mortality (Table 3).

Mean age, EHA administration, mortality and days of stay in ICU were statistically significantly higher in patients developing AKI compared to patients not developing AKI (p=0.0001). There was no statistically significant difference observed in terms of gender distribution between patients with and without AKI (p=0.690) (Table 4).

Logistic regression analysis was performed to determine the factors such as age, EHA administration and days of stay in ICU, affecting AKI development. Advanced age (p=0.0001) and EHA administration (p=0.0001) were determined to be factors affecting AKI development (Table 5).

Discussion

According to the results of our study, advanced age and EHA application in hypoalbuminemic patients were determined as important factors effective in the development of AKI. At the same time, EHA application and advanced age were among

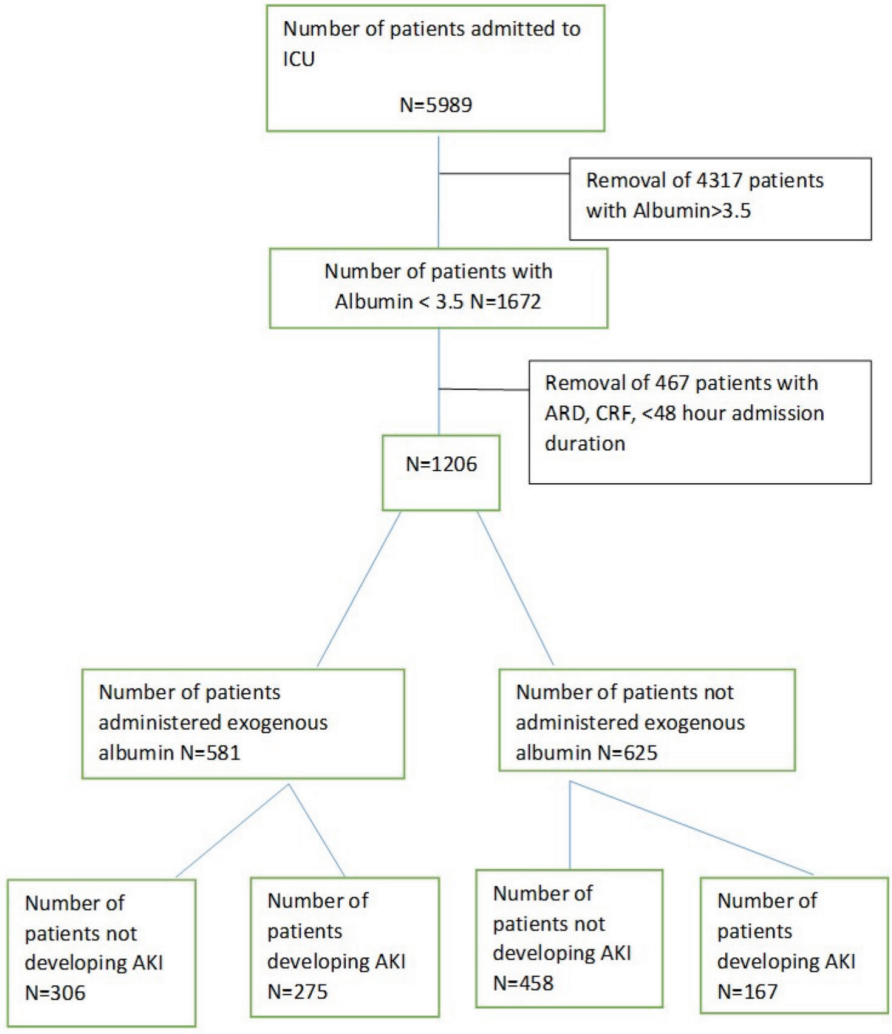

Figure 1. Patient selection algoritm

ICU: Intensive care unit, ARD: Acute renal disease, CRF: Chronic renal failure, AKI: Acute kidney injury

Table 1. Age, gender, AKI development rates, serum albumin values, mortality and number of days admission in the groups

Variables		Group NHA n=625		Group HA n=581		p
Age		59.7±19.95		65.95±17.19		**0.0001***
Gender	**Female**	289	46.24%	239	41.14%	0.074+
	Male	336	53.76%	342	58.86%	
AKI	**No**	458	73.28%	306	52.67%	**0.0001+**
	Yes	167	26.72%	275	47.33%	
Serum albumin value		2.75±0.52		2.36±0.63		**0.0001***
Mortality	**No**	485	77.60%	220	37.87%	**0.0001+**
	Yes	140	22.40%	361	62.13%	
Days of admission		7.54±5.98		14.4±13.43		**0.0001***

*Independent t-test, +Chi-square test
AKI: Acute kidney injury, p<0.05, NHA: None human albumin, HA: Human albumin

Table 2. Mean ages, AKI development rate, exogenous human albumin administration and number of days in ICU of surviving and mortal patients

Variables		Surviving patients n=705		Dead patiens n=501		p
Age		59.6±19.93		67.1±16.47		**0.0001***
Gender	**Female**	317	44.96%	211	42.12%	0.326+
	Male	388	55.04%	290	57.88%	
AKI	**No**	555	78.72%	209	41.72%	**0.0001+**
	Yes	150	21.28%	292	58.28%	
Serum albumin value		2.69±0.55		2.39±0.64		**0.0001***
Exogenous human albumin administration	**No**	485	68.79%	140	27.94%	**0.0001+**
	Yes	220	31.21%	361	72.06%	
Days of admission		10±11.25		12.03±10.08		**0.001***

*Independent t-test, +Chi-square test
AKI: Acute kidney injury, p<0.05, ICU: Intensive care unit

Table 3. Logistic regression analysis to determine the factors affecting mortality

Factors	p	OR	95.0% CI for OR	
			Lower limit	Upper limit
Age	**0.0001***	1.02	1.01	1.02
AKI development	**0.0001***	0.23	0.17	0.30
Days of admission	0.170	0.99	0.98	1.00
Exogenous human albumin administration	**0.0001***	0.20	0.15	0.26

AKI: Acute kidney injury
*p<0.05, CI: Confidence interval, OR: Odss ratio

the factors affecting mortality, along with the duration of hospitalization in ICU. Hypoalbuminemia incidence among patients admitted to ICU is reported as 21% (3). We found this rate to be 27.91%. Hypoalbuminemia alone is an independent risk factor for mortality (5). The literature notes a strong link between developing morbidity and mortality and serum albumin in patients with underlying acute and chronic disease (3). Each 1 g/dL reduction in serum albumin concentration is reported to increase AKI rates by 137% and mortality rates by 147% and lengthen stay in ICU by 28% (4,9). Findik et al. (10) reported that patients with low preoperative serum albumin had higher renal replacement treatment requirements and mortality.

In our study, Group HA with low serum albumin levels had higher mortality and longer stay in ICU, in accordance with the literature.

Serum albumin level is known to decrease with age (11). The mean serum albumin value in Group NHA was 2.75±0.52,

Table 4. Mean ages, gender, exogenous human albumin administration, mortality and number of days in ICU of patients with and without AKI

Variables		AKI (-) n=763		AKI (+) n=167		p
Age		60.34±19.67		66.74±16.79		**0.0001***
Gender	**Female**	338	44.24%	190	43.00%	0.690+
	Male	426	55.76%	252	57.00%	
Exogenous human albumin administration	**No**	458	59.94%	167	37.80%	**0.0001+**
	Yes	306	40.06%	275	62.20%	
Mortality	**No**	555	72.64%	150	33.90%	**0.0001+**
	Yes	209	27.36%	292	66.10%	
Days of admission		10.32±9.55		11.74±12.68		**0.028***

*Independent t-test, +Chi-square test
AKI: Acute kidney injury, p<0.05, ICU: Intensive care unit

while it was 2.36±0.63 in Group HA with a higher mean age. We found that this small mathematical difference increased mortality by a significant rate and lengthened ICU admission at a significant rate. This situation may be explained as advanced age causing hypoalbuminemia and increased comorbidities that increase mortality in critically ill patients (3,5). Additionally, we think it will be beneficial to conduct prospective comparative studies on patients with different albumin values.

The EHA has been used as a therapeutic agent in ICUs for more than 50 years. However, harmful effects were reported in the 1990s, and its use began to be debated (4). The main use of EHA is the field of hepatology. The most controversial results related to EHA replacement treatment were obtained in patients with liver failure and renal function disorder. It was reported that EHA use should be limited to high-risk patients based on obtained data (12). Schortgen et al. (13) reported negative effects of hyperoncotic albumin use on the kidneys in critically ill patients and an increased risk of death. However, Lee et al. (14) reported that 20% EHA replacement treatment before coronary artery bypass surgery in patients with albumin levels lower than 4.0 g/dL increased urine output during surgery and reduced the risk of AKI development. Yu et al. (15) reported EHA administration might contribute to renal amelioration after AKI developed. In the current study, we found that AKI developed at higher rates in group HA. In our logistic regression analysis to determine factors affecting AKI development, we determined administration of EHA in addition to advanced age were risk factors for the development of AKI.

Tablo 5. Logistic regression analysis to determine the factors affecting AKI development

Factors	p	OR	95.0% CI for OR	
			Lower limit	Upper limit
Age	**0.0001***	1.02	1.01	1.03
Exogenous human albumin administration	**0.0001***	0.47	0.35	0.64
Days of admission	0.699	1.00	0.98	1.01

*p<0.05, CI: Confidence interval, OR: Odds ratio, AKI: Acute kidney injury

In elderly kidneys, structural and physiological changes, such as loss of nephron mass, vascular and glomerular tubular degeneration at the microscopic level, reduced glomerular filtration rates and a tendency toward cellular apoptosis may be observed. These physiological changes in renal functions may explain the tendency toward AKI development among elderly patients (16). Male gender is a known risk factor for AKI development (2). However, we found no difference regarding gender among factors affecting AKI development, contrary to the literature. Mortality was higher, and stay in ICU was longer among patients developing AKI.

A variety of studies researching the correlation between EHA administration and mortality have been carried out (17-20). Patel et al. (17) reported that the use of EHA as a part of severe volume resuscitation in sepsis, whether hypoalbuminemia was present or not, might be ineffective in reducing mortality. Additionally, Caironi et al. (18) reported that EHA replacement ameliorated hypoalbuminemia in sepsis but did not improve outcomes. Another study found there was no evidence that EHA administration reduced mortality in critically ill patients compared to cheaper alternatives like saline for patients with hypovolemia in addition to hypoalbuminemia; however, they suggested that EHA might be indicated in a selected critically ill patient population (19). Offringa et al. (20) reported that mortality was higher in critically ill patients given EHA. Our results agree with the literature. Our logistic regression analysis found that advanced age, AKI presence and EHA administration were among factors affecting mortality, while mortality was not affected by the length of stay in ICU.

Some authors have emphasised the need for additional strengthened controlled-randomised studies to further explain the underlying physiological and molecular reasoning (11,20).

Study Limitations

The main points in this study were that, albumin administration did not affect prognosis and might have side effects that would limit its administration and cost benefits. Our study had some limitations, such as being retrospective, including a heterogenous patient group and not being able to research and diagnose accompanying pathologies that might affect the process in

patients. Additionally, another limitation was that we could not identify data related to the dose, frequency and duration of EHA administration.

Conclusion

In conclusion, although we found that EHA administration in hypoalbuminemic patients increased AKI development, mortality and lengthened stay in ICU. The main points in this study were that, albumin administration did not affect prognosis and might have side effects that would limit its administration and cost benefits. We believe that there is a need for prospective randomised controlled studies researching the effects of EHA replacement treatment on AKI.

Ethics

Ethics Committee Approval: This study was a single-centre retrospective study and was completed after receiving approval from Hospital Institutional Ethics Committee (date: 02/10/2018, no: 18/235).

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.T., Design: H.D., M.T., Data Collection or Processing: S.Y., C.G., İ.S., H.U., H.D., M.T., K.K., Analysis or Interpretation: S.Y., C.G., İ.S., H.U., H.D., M.T., K.K., Literature Search: S.Y., H.D., M.T., Writing: S.Y., M.T., K.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2020.4733
Bezmialem Science 2022;10(2):150-6

Association between Omega-6 and Omega-3 Polyunsaturated Fatty Acids Intake and *IL-6* G(-174)C Polymorphism with Hs-CRP Level in Healthy Subjects

Sağlıklı Bireylerde Omega-6 ve Omega-3 Çoklu Doymamış Yağ Asitleri Tüketimi ve *İL-6* Geni G(-174)C Polimorfizmlerinin Yd-CRP Düzeyi ile İlişkisi

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ABSTRACT

Objective: Studies have shown that omega-3 and omega-6 polyunsaturated fatty acids play a major role in the prevention of inflammation, and interleukin-6 (IL-6) levels can be associated with inflammation. The aim of this study was to investigate the relationship between G(-174)C polymorphism of *IL-6* gene with dietary omega-3 and omega-6 intake and high sensitive C-reactive protein (CRP) (hs-CRP] level.

Methods: This retrospective studywas carried out with a total of 302 healthy individuals, 152 women and 150 men, with a mean age of 50.1 years. Food consumption records, biochemical measurements, and genetic analyzes of individuals were recorded from patient files. Daily energy, fat, cholesterol, omega-6 and omega-3 intakes were evaluated from food consumption records.

Results: The CC variation was observed in 7%, GC variation in 31%, and GG variation in 62% of the subjects. Dietary cholesterol intake of individuals with CC variation and omega-6/omega-3 intake of individuals with GG variation were found to be positively and significantly correlated with hs-CRP (r=0.155; p=0.035).

Conclusion: Inflammation is the complete response of the organism to the stimuli. In order to prevent the formation of inflammation, it is recommended that individuals with GG variation increase their omega-6/omega-3 consumption rates in their diets towards omega-3.

Keywords: IL-6, G(-174)C, CRP, omega-3, omega-6

ÖZ

Amaç: Yapılan çalışmalar, enflamasyonun önlenmesinde omega-3 ve omega-6 çoklu doymamış yağ asitlerinin büyük rol oynadığını ve interlökin-6 (İL-6) seviyelerinin enflamasyonla ilişkilendirilebileceğini ortaya koymuştur. Bu araştırmanın amacı, *İL-6* geninin G(-174)C polimorfizminin diyetle omega-3 ve omega-6 alımı ve yüksek duyarlı C-reaktif protein (CRP) yd-CRP seviyesi ile ilişkisini araştırmaktır.

Yöntemler: Retrospektif olarak yürütülen bu araştırma, özel bir sağlık kurumuna başvurmuş olan yaş ortalaması 50,1±12,4 yıl olan 152 kadın, 150 erkek, toplam 302 sağlıklı birey ile gerçekleştirilmiştir. Bireylerin besin tüketim kayıtları, biyokimyasal ölçümleri ve genetik analizleri hasta dosyalarından kaydedilmiştir. Besin tüketim kayıtlarından günlük enerji, yağ, kolesterol, omega-6 ve omega-3 alım miktarları hesaplanmıştır.

Bulgular: Bireylerin %7'sinde CC varyasyonu, %31'inde GC varyasyonu ve %62'sinde GG varyasyonu bulunmuştur. CC varyasyonu görülen bireylerin diyet kolesterol alımları ve GG varyasyonu görülen bireylerin omega-6/omega-3 alımları ile yd-CRP arasında pozitif yönde ve anlamlı bir ilişki olduğu belirlenmiştir (r=0,155; p=0,035).

Sonuç: Enflamasyon, organizmanın uyaranlar karşısında başlatmış olduğu yanıtların tamamıdır. Enflamasyon oluşumunun engellenmesi için GG varyasyonuna sahip bireylerin diyetlerindeki omega-6/omega-3 tüketim oranlarını omega-3 yönünde artırmaları önerilmektedir.

Anahtar Sözcükler: İL-6, G(-174)C, CRP, omega-3, omega-6

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Cite this article as: Girgin E, Öngün Yılmaz H. Association between Omega-6 and Omega-3 Polyunsaturated Fatty Acids Intake and *IL-6* G(-174)C Polymorphism with Hs-CRP Level in Healthy Subjects. Bezmialem Science 2022;10(2):150-6

Received: 02.06.2020
Accepted: 30.10.2020

Introduction

According to the report published by the World Health Organization in 2016, 71% of the causes of death worldwide are cardiovascular diseases, cancer, respiratory system diseases and diabetes (1). It is stated that the basis of these diseases is the inflammatory response process (2).

All of the responses initiated by the organism against exogenous and endogenous stimuli and necessary for vital continuity are considered as inflammatory responses (3). Numerous single point polymorphisms (single nucleotide polymorphism) have been defined in the interleukin (*IL*)-*6* gene in scientific studies (4-7). It is stated that IL-6 G(-174)C polymorphism is related to the formation of systemic inflammatory response. Specific inflammatory mediators such as IL-6 and C-reactive protein (CRP) are closely associated with inflammation (8). CRP is an acute phase protein, level of which increases immediately in case of inflammation and is synthesized in the liver under the control of IL-6. High sensitivity CRP (hs-CRP) is a value that is not different from normal CRP, only a more sensitive measurement system is used. Hs-CRP, which is more sensitive than standard CRP test, can detect inflammation that CRP cannot detect (9).

Inflammation, which is one of the basic components of the immune system, plays a critical role in the daily diet nutrients. Therefore, nutrition is defined as meeting the energy and nutritional needs of the individual, as well as strengthening the immune system and turning the inflammatory response in its favor (10).

It is known that fatty acids, which are an important component of nutrition, have an effect on immune functions both pathologically and physiologically (11). A diet rich in omega-3 polyunsaturated fatty acids suppresses inflammatory metabolism, while excessive consumption of omega-6 polyunsaturated fatty acids plays a role in increasing inflammation (12). Although there are various recommendations regarding the omega-6/omega-3 ratios that should be taken in the daily diet, it has been shown that inflammation markers decrease as the ratio approaches 1/1 (13). For this reason, it is extremely important to increase omega-6/omega-3 consumption in the daily diet in the direction of omega-3 (12).

According to the hypothesis of the research, high dietary omega-6/omega-3 consumption ratio increases yd-CRP levels with G(-174)C polymorphisms of *IL-6* gene. In this direction, the aim of the study was to investigate the relationship between G(-174)C polymorphisms of the *IL-6* gene and dietary omega-3 and omega-6 ratios and hs-CRP levels in individuals who were admitted to a health institution for genetic analysis.

Methods

The research was planned and conducted retrospectively. Inclusion criteria for the study were determined as being over the age of 18, not having a chronic disease, not using non-steroidal anti-inflammatory drugs (NSAIDs), having genetic tests done, and volunteering to use the registered information for research purposes. The research was conducted with a total of 302 healthy individuals, 152 women and 150 men, with an average age of 50.1±12.40 years, who were admitted to a private genetic testing center in Istanbul and had their genetic tests done between January 2015 and February 2018. Since the research was carried out retrospectively and with recorded data, the entire universe was included in the study without making a sample calculation.

This research was conducted after it was approved by the Ethics Committee with the decision number 9 at the meeting of the Ethics Committee of Okan University, dated 12.03.2018 and numbered 92. Institutional approval was obtained from the genetic testing center in order to conduct the research. It was conducted on a voluntary basis with individuals who agreed to participate in the research. When individuals were first admitted to the genetic testing center, approval was obtained that they would allow the use of their information for research purposes. After obtaining the consent of the individuals, the information form filled face to face by the dietician included the personal information of the individuals, anthropometric measurements, food consumption, exercise status, biochemical measurements and genetic analysis. Food consumption frequency, serum hs-CRP values and IL-6 G(-174)C analysis results were obtained from the information form of the individuals and used in the study.

While recording the frequency of food consumption, the foods consumed by the individuals on average for a year were questioned with the help of the food atlas and recorded in the patient information form. The frequency of food consumption from archived files was mathematically converted into one-day amounts. Daily energy, fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, and omega-6 and omega-3 intakes were analyzed in the Nutrition Information System 7.2 (Pacific Company) program. These determined values were evaluated according to the recommended intake levels with reference to the Turkish Dietary Guidelines (TÜBER-2015). Presence of IL-6 G(-174)C polymorphism analyzed by using mass spectrometry (MassSpectrometer, MS) method and hs-CRP levels measured by using immunoturbidimetric method with Cobas 6000 (Roche Diagnostics International Ltd., Switzerland) device were taken from archived patient files.

Statistical Analysis

The data were evaluated with SPSS (v22.0) statistical program. Categorical variables were expressed as numbers (n) and percentage (%), continuous variables were expressed as mean ($\bar{X}$), standard deviation (SD). Shapiro-Wilks, one of the normal distribution tests, was used in the analysis of the data, and parametric tests were used because the data showed normal distribution. The means of more than two independent groups were compared with the "one-way ANOVA test". Pearson correlation coefficient was calculated for the relationship between numerical measurements. A p value <0.05 was considered statistically significant.

Results

A total of 302 individuals were included in the study to examine the effects of omega-6/omega-3 intake and *IL-6* gene G(-174)

C polymorphism on hs-CRP levels in healthy individuals. The mean age of the individuals was 50.1±12.40 years, and the mean body mass index was 27.3 kg/m^2 (lower-upper: 16.4-46.1 kg/m^2). The mean hs-CRP value was found to be 2.9±4.2 mg/L. Of the individuals 7.0% had the CC, 31.0% CG and 62% GG genotypes (not shown in the Table).

The comparison of hs-CRP levels according to *IL-6* gene variations in individuals included in the study is given in Table 1. There was no statistically significant difference between the mean hs-CRP values of the individuals and the polymorphism groups (p>0.05).

Comparison of energy and fat intake values according to *IL-6* gene variations is shown in Table 2. When the differences between the polymorphism groups in terms of the energy and fat intakes from the daily foods were evaluated, there was no significant difference between the mean daily dietary fat intake of individuals (p>0.05), only the saturated fat (%) intake differed significantly between polymorphism groups (p<0.05). The mean values of saturated fat (%) of individuals with CC polymorphism were significantly higher than the mean values of individuals with GC and GG polymorphisms (Table 2).

The correlation between daily energy and fat intake and blood hs-CRP levels of individuals with *IL-6* gene CC variation is shown in Table 3. There was a positive and significant relationship between individuals' cholesterol intake and hs-CRP level (p<0.05). As individuals' cholesterol intake increased, hs-CRP levels increased at the same rate (Table 3).

As shown in Table 4, there was no statistically significant correlation between daily energy and fat intake and blood hs-CRP levels of individuals with *IL-6* gene GC variation (p<0.05).

The correlation between daily energy and dietary fat intakes and blood hs-CRP levels of individuals with *IL-6* gene GG variation is shown in Table 5. There was a positive and significant relationship between individuals' omega-6/omega-3 intake ratio and hs-CRP level (p<0.05). As the omega-6/omega-3 intake ratio increased, the hs-CRP level also increased (Table 5).

Discussion

Within the scope of this study, the relationship between *IL-6* gene G(-174)C polymorphisms and dietary omega-3/omega-6 ratios and hs-CRP levels were investigated in individuals who did not use NSAIDs.

When hs-CRP levels of individuals were compared with daily energy and fat intake values, cholesterol intake (p<0.05) in CC

Table 1. Comparison of *IL-6* gene variations and hs-CRP levels

	SNP	S	χ^2	SS	F	p
hs-CRP (mg/L)	CC	21	3.4	5.1	1.002	0.368
	GC	94	3.3	5.3		
	GG	187	2.6	3.4		

One-Way ANOVA test. p>0.05
hs-CRP: High-sensitive C-reactive protein, F: Frequency

Table 2. Comparison of *IL-6* gene variations and energy and fat intake values

Energy and fat intake	*IL-6* gene	S	χ^2	SS	F	p
Energy (kcal)	CC	21	2.127	567.6	0.273	0.761
	GC	94	2.070	644.4		
	GG	187	2.032	637.3		
Fat (g)	CC	21	97.5	31.9	0.523	0.593
	GC	94	103.9	35.8		
	GG	187	99.7	36.7		
Fat %	CC	21	41.4	8.2	1.856	0.158
	GC	94	44.8	8.2		
	GG	187	43.9	7.0		
SFA (g)	CC	21	28.9	8.6	1.375	0.254
	GC	94	33.2	13.0		
	GG	187	31.1	13.2		
SFA (%)	CC	21	12.4	2.9	4.493	0.012
	GC	94	14.7	4.1		
	GG	187	13.7	3.3		
MUFA (g)	CC	21	40.3	16.5	0.606	0.546
	GC	94	41.7	16.5		
	GG	187	39.5	15.8		
MUFA (%)	CC	21	17.1	4.8	1.100	0.334
	GC	94	18.1	4.1		
	GG	187	17.4	3.8		
PUFA (g)	CC	21	22.1	9.7	0.325	0.723
	GC	94	23.2	10.1		
	GG	187	22.3	10.1		
PUFA (%)	CC	21	9.3	2.9	0.841	0.432
	GC	94	10.2	3.5		
	GG	187	9.9	3.2		
Cholesterol (mg)	CC	21	335.3	142.7	0.310	0.734
	GC	94	366.8	158.4		
	GG	187	362.0	172.0		
Omega-3 (mg)	CC	21	2.5	0.9	1.403	0.247
	GC	94	2.8	1.4		
	GG	187	2.6	1.2		
Omega-6 (mg)	CC	21	19.6	9.2	0.182	0.834
	GC	94	20.4	9.0		
	GG	187	19.8	9.3		
Omega-6/ omega-3	CC	21	8.0	3.2	0.592	0.554
	GC	94	7.7	2.7		
	GG	187	8.1	3.1		
ALA (mg)	CC	21	1.5	0.6	2.806	0.062
	GC	94	2.0	0.9		
	GG	187	2.1	1.0		
EPA+DHA (mg)	CC	21	0.4	0.3	2.570	0.078
	GC	94	0.6	0.6		
	GG	187	0.5	0.4		

ALA: Alpha linolenic acid, DHA: Docosahexanoic acid, EPA: Eicosapentaenoic acid, F: Frequency, g: Grams, kcal: Kilocalorie, mg: milligrams, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, SFA: Saturated fatty acid

genotype and omega-6/omega-3 intake ratio ($p<0.05$) in GG genotype were found be significantly different. It was found that hs-CRP levels increased as individuals' cholesterol and omega-6/omega-3 intake ratios increased.

Karaman (14) investigated the relationship between *IL-6* gene variants and hypertension in the population of Adıyaman. It was stated that 63.9% of the population had GG, 31.5% GC and 4.6% CC variations of IL-6 G(-174)C polymorphism in the healthy group. Ferrari et al. (15) investigated the relationship between IL-6 promoter polymorphisms, diet and lifestyle factors, bone mass and osteoporosis, and it was reported that 13.7% of the participants had CC variation, 50% CG variation, and 36.3% GG variation. Lacopetta et al. (16), on the other hand, stated that they detected the CC allele in 21%, CG in 44%, and GG in 35%, according to the results of their study only on women. Examining the relationship between IL-6 and CRP gene polymorphisms in obese children with metabolic syndrome, Todendi et al. (17) showed that 45.3% of them had GG, 43.8% GC, and 10.9% CC variation in their study with 470 students in Brazil. Although genetic polymorphisms of IL-6 have different effects on diseases, they may have different allele structures in each population and may not be a determining factor for the population.

Table 3. Correlation between diet, daily energy and fat intake and blood hs-CRP levels of individuals with CC variation (n=21)

Energy and fat intake	χ^2 ± SD		hs-CRP
Energy (kcal)	2127.5 (567.6)	r	0.336
		p	0.136
Fat (g)	97.5 (31.9)	r	0.144
		p	0.533
Fat %	41.4 (8.2)	r	-0.256
		p	0.262
SFA (g)	28.9 (8.6)	r	0.153
		p	0.508
SFA(%)	12.4 (2.9)	r	-0.190
		p	0.409
MUFA(g)	40.3 (16.5)	r	0.133
		p	0.566
MUFA (%)	17.1 (4.8)	r	-0.178
		p	0.440
PUFA (g)	22.1 (9.7)	r	0.051
		p	0.827
PUFA (%)	9.3 (2.9)	r	-0.219
		p	0.341
Cholesterol (mg)	335.3 (142.7)	r	0.502*
		p	0.020
Omega-3 (mg)	2.5 (0.9)	r	0.106
		p	0.648
Omega-6 (mg)	19.6 (9.2)	r	0.043
		p	0.852
Omega-6/omega-3	8 (3.2)	r	-0.086
		p	0.709
ALA (mg)	1.5 (0.6)	r	0.073
		p	0.752
EPA+DHA (mg)	0.4 (0.3)	r	0.143
		p	0.537

ALA: Alpha linolenic acid, DHA: Docosahexanoic acid, EPA: Eicosapentaenoic acid, g: Grams, kcal: Kilocalorie, mg: milligrams, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, SFA: Saturated fatty acid, SD: Standard deviation, hs-CRP: High sensitivity C-reactive protein

Table 4. Correlation between dietary, daily energy and fat intake and blood hs-CRP levels of individuals with GC variation (n=94)

Energy and fat intake	χ^2 ± SD		hs-CRP
Energy (kcal)	2070.2 (644.4)	r	0.057
		p	0.583
Fat (g)	103.9 (35.8)	r	-0.056
		p	0.594
Fat %	44.8 (8.2)	r	-0.167
		p	0.108
SFA (g)	33.2 (13)	r	-0.091
		p	0.382
SFA (%)	14.7 (4.1)	r	-0.151
		p	0.146
MUFA (g)	41.7 (16.5)	r	-0.009
		p	0.929
MUFA (%)	18.1 (4.1)	r	-0.116
		p	0.267
PUFA (g)	23.2 (10.1)	r	-0.082
		p	0.430
PUFA (%)	10.2 (3.5)	r	-0.168
		p	0.105
Cholesterol (mg)	366.8 (158.4)	r	-0.007
		p	0.945
Omega-3 (mg)	2.8 (1.4)	r	-0.193
		p	0.063
Omega-6 (mg)	20.4 (9)	r	-0.065
		p	0.536
Omega-6/omega-3	7.7 (2.7)	r	0.162
		p	0.120
ALA (mg)	2 (0.9)	r	0.026
		p	0.802
EPA + DHA (mg)	0.6 (0.6)	r	0.056
		p	0.590

ALA: Alpha linolenic acid, DHA: Docosahexanoic acid, EPA: Eicosapentaenoic acid, g: Grams, kcal: Kilocalorie, mg: milligrams, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, SFA: Saturated fatty acid, SD: Standard deviation, hs-CRP: High sensitivity C-reactive protein

In the study of Aslan (18) in which they compared serum hepcidin levels with CRP and IL-6 levels in neonatal sepsis, they found statistically strong and significant relationships between IL-6 and CRP values. Tonet et al. (19) investigated the relationship between the G(-174G)C polymorphism of the *IL-6* gene and cardiovascular disease risk factors in elderly women in Brazil and stated that GG homozygotes had higher serum IL-6 and yd-CRP levels compared to carriers of the C allele. It has also been emphasized that GG homozygotes are more prone to inflammatory diseases than other alleles. According to another study investigating the relationship between the alleles of CRP and IL-6, it was stated that the IL-6 -174CC genotype was associated with significantly worse overall survival compared to the GG or GC genotypes. It has been shown that people with the CC allele are more prone to inflammation than those with the CG and GG alleles (16). However, in this study, mean hs-CRP values of individuals did not differ significantly between polymorphism groups (p>0.05) (Table 2).

Table 5. Correlation between diet, daily energy and fat intake and blood hs-CRP levels of individuals with GG variation (n=187)

Energy and fat intake	χ^2 ± SD		hs-CRP
Energy (kcal)	2032.7 (637.3)	r	0.019
		p	0.800
Fat (g)	99.7 (36.7)	r	-0.016
		p	0.831
Fat %	43.9 (7)	r	-0.103
		p	0.162
SFA (g)	31.1 (13.2)	r	-0.044
		p	0.548
SFA (%)	13.7 (3.3)	r	-0.092
		p	0.211
MUFA (g)	39.5 (15.8)	r	-0.022
		p	0.760
MUFA (%)	17.4 (3.8)	r	-0.064
		p	0.384
PUFA (g)	22.3 (10.1)	r	0.047
		p	0.520
PUFA (%)	9.9 (3.2)	r	0.025
		p	0.732
Cholesterol (mg)	362 (172)	r	0.062
		p	0.403
Omega-3 (mg)	2.6 (1.2)	r	-0.062
		p	0.399
Omega-6 (mg)	19.8 (9.3)	r	0.053
		p	0.475
Omega-6/omega-3	8.1 (3.1)	r	0.155*
		p	0.035
ALA (mg)	2.1 (1)	r	-0.034
		p	0.644
EPA+DHA (mg)	0.5 (0.4)	r	0.045
		p	0.540

ALA: Alpha linolenic acid, DHA: Docosahexanoic acid, EPA: Eicosapentaenoic acid, g: Grams, kcal: Kilocalorie, mg: milligrams, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, SFA: Saturated fatty acid, SD: Standard deviation, hs-CRP: High sensitivity C-reactive protein

As researches on the benefits of omega-6 and omega-3 fatty acids to human health increase, the importance of them has increased in the society (20). Moertl et al. (21) investigated the effect of omega-3 intake on systolic left ventricular function, endothelial function and inflammation markers, and found that omega-3 supplementation dose-dependently increased the left ventricular ejection fraction, but when a dose of 4 g was reached, there was a significant decrease in IL-6 level. Reinders et al. (22) showed in a study on men that the blood value of omega-3 fats was inversely related to hs-CRP levels. Saravanan et al. (23) defined omega-3 fatty acids as pleiotropic agents with beneficial effects on the cardiovascular system and stated that the most important effect was to reduce mortality after myocardial infarction. Kalogeropoulos et al. (24) found in a study conducted in healthy individuals that the ratio of omega-6/omega-3 fatty acids had a strong positive correlation with inflammatory markers. Reinders et al. (22) found in a study on men that the value of omega-3 fatty acids in the blood was inversely proportional to the CRP levels. It is recommended to increase the intake of omega-3 fatty acids and decrease the intake of omega-6 in the treatment of diseases and reducing the effect of inflammation (12). In this study, it was observed that there was a positive and significant relationship between the dietary omega-6/omega-3 ratio and hs-CRP in individuals with GG variation (p<0.05). As the omega-6/omega-3 ratio of people with this genotype increased, the hs-CRP value also increased. However, there was no significant relationship between omega-6/omega-3 ratio and hs-CRP values of individuals with GC and CC variations (p>0.05). When the relationship between omega-3 alone and hs-CRP was evaluated between polymorphism groups, no significant relationship was found (p>0.05).

Study Limitations

The research had some limitations. The findings obtained as a result of the research should be evaluated with these limitations. The first limitation was that the sample of the study consisted of individuals who were admitted to a single center and had their genetic analysis done. Therefore, the results of the study could not be generalized to the general population. Other important limitations of the study were that the data were obtained retrospectively from the records and the daily amount of food consumption was calculated mathematically based on the frequency of food consumption.

Conclusion

As a result of this study, it was found that as omega-6/omega-3 intake ratios of individuals with IL-6 G(-174)C GG variation increased, hs-CRP levels increased and inflammation increased. For this reason, it is recommended that people with this variation

increase their omega-3 consumption and reduce their omega-6 consumption. In this direction, increasing the consumption of foods such as mackerel, salmon, trout, herring and sardines in order to increase omega-3 consumption. In order to reduce omega-6 intake, it is recommended to limit the consumption of vegetable oils rich in omega-6, such as sunflower oil and corn oil.

Ethics

Ethics Committee Approval: This research was conducted after it was approved by the Ethics Committee with the decision number 9 at the meeting of the Ethics Committee of Okan University, dated 12.03.2018 and numbered 92.

Informed Consent: Institutional approval was obtained from the genetic testing center in order to conduct the research.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: E.G., Concept: E.G., H.Ö.Y., Design: E.G., H.Ö.Y., Data Collection or Processing: E.G., Analysis or Interpretation: E.G., H.Ö.Y., Literature Search: E.G., H.Ö.Y., Writing: E.G., H.Ö.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5145
Bezmialem Science 2022;10(2):157-62

Statistical Errors in Medical Residency Theses

Tıpta Uzmanlık Tezlerinde Yapılan İstatistiksel Hatalar

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ABSTRACT

Objective: The aim of this study is to evaluate the theses of residency in medicine in terms of statistical errors made and thus to contribute to the production of quality scientific publications by ensuring that scientific publishers in the field of medicine are sensitive and careful about statistics when doing their work.

Methods: In this study, we investigated 321 thesis theses which are defended from 6 different universities are obtained from the database of the Turkish Higher Education Council. The investigation is was conducted in terms of "Errors Related to p-values", "Errors Related to Tests", "Mathematical Notation Errors", "Statistical Symbol Errors", "Inappropriate Interpretation", "Presentation of The the Statistical Method Analysis and Results in The the Incorrect Section of The the Manuscript", "Errors in Summarizing Data","Incomprehensible Statistical Terms" and "Errors in Statistical Terminology"

Results: There was at least one statistical error in all 321 medicine residency theses examined. The most common error was "errors in summarizing data" with a ratio of 70.1% (n=225), while the least common error was "incomprehensible statistical expressions" with a ratio of 14.3% (n=46).

Conclusion: As a result, both researchers and consultants who undertake scientific studies have a responsibility to minimize these errors. To prevent statistical errors, students who are doing residency in medicine are required to receive the necessary training in statistical literacy, to have basic statistical knowledge, and to receive consultancy from a biostatistics expert for statistical evaluations. Students who residency in medicine in preventing statistical errors

ÖZ

Amaç: Bu çalışmanın amacı tıpta uzmanlık tezlerini yapılan istatistiksel hatalar bakımından değerlendirmek ve tıpta uzmanlık öğrencilerinin istatistik yeterliliklerini değerlendirmektir. Böylece tıp alanında bilimsel yayın yapan araştırmacıların istatistik konusunda duyarlı ve dikkatli olmasını sağlayarak kaliteli bilimsel yayınların üretilmesine katkıda bulunmaktır.

Yöntemler: Çalışmamızda Yüksek Öğretim Kurulu veri tabanı taranarak en az 40 yıllık geçmişe sahip 6 farklı tıp fakültesinin farklı anabilim dallarına ait 321 uzmanlık tezi rastgele seçilmiştir. Seçilen uzmanlık tezleri, Biyoistatistik uzmanı olan beş araştırmacı tarafından "p-değerleriyle ilgili hatalar", "uygulanan testlerle ilgili hatalar", "Matematiksel gösterim hataları", "İstatistiksel sembol hataları", "Uygun olmayan yorumlama", "Verilerin özetlenmesindeki hatalar" , "Anlaşılmayan istatistiksel ifadeler" ve "İstatistik terminolojisindeki hatalar" bakımından değerlendirildi.

Bulgular: İnceleme 321 tezin tamamında en az 1 tane istatistiksel hata mevcuttu. En sık karşılaşılan hata, %70,1 (n=225) oran ile "verilerin özetlenmesindeki hatalar", en az karşılaşılan hata ise %14,3 (n=46) oran ile "anlaşılmayan istatistiksel ifadeler" olduğu görüldü.

Sonuç: Sonuç olarak, istatistiksel hataları önleme konusunda tıpta uzmanlık öğrencilerinin istatistik okuryazarlığı konusunda gerekli eğitimi almaları, temel istatistik bilgisine sahip olmaları ve istatistiksel değerlendirme konusunda da bir biyoistatistik uzmanından danışmanlık almaları gerekmektedir. Danışmanların ise tez değerlendirme sürecinde istatistik konusunda daha hassas

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Received: 14.07.2020
Accepted: 14.02.2021

Cite this article as: Yabacı Tak A, Can FE, Kaskır Kesin F, Ahmadian R, Ercan İ. Statistical Errors in Medical Residency Theses. Bezmialem Science 2022;10(2):157-62

are required to receive the necessary training in statistical literacy, to have basic statistical knowledge, and to receive consultancy from a biostatistics expert for statistical evaluations.

Keywords: Statistical errors, residency in medicine thesis, statistical review

olmaları ve tıpta uzmanlık öğrencilerinin tezlerinin yürütülmesi sırasında bir biyoistatistik uzmanından danışmanlık almalarını sağlamalıdırlar.

Anahtar Sözcükler: İstatistiksel hatalar, tıpta uzmanlık tezleri, istatistiksel inceleme

Introduction

Statistical science is used in all stages of scientific research, from the preparation stage of a studyies through to the reporting stage. It is known that the correct use of statistics and statistical procedures is extremely important in scientific studies today. Medical authorities also stress the importance of statistics and note that physicians should be a good reader of statistics, both in scientific studiesy and in the conduct of specialized theses. Researchers who lack adequate statistical knowledge may make error in using statistical science at any stage of the research, including data planning, design, execution, analysis and presentation. When the medical literature was examined, it was observed that the majority of studies had errors in terms of statistical procedures. Some of these errors have had a major effect on the results by directly affecting the results, while others are were presentation errors in terminology and dido not have a major effect on the outcome (1-3).

Statistical errors can be seen in the articles as well as in the theses of residency in medicine. Although the authors are responsible for the errors made in the thesis work as majors, the thesis advisors are also responsible for the academic prestige of the work.

Subsequent publication of thesis work bywith statistical errors would also result in a loss of academic confidence of the thesis authors and their thesis advisor. Therefore, consultants in medical residency training are required to guide their students for their statistical knowledge proficiency and to direct them to receive biostatistical counseling in their studies. Many researchers seeking to draw attention to errors and deficiencies in statistics and methodology have emphasized since the 1960s the importance of accurate use of statistics in scientific publications in medical journals since the 1960s (4,5). When studies in literature studies were examined, it was found that some subject matter experts looked at the research design used, the design flaws and mistakes, and the inappropriate use of design in medical studies. Some subject matter experts have examined the statistical tests used, the use of unsuitable statistical methods, and the failure of statistical tests used in medical studies to be specified in the study (6,7). Similarly, some subject matter experts have worked on errors in summarizing data and errors in statistical terminology (8-11). Some subject matter experts have emphasized the knowledge of statistics and statistical training for clinicians in their work (11-14). It is observed that statistical errors were made in medical studies due to the lack of statistical knowledge of researchers lack of statistical knowledge and their failure to consult a biostatistics expert. For this reason, the first step of the medical residency theses should be checking statistical errors in the requirement assessment. For this reason, the first step of the medical residency theses of the scientific study of physicians occurs in terms of statistical errors in the requirement assessment. It is important to receive advice from a biostatistics expert in the planning phase, analysis phase, reporting phase, as well as consulting for statistical review and evaluation of statistical quality prior to publication of articles (5,12,15-17).

The aim of this study is to statistically evaluate the theses of medical residency students. In this way, the statistical competencies of the specialized students will be evaluated.

Methods

When literature research on statistical errors were analyzed, it was found that in most of the researches at least one statistical error was present. In the study of statistical errors in psychiatry journals, it was reported that 40% of 164 studies had statistical errors (18). The error rate was found to be 50% in the study conducted by Glantz (15). In 52% of 62 scientific papers reviewed by Gore et al. (8), at least one statistical error has beenwas found. Lukić and Marušić (19) reported that statistics were not reliable in 63% of 144 articles published in medical journals 63% of the 144 articles published by Lukić and Marušić (19) in medical journals reported that the statistics were not reliable. Šimundić and Nikolac (20) reported at least one error in 87% of 55 articles submitted to the medical journal. Ercan et al. (21) reported statistical errors in 96% of 181 studies submitted to the Medical Sciences journal Medical Sciences reported statistical errors. Statistical errors were found in 93% of 157 articles reviewed by Günel Karadeniz et al. (2) in radiology journals. When the studies mentioned above are were taken as reference, the median ratio determined according to the studies is was 0.63 (0.40-0.96). In the light of this knowledge, it was decided to review 321 medical residency theses in terms of statistical errors for α=0.05 significance level and d=0.052 margin of error.

In our study, by scanning the database of the Turkish Higher Education Council (THEC), 321 medical residency theses belonging to different departments of 6 different medical faculties with at least 40 years of experience were randomly

selected. The selected medical residency theses were examined in terms of statistical errors by five researchers (A. Yabacı Tak, F.E. Can, F. Kaskır Kesin, R. Ahmadian ve I. Ercan) who were experts in Biostatistics. The medical residency theses were first evaluated individually by subject matter experts, and then evaluated together by five researchers. The classification of the statistical errors found in the residency theses was done in accordance with the statistical error groupings specified in the studies of Ercan and Demirtas (1) and Ercan et al. (7). Accordingly, statistical errors were classified as follows;

Errors relating to the p-value;

• p-values given in closed form ($p<0.01$, $p<0.05$, $p>0.05$,etc.),

• Non-reported p-values,

• Incorrect p-values,

• Incorrect demonstration of p-values ($p=0.000$, $p<0.0005$, etc.).

Errors relating to the statistical tests;

• Statistical technique defined but not used,

• Incorrect name for the statistical test,

• Undefined statistical test,

• Use of incorrect test,

• Statistical analysis required but not performed.

Other errors;

• Mathematical demonstration errors,

• Statistical symbol errors,

• Incomprehensible statistical terms,

• Inappropriate interpretation,

• Errors in (statistical) terminology,

• Errors in summarizing data,

• Presentation of statistical method-analysis and results in the incorrect section of the manuscript.

Statistical Analysis

Statistical errors identified by each researcher were confirmed by the study team. In this way, a complete harmony between the researchers was achieved. Therefore, the inter- rater reliability criterion was not calculated. Statistical errors were presented as frequency and percentage, taking into account the number of medical residency theses studied. IBM SPSS Statistics 22.0 was used to analyze the data.

Results

There were at least one statistical error in all 321 medical residency theses. The most common error was "errors in summarizing data" with a ratio of 70.1% (n=225), while the least common error was "incomprehensible statistical expressions" with a ratio of 14.3% (n=46). The details of the distribution of statistical errors made in the medical residency theses examined are given in Table 1.

Discussion

In this study, statistical errors made in medical residency theses were examined. In our literature research, while there were articles on statistical errors in the publications, there was no study on statistical errors in in which studies on residency theses in medicine were conducted.

When a residency thesis it is turned into a scientific publication after the residency theses, it is used as a reference by scientists and offered to human service. Therefore, the accuracy and reliability of residency thesis studies is very important. Statistics is one of the most important factors for the accuracy and reliability of a scientific study, from the planning stage of the study to the reporting stage. Studies of medicine residency thesis studies is are the first step of academic studies for clinicians. For this reason, in this study, it was investigated whether there are were statistical errors in residency theses in medicine and what kinds of statistical errors, if any. Statistical errors in studies are generally classified as errors related to p-value, errors related to statistical tests and other errors. When 321 medical residency theses obtained randomly from THEC database were examined, errors related to p-value were found to be relatively high. It is known that this error is an important error considering the importance of p-value. In the medicine residency theses examined, the most common mistake about p-value was the wrong display of p-value with a rate of 47.4%, followed by "p-value with a closed form" of with a rate of 46.4%. When similar studies in the literature were examined, this ratio was found to be 18.43% and 37.25% respectively in the studies of Ercan et al. (21) in 2015 and 2017, respectively. In the study conducted by Günel Karadeniz et al. (2), this rate is was 42.04%. Although it is not considered an error by some readers to give the p-value in closed form, it prevents the actual information obtained from statistical testing from being reached. For example, it is error to state $p>0.05$, instead of $p=0.058$. At the same time, actual values of the p-value are needed in studies such as meta-analysis (22). One of the most common errors encountered when examining p-value errors is was that the p-values are were not correct with a ratio of 34.6%. When analyzed in other studies in the literature; this rate was 13.36% in the study of, Ercan et al. (21) in (2015 and it was 8.82% in the study of Ercan et al. (7) in2017.) this rate is 13.36%, 8.82% and Ercan et al. (21) reported this rate as 17.83%. The authors emphasized that this rate is was obtained only from articles where p-value can could be controlled, so this ratio may might actually be higher.

When the errors for statistical tests are examined, the most common error is the use of an undefined statistical test. This rate was obtained as 44.2% in our study. The least mistake

made in this classification is was not to apply the statistical test defined in the method section with a rate of 18.7% in our study. This rate was found to be 11.52% in the study by Ercan et al. (21) In the studies in which Hanif and Ajmal (23) evaluated 80 research articles published in local journals indexed and recognized in Pakistan, the rate was 26.25%. In our study, we found that the name of the statistical test was incorrectly given in 25.2% of medicine residency theses. While this rate was 3.23% in medical journals in the study of Ercan et al. (21), This this rate was 12.50% in the study of Hanif and Ajmal (23) and this rate was 12.66% in the studies of Günel Karadeniz et al. (2). Another important error related to statistical tests is the use of the incorrect statistical test. While this rate was 28.3% in medicine residency theses, theseis rates wereas 7.83% and 10.78%, respectively, in the studies of Ercan et al. (7,21) in 2015 and-2017. In the study of Hanif and Ajmal (23) study, this rate was 28.75% while in the study of Günel Karadeniz et al. (2), it was 6.33%. In some cases, clinicians evaluate the situation subjectively in their studies and make comments without statistical testing. However, a scientific result cannot be achieved without statistical testing. While the rate of this kind of error rate was 21.8% in medicine residency theses, when Ercan et al. (7,21) reported this rate as 17.51% in their studyies in 2015 and 1.96% in their studyies in 2017, and Günel Karadeniz et al.(2) reported this rate as 8.86%.

In the 321 medicine residency thesis theses examined for the errors classified as "Other Errors", it was seen that the most common error was the "errors made in summarizing the data" with a rate of 70.1%. For example, regardless of the distribution of numerical data, it was observed that mean, standard deviation or median, minimum and maximum values were given in theses. The rate of "Errors in summarizing data" wasere 26.73% in their study of medicine articles inin the study of Ercan et al. (7) and it was 57.84% in their studythe study of Ercan et al. (21). In the study of Hanif and Ajmal (23), it was 16.25%, while in the study of Günel Karadeniz et al. (2), it was 64.56%.

Another error addressed is notation errors. Notation errors were classified as mathematical notation errors and statistical symbol errors. In medicine residency theses, these rates were 51.4% and 19.6%, respectively. In similar studies in the literature, Ercan et al. (7) reported 6.91% and 3.23%, respectively, while Ercan et al. (21) reported 2.94% and 3.43%, respectively, and Günel Karadeniz et al. (2) reported 16.46% and 3.80%, respectively. Errors in notation are known to mislead the reader in scientific studies and lead to a misunderstanding of the results.

In some studies, it was observed that the researchers used statistical expressions that were not understood in a manner contrary to statistical language and that there were errors in statistical terminology. In medicine residency theses, these rates was 14.3% and 28.7%, respectively. Accordingly, it was observed that comments that were not in accordance with statistical language were contradictory in the interpretation of results in scientific research. In our study, this rate was 23.1%. In the study of Ercan et al. (7) study, the rate of errors of incomprehensible statistical terms was 4.15%, while in their 2017 study, this rate was 0.49%. Likewise, the rate of inappropriate interpretation errors was reported as 8.76% in the study of Ercan et al. (21) in 2015 and 14.71% in the study of Ercan et al. (7) in 2017. In the study of Günel Karadeniz et al. (2), the rate of errors of incomprehensible statistical terms errors was 22.78%, while the rate of errors of inappropriate interpretation errors was of the statistical analysis in the discussion section. The rate of

Table 1. Distribution of statistical errors in medical residency thesis theses

Source of errors		n (%)
Errors relating to the p-value	p-values given in closed form	149 (46.4%)
	Non-reported p-values	92 (28.7%)
	Incorrect p-values	111 (34.6%)
	Incorrect demonstration of p-values	152 (47.4%)
Errors relating to the statistical tests	Undefined statistical test	142 (44.2%)
	Incorrect name for the statistical test	81 (25.2%)
	Statistical technique defined but not used	60 (18.7%)
	Use of incorrect test	91 (28.3%)
	Statistical analysis required but not performed	70 (21.8%)
Errors in summarizing data	225 (70.1%)	
Mathematical demonstration errors	165 (51.4%)	
Statistical symbol errors	63 (19.6%)	
Incomprehensible statistical terms	46 (14.3%)	
Inappropriate interpretation	74 (23.1%)	
Errors in (statistical) terminology	92 (28.7%)	
Presentation of statistical method-analysis and results in the incorrect section of the manuscript	135 (42.1%)	

this error was 42.1% in our study. When similar studies were examined, this rate was 6.91% and 10.13% (7,21).

Study Limitations

According to the results obtained from our study, they we showed that the researchers were insufficient in statistics or did not show the necessary care in this regard. Statistical errors can affect the results either directly or indirectly. Considering the examined theses, it is was concluded that the rate of errors both directly and not directly affecting the result is was not very low. The reasons for these errors can be considered as, not consulting a biostatistics expert on the subject, assuming that they are sufficient in statistics, but not having sufficient knowledge, and carelessness (1,21). In the evaluation process of medicine residency theses, it would be more useful to have statistical evaluation earlier than other expert evaluations. Because, an error found as a result of a statistical evaluation that needs to be corrected can affect the results and therefore the discussion of the study. This will lead to a waste of time and extend the research process.

Conclusion

As a result, both researchers and consultants who undertake scientific studies have a responsibility to minimize these errors. To prevent statistical errors, students who are doing residency in medicine are required to receive the necessary training in statistical literacy, to have basic statistical knowledge, and to receive consultancy from a biostatistics expert for statistical evaluations.Students who residency in medicine in preventing statistical errors are required to receive the necessary training in statistical literacy, to have basic statistical knowledge, and to receive consultancy from a biostatistics expert for statistical evaluations. The thesis advisor should be more sensitive about statistics during the thesis evaluation process and ensure that medical students receive consultancy from a biostatistics expert during the execution of their thesis.

Ethics

Ethics Committee Approval: The theses in our article have been examined by scanning the Thesis Center of the Council of Higher Education (YÖK), and an Ethics Committee approval is not required as there is no interventional or clinical situation.

Informed Consent: Patient consent information is also not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

Data Collection or Processing: A.Y.T., F.E.C., F.K.K., R.A., İ.E., Analysis or Interpretation: A.Y.T., F.E.C., F.K.K., R.A., İ.E., Literature Search: A.Y.T., F.E.C., F.K.K., R.A., İ.E., Writing: A.Y.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.3931
Bezmialem Science 2022;10(2):163-7

Ischemic Stroke Severity, Stroke Types and Early Mortality in Patients with End-stage Renal Disease

Son Dönem Böbrek Yetmezliği Olan Hastalarda İskemik İnme Şiddeti, İnme Tipleri ve Erken Dönem Mortalite

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ABSTRACT

Objective: To compare the demographic characteristics, vascular risk factors, stroke subtypes and early prognosis of patients with end-stage renal disease (ESRD)/normal renal function (NRF) with acute ischemic stroke (IS).

Methods: Demographic data and vascular risk factors of patients with acute stroke between August 1 2013-April 1 2015 were determined. Patients diagnosed with ESRD who were receiving dialysis treatment with a glomerular filtration rate below 15, were included in the study. Stroke severity was determined by using the National Stroke Health Stroke scale. To determine the types of IS, "Trial of Org 10172 in Acute Stroke Treatment" TOAST criteria were used. Deaths during hospitalization were used to determine early mortality.

Results: Of the 132 patients included in the study, 33.3% had ESRD and 66.6% had normal renal functions (NRF). Demographic characteristics and risk factors did not differ between ESRD and NRF groups. When stroke types were compared, it was determined that stroke with undetermined etiology with more than one etiology was higher in ESRD patients. Of all patients 10.6% died in hospital and this rate was 20.5% in ESRD patients. In the regression analysis, the presence of ESRD increased hospital mortality significantly (risk ratio: 11.6, 95% confidence interval 1.7-77, p=0.011).

Conclusion: Our results showed that more than one etiology was seen in acute IS patients with ESRD and ESRD significantly increased the risk of hospital mortality.

ÖZ

Amaç: Çalışmamızda akut iskemik inme (İİ) saptanmış son dönem böbrek yetmezliği (SDBY) olan ve böbrek fonksiyonu normal saptanan hastaların demografik özelliklerinin, vasküler risk faktörlerinin, inme alt tiplerinin ve erken dönem prognozunun karşılaştırılması amaçlanmıştır.

Yöntemler: Akut iskemik inme tanısıyla 1 Ağustos 2013-1 Nisan 2015 arasında başvuran hastaların demografik verileri ve vasküler risk faktörleri belirlendi. Glomerüler filtrasyon hızı 15 altında olan ve diyaliz tedavisi alan SDBY tanılı hastalar belirlenerek çalışmaya dahil edildi. İİ tipinin belirlenmesinde "Trial of Org 10172 in Acute Stroke Treatment" TOAST kriterleri kullanıldı. Hastaların inme şiddeti Ulusal İnme Sağlık ölçeği (NIHSS) ile belirlendi. Erken dönem mortalitenin belirlenmesinde hastane yatışı sırasındaki ölüm oranları kullanıldı.

Bulgular: Çalışmaya dahil edilen 132 hastanın %33,3'ünde SDBY varken geriye kalan %66,6'sında böbrek fonksiyonları normal bulundu. SDBY olan ve olmayan grup arasında demografik özellikler ve risk faktörleri farklılık göstermedi. İnme tipleri karşılaştırıldığında ise, SDBY olanlarda birden fazla etiyolojiye bağlı sebebi bilinmeyen inmenin daha yüksek oranda olduğu belirlendi. Tüm hastaların %10,6'sı hastanede eks olmuş olup, bu oran SDBY olanlarda %20,5 olarak bulundu. Regresyon analizinde SDBY varlığı hastanede ölüm oranını anlamlı derecede yükseltmekteydi (risk oranı: 11,6, %95 güven aralığı 1,7-77, p=0,011).

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Received: 28.11.2019
Accepted: 15.02.2021

Cite this article as: Özkan S, İlgen Uslu F, Yoldaş TK. Ischemic Stroke Severity, Stroke Types and Early Mortality in Patients with End-stage Renal Disease. Bezmialem Science 2022;10(2):163-7

Keywords: Stroke, end-stage renal disease, risk factors, stroke subtypes, early prognosis

Sonuç: SDBY olan akut İİ hastalarında birden fazla etiyolojinin daha fazla görüldüğünü ve SDBY'nin hastanede ölüm riskini anlamlı derecede yükselttiğini göstermiştir.

Anahtar Sözcükler: İnme, son dönem böbrek yetmezliği, risk faktörleri, inme alt tipleri, erken dönem prognoz

Introduction

Renal failure is a strong risk factor for cerebrovascular disease [(CVD), stroke], which is one of the most important causes of mortality and disability worldwide. It has been reported that patients with end-stage renal disease (ESRD), especially receiving dialysis treatment, have stroke 5-30 times more than the population without kidney disease. Although the case fatality rate differs among stroke subgroups, it reaches up to 90% (1). It has been stated that the increased risk of stroke in chronic renal failure (CRF) is associated with the interaction of renal failure-specific factors such as vascular comorbidities, malnutrition-inflammation-atherosclerosis complex, fluid overload, toxic molecules that cannot be excreted, and disruption of the coagulation mechanism. The reason why one third of the developing strokes are seen in the first 30 days following the start of dialysis has been attributed to the more frequent observation of risk factors such as diabetes, hypertension (HT), previous CVD and hyperlipidemia (HL) (1,2). For a disease such as CVD with a high incidence, disability and mortality, determining the risk factors and knowing the modifiable or non-modifiable factors that may affect the early and late prognosis gain importance in terms of both early treatment and preventive medicine. In our study, it was aimed to compare the demographic characteristics, risk factors, ischemic stroke (IS) subtypes, stroke severity, stroke subgroups and early death in acute IS patients with and without ESRD, and to investigate the effect of ESRD on stroke severity, subtype and early mortality.

Methods

In our study, all patients who were hospitalized, examined and treated in the neurology service and/or intensive care unit with the prediagnosis of IS in Van Regional Training and Research Hospital between January 1 2014 and August 1 2015, were retrospectively scanned. Being over the age of 18 was accepted as an inclusion criterion. The diagnosis of stroke was made by using computed brain tomography and diffusion-weighted imaging and apparent diffusion coefficient magnetic resonance imaging findings at the first admission. Patients with no signs of stroke in their cranial imaging and those with metabolic pathologies mimicking stroke (hypoglycemia, hyponatremia, etc.) were excluded from the study. Demographic data and risk factors of the patients were recorded. The following criteria were used to determine risk factors: systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or using antihypertensive medication for HT; fasting blood glucose >140 mg/dL in 2 consecutive measurements or >200 mg/dL in glucose challenge test or using antidiabetic medication for diabetes mellitus (DM); stable or unstable angina pectoris, previous MI, diagnosis of coronary artery disease (CAD) with coronary angiography and therefore taking medication for CAD; total cholesterol >200 mg/dL, triglyceride >150 mg/dL low density lipoprotein cholesterol ≥100 mg/dL, high density lipoprotein cholesterol <40 mg/dL; or the use of antihyperlipidemic drugs for HL. Patients receiving dialysis treatment were patients with a calculated glomerular filtration rate (GFR) of less than 15 during their clinical follow-up or prior to hospitalization and undergoing dialysis.

All investigations for the etiology of stroke were reviewed. Stroke types of patients according to the TOAST criteria (3) were as follows: large artery atherosclerosis (LAA), small vessel occlusion (SVO), cardioembolism (CE), stroke of other determined etiology (SODE), and stroke of undetermined etiology (SUE). LAA was defined as more than 50% stenosis or occlusion in large vessels in MR angiography, CT angiography, or carotid and vertebral arteries doppler ultrasonography ipsilateral to the brain region where symptomatic stroke occurred. The diseases with or without atrial fibrillation (AF) leading to thrombus formation in the heart such as rheumatic heart disease, nonvalvular valve disease, sick sinus syndrome, recent heart attack, cardiac vegetation, akinetic left ventricle, atrial myxoma, dilated cardiomyopathy, prosthetic heart valve, and paradoxical embolism were accepted as sources of CE. Patients with one of the lacunar syndromes characterized by clinical findings, infarcts of less than 1.5 cm in neuroimaging and exclusion of CI and LAA were classified as having SVO. Rare diseases such as vasculopathies and hematological disorders without atherosclerosis, carotid and vertebral artery dissection, arteritis, migraine, diseases such as substance abuse vasculopathy, fibromuscular dysplasia, radiation arteritis, protein C and protein S deficiencies, antithrombin 3 deficiency, and IS due to other coagulation disorders were designated as SODE. The SUE group, on the other hand, was considered as those in whom no etiology could be found despite further evaluations, or those who were found to have two or more causes or those who were examined insufficiently.

The stroke severity of the patients was determined according to the The National Institutes of Health Stroke scale (NIHSS). Length of stay and hospital mortality status of all patients were noted. In order to determine the functional status of patients after stroke, the modified Rankin scale (mRS) score was recorded at the first admission and on the 15th day (4,5).

Statistical Analysis

The study was approved by the local ethics committee. After all data were collected in the SPSS 20 database, patients with ESRD and patients with normal renal functions (NRF) were compared in terms of demographic characteristics, vascular risk factors, IS etiology, stroke severity, and early functional outcome. Most of

the results were evaluated by descriptive statistical methods. Chi-square test was used in the analysis of ordinal variables. In the analysis of numerical variables, the Student's t-test was used for normally distributed data and the results were given as mean ± standard deviation, while the data were given as interquantile range when the Mann-Whitney U test was used for data that did not show normal distribution. Binary logistic regression model was used for multivariate analysis. The results were considered significant at the 95% confidence interval and significance level of $p<0.05$.

Results

A total of 132 patients (68 men, 51.5%) were included in the study, including 44 patients undergoing dialysis for ESRD and 88 patients with NRF. The mean age of all patients was 69.5±11.7 (minimum-maximum: 31-88). The mean age of the ESRD group was not different from the NRF group (71 and 73, p=0.395). Gender distribution did not differ between the groups. The distribution of risk factors in groups with and without ESRD is shown in Table 1. The history of stroke was more common in the ESRD group than the NRF group, but the difference did not reach statistical significance.

Comparison of the etiologies of IS is given in Table 2. It was seen that the SUE subtype was the most common in the ESRD group and the CE subtype was the most common in the NRF group (Table 2). Nineteen (43.2%) of the SUE in the ESRD group were due to the coexistence of more than one etiology.

When groups with and without ESRD were compared, the NIHSS scores at admission to hospital [9 (6) and 8 (8.8), p=0.396], and length of stay [8 (7.5) and 8 (4), p=0.234] did not differ.

Of patients with ESRD 20.5% (9 patients) and 5.7% (5 patients) of patients without ESRD died in hospital, and this difference was statistically significant (p=0.015). Initial NIHSS scores of patients who died in the hospital were significantly higher than those who did not die [16 (6) and 8 (8), respectively, p<0.0001]. Of the vascular risk factors, only HT differed significantly between the groups with and without mortality (13.6% and 0%, respectively, p=0.039). Age and gender adjusted regression analysis revealed that the presence of ESRD was significantly associated with hospital mortality (hazard ratio: 11.6, 95% CI 1.7-77, p=0.011).

Discussion

In this study, patients with ESRD presenting with IS were examined in terms of demographic characteristics, vascular risk factors, stroke severity and type, length of hospital stay and hospital mortality, and the results were compared with IS patients without ESRD.

IS patients with ESRD included in our study did not differ in terms of age, gender and vascular risk factors compared to patients without ESRD. Although there are many publications in the literature reporting a higher incidence of IS in hemodialysis (HD) patients, it is noteworthy that hemorrhagic stroke is mostly seen in this patient group. The reason for this difference is explained by genetic predisposition and uncontrolled high blood pressure, which rapidly decreases the GFR value and creates a situation that is unprotected against changes in blood pressure by reducing the resistance in the brain and renal vascular bed (6-8). Independent risk factors for stroke in the CRF population include African-American race, old age, DM, elevated systolic and diastolic blood pressure, left ventricular hypertrophy, GFR below 60 mL/min/1.73 m^2, male gender, and positive family history (9). In a 22-year single center study comparing CRF and stroke patients with stroke patients with NRF, it was reported that CRF patients were younger (mean age= 65.3; 70.5 years) (7).

In a study examining gender differences in CRF in the literature, it was reported that women had protective factors against CRF during the reproductive age and they had a tendency to develop CRF 10 years later than men. The studies of Wang et al. (10) and Ovbiagele (11) also supported that stroke was more common in men. Differently, in the study of J. Mattana et al. (12) in which they compared CRF and stroke patients with stroke patients with NRF, the male/female ratio was reported to be 50% in both groups. Age can also be evaluated as a strong long-term predictor for death after stroke. In the study of McWalter et al. (13), it was reported that mortality was 8 times more in patients older than 85 years of age who had a stroke compared to the group under 60 years of age. In HD patients, HT is a factor that increases the risk of both IS and hemorrhagic stroke, and it is thought that high

Table 1. Comparison of risk factors between groups with and without ESRD

	ESRD (n=44)	NRF (n=88)	p
HT, n (%)	38 (86.4)	65 (73.9)	0.122
DM, n (%)	16 (36.4)	30 (34.1)	0.847
CAD n (%)	14 (31.8)	33 (37.5)	0.567
HL n (%)	22 (50)	38 (43.2)	0.466
AF n (%)	9 (20.5)	30 (34.1)	0.156
Smoking n (%)	6 (13.7)	11 (12.5)	0.470
History of stroke n (%)	18 (40.9)	22 (25)	0.072

HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, HL: Hyperlipidemia, AF: Atrial fibrillation, ESRD: End-stage renal disease, NRF: Normal renal function

Table 2. Comparison of stroke types between groups with and without ESRD in IS patients

TOAST classification	ESRD (n=44)	NRF (n=88)	p
1. Large artery atherosclerosis	3 (6.8)	9 (10.2)	0.067
2. Cardioembolism	7 (15.9)	31 (35.2)	
3. Small vessel occlusion	5 (11.4)	10 (11.5)	
5. Stroke of undetermined etiology (including more than one etiology)	29 (65.9)	38 (43.2)	

ESRD: End-stage renal disease, NRF: Normal renal function, IS: Ischemic stroke

BP, which is difficult to control at the time of admission, may be a predisposing factor in the development of stroke (11). Other modifiable atherosclerotic risk factors such as smoking, HL and AF also increase the risk of IS. The facts that our study was a retrospective and single-center study and had a low number of patients maked it difficult to interpret our results.

In the literature, there are publications reporting that the frequency of HT in HD patients diagnosed with stroke reaches 92%. In the publications, it was reported that the most common comorbidity in both HD patients and non-HD patients with stroke was HT, and uncomplicated DM was the second most common (14,15). In our study, the frequency of HT was 88.3% in the ESRD group and 73% in the NRF group, and it was lower when compared to the literature. It has been reported that AF develops more in patients with CRF than in the normal population, and CRF develops more in patients with AF than in the normal population (16). AF was a risk factor on its own for stroke, and it was found that 24% of our patients had a history of AF and there was no difference between groups. It has been reported that HT is an important risk factor leading to the development of CAD in patients with CRF. It has been reported that DM also accelerates the development of CAD in patients with CRF (17). In our data, 1/3 of all patients had a history of CAD and there was no significant difference between the groups. Supporting the literature, HT was the most common comorbid disease in our patients, and HL and DM were also common. The presence of risk factors did not differ between the groups.

In our study, when evaluated according to IS subtypes in patients with ESRD, the most common one was SUE due to more than one risk factor in the patients. In patients with NRF, CE was the most common etiology. It was thought that the frequent observation of the SUE subtype might result from the coexistence of LAA and CE risk factors in patients receiving dialysis treatment, and the absence of a single etiologic cause. In a study in the literature examining CVD developing in ESRD patients receiving intermittent HD treatment, the distribution of IS subtypes was reported as 28% CE, 23% SUE, 20% SVO, 18% SODE, and 11% SUE (18). Tusukamoto et al. (19) examined the relationship between stage 3-4 kidney disease and IS. They found that 46% of patients with IS with CRF, 36% of patients with LAA, 31% of patients with SVO, and 26% of patients with SUE and SODE had stage 3-4 renal failure. Stage 4 CRF in this study included stage 4 and 5 CRF levels in our study. The increase in the frequency of AF in advanced ages is the most important factor that makes CE play an important role in the etiology of IS. Observation of CRF more frequently in patients with AF also increases the incidence of CE. While CE is more closely related to the degree of CRF, LAA increases significantly as the stage of CRF increases. The reason for the increased frequency of LAA is associated with an increase in carotid intima and media thicknesses independent of atherosclerotic risk factors in HD patients, and especially this increase in those with carotid stenosis decreases blood flow velocity in middle cerebral artery and impairs brain perfusion (19). Sandsmark et al. (20) examined 3,939 patients for the presence of stroke in CRF and reported that lacunar infarcts and white matter lesions were observed more frequently in cranial MR imaging as the GFR value decreased, and this increase was higher in those with albuminuria. Contrary to all these studies, we found significantly more critical stenosis and atherosclerotic changes in the NRF group in our own patients. One of the important factors in this might be that this group was older in our study.

Our results did not show any difference in terms of stroke severity between patients with and without ESRD. In a study in which HD patients with stroke were evaluated in the literature, the mean NIHSS score at admission was reported as 5.33 (21). In the study conducted by Ülker et al. (22) in our country, it was reported that the initial NIHSS score was very valuable in determining the early prognosis, and that the prognosis was much better after the second week in approximately 90% of patients with NIHSS ≤6 compared to those with NIHSS ≥16. In most studies with stroke patients, the presence of CRF has been found to be associated with increased stroke severity, 3-month mortality, and poor short-term prognosis, with more sequelae than strokes with other mechanisms. There are conflicting data regarding the post-stroke mortality rate in HD patients. For example, while this rate was reported as 8-9% in some studies in the literature, Mattana et al. (12) reported this rate as 23% (11). In our study, 10.6% of all patients died during hospitalization, and the mortality rate was found to be low compared to other studies. In patients with ESRD, this rate increased to 20.5% in our study. In the study of Ovbiagele (11), they found that younger patients with hemorrhagic strokes and women died at a higher rate in 25 patients with ESRD and stroke. In our study, patients with and without ESRD were included and only hospital mortality rates were evaluated. It was observed that the presence of ESRD significantly increased the risk of in-hospital mortality in patients with IS.

In the literature, it has been reported that the hospitalization period of patients with CRF is longer than that of patients with NRF (10,11). The reason for this is that there are often accompanying complex medical problems, infections and/or sepsis, and complications are more common in these patients. For example, in the study conducted by Ovbiagele (11), the average hospitalization time was reported as 3.9 days in the CRF group and 2.7 days in the other group. There was no difference between the groups in terms of the total length of hospital stay of the patients in our study. Even when the patients who died were excluded for more objective evaluation, there was no difference in terms of length of stay between the two groups. This can be attributed to the fact that patients with ESRD and undergoing HD are tried to be discharged as soon as possible before complications occur and infection is detected.

Study Limitations

The limitations of our study were that it was a retrospective and single-center study. Multicenter studies are still needed to determine stroke types in ESRD patients and to understand their effects on early and late prognosis.

Conclusion

In conclusion, in our study, it was shown that IS with more than one etiology was seen more frequently in patients with ESRD, and ESRD significantly increased the risk of mortality in the hospital. It is recommended to take more effective measures to prevent mortality during the treatment of IS patients with ESRD.

Ethics

Ethics Committee Approval: The study was approved by the Van Regional Training and Research Hospital Local Ethics Committee.

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: S.Ö., T.K.Y., Design: S.Ö., Data Collection or Processing: S.Ö., F.İ.U., Analysis or Interpretation: S.Ö., F.İ.U., T.K.Y., Literature Search: S.Ö., F.İ.U., Writing: S.Ö., F.İ.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5543
Bezmialem Science 2022;10(2):168-73

Chloroquine Decreased Kir6.2 Immunoreactivity in Chronic Hypoxic Heart

Klorokin Kronik Hipoksik Kalpte Kir6.2 İmmünoreaktivitesini Azaltır

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ABSTRACT

Objective: Experimental and clinical studies indicate that cardiovascular system diseases have the highest mortality rate. One of the major factors underlying this high mortality rate is hypoxia. The inward rectifying potassium channel 6.2 (Kir6.2) plays a key role in the adaptation and metabolic regulation of cardiac tissue to hypoxia. In this study, the effect of chloroquine on Kir6.2 expression in heart tissue exposed to moderate chronic hypoxia was investigated.

Methods: In this study, 32 8-12-week-old male Wistar albino rats weighing 200-300 g were used in 4 groups. Accordingly, the first group exposed to a normoxic (at 21% O_2 concentration) environment, the second group was administered daily chloroquine (50 mg/kg intraperitoneal) injection in a normoxic environment, the third group exposed to a moderately hypoxic environment (10% O_2 concentration), and the fourth group in the hypoxic environment had chloroquine (50 mg/kg intraperitoneal) injection. At the end of the 28-day period, the heart tissues were dissected with anesthesia. Immunohistochemical analysis was performed for Kir6.2 channels.

Results: In hypoxic heart tissue, a significant decrease in Kir6.2 immunoreactivity was observed due to chloroquine administration compared to the control group ($p<0.05$). In addition, in the hypoxia

ÖZ

Amaç: Deneysel ve klinik çalışmalar, kardiyovasküler sistem hastalıklarının son yıllarda ölüm oranı en yüksek hastalık grubu olduğunu göstermektedir. Bu yüksek ölüm oranının altında yatan en büyük etkenlerden birisi hipoksidir. İçeri doğrultucu potasyum kanalı 6.2 (Kir6.2) kardiyak dokunun hipoksiye adaptasyonunda ve metabolik düzenlenmesinde rol oynamaktadır. Bu çalışmada, klorokin maddesinin orta şiddette kronik hipoksiye maruz kalan kalp dokusundaki Kir6.2 ifadesine olan etkisi incelenmiştir.

Yöntemler: Bu çalışmada 32 adet 8-12 haftalık, 200-300 g ağırlığında yetişkin Wistar albino cins erkek sıçanlar, 4 grupta incelenmek üzere kullanıldı. Bu doğrultuda ilk grup normoksik (%21 O_2 konsantrasyonunda) ortama, ikinci grup normoksik ortamda günlük klorokin (50 mg/kg intraperitoneal) enjeksiyonuna, üçüncü grup orta şiddette hipoksik (%10 O_2 konsantrasyonunda) ortama, dördüncü grup ise hipoksik ortamda klorokin (50 mg/kg intraperitoneal) enjeksiyonuna maruz bırakıldı. Yirmi sekiz günlük sürecin sonunda anestezi altında kalp dokuları sakrifiye edilerek Kir6.2 için immünohistokimyasal analiz yapıldı.

Bulgular: Hipoksik kalp dokusunda, klorokin uygulamasına bağlı olarak kontrol grubuna kıyasla Kir6.2 immünoreaktivitesinde anlamlı azalma gözlemlendi ($p<0,05$). Ayrıca, yine klorokin

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Cite this article as: Üner AK, Kaymak E, Doğanyiğit Z, Akın AT, Başaran KE, Özdamar S, Yakan B, Akyüz E. Chloroquine Decreased Kir6.2 Immunoreactivity in Chronic Hypoxic Heart. Bezmialem Science 2022;10(2):168-73

Received: 08.11.2020
Accepted: 31.03.2021

group, which was also treated with chloroquine, hemorrhagic areas were significantly reduced compared to the control group (p<0.001).

Conclusion: Data may demonstrate the potential protective and adaptive effects of chloroquine in hypoxic heart. Further molecular and functional studies are required to confirm the Kir6.2 related mechanism.

Keywords: Kir6.2, KATP channel, ischemia, heart

uygulanan hipoksi grubunda, hemorajik alanlar kontrol grubuna kıyasla anlamlı olarak azaldı (p<0,001).

Sonuç: Bu bulgular, klorokinin hipoksik kalpte potansiyel koruyucu ve adaptif etkilerini göstermiştir. Bununla beraber, Kir6.2 ilişkili mekanizmanın doğrulanması için daha ileri moleküler ve fonksiyonel çalışmalar yapılması gerekmektedir.

Anahtar Sözcükler: Kir6.2, KATP kanalı, iskemi, kalp

Introduction

Oxygen is essential for normal aerobic metabolism in mammals. The key reaction occurs in the mitochondria of the cells. With this reaction, the energy need is provided by adenosine triphosphate (ATP) (1). Hypoxia is the presence of oxygen at low levels and at low partial pressure in the cell. Depending on cell types, metabolic demands, their ability to adapt to hypoxia, and response to various levels of hypoxia range from adaptation to cell death (2).

Hypoxic states of the cardiovascular system are caused by an imbalance between the amount of oxygen delivered to the cardiac cell and the amount required. The degree of hypoxic damage depends not only on the intensity and duration of the hypoxic stimulus, but also on the tolerance of the cardiac system to oxygen deprivation (3). The adaptation to metabolic stress and hypoxia in cardiac tissue can be provided by Kir6.1 and Kir6.2 channels, which are ATP-dependent and sub-members of inward rectifying potassium channels (Kir) (4). However, the adaptation mechanisms have still not been fully known (5).

The ATP sensitive K^+ (K_{ATP}) channels include the Kir channel family and Sulfonylurea subunits. K_{ATP} channels are complex structures formed by subunits (Kir6.1 and Kir6.2 encoded by *KCNJ8* and *KCNJ11*, respectively) and Sulfonylurea receptors that form 4 pores. In addition, K_{ATP} channels function in the physiological process depending on the cellular ATP/ADP balance (6). K_{ATP} channels play a protective role in cardiac myocytes during ischemia or hypoxia (7).

In cardiac muscle cells, cell protection mechanisms are associated with the activation of sarcolemma and mitochondrial K_{ATP} channels in conditions such as ischemia/hypoxia (8-11). In addition, K_{ATP} channels maintain the balance between oxygen delivery and demand during heart failure (12). The number of K_{ATP} channels is critical at determinant of myocardial membrane excitability. Optimization of cardiac energy expenditure may cause a stress in cardiac metabolism (13). K_{ATP} channels regulate the blood flow, especially in cardiomyocytes (14). Investigating the possible effects of various agents on mechanisms and especially the widespread use of chloroquine in recent years can be considered as an important goal.

Chloroquine has been used as a drug in the prophylaxis and treatment of malaria for years. It has also been used in the treatment of autoinflammatory diseases such as rheumatoid arthritis (15). In addition, there are evidences that chloroquine may regulate vascular tone (16,17). The effects of chloroquine on cardiac tissue and its role in hypoxia adaptation are of critical importance.

In summary, with this study, we assessed the Kir6.2 expression in chloroquine-applied hypoxic heart tissue for the first time and we nominated for the relevant mechanism (Figure 1).

Method

Thirty two male adult Wistar albino rats, which were 8-12-week-old and weighing 200-300 g, were raised in Kayseri Erciyes University Experimental and Clinical Research Center (DEKAM). The rats kept in cages were provided with water and nutrients in the normal order of the day at 21 °C and 12 hours in light/dark environment. Experimental groups were formed by weighing the subjects and bringing those with similar weights together. At the end of the experiment, the animals were sacrified under combining ketamine and xylazine anesthesia were and their heart tissues were removed. The experimental protocol of this study was approved by the Animal Ethics Committee of Kayseri Erciyes University (ethics committee decision number: 2018/027).

Eight rats from each group to be used for the experimental groups were placed in normobaric rooms (BioSpherix, NY, USA) with their own cages. Groups to be placed in a normobaric room were exposed to 21% O_2 for sham (normoxic) and 10% O_2 for moderate hypoxia for 28 days and kept in a normobaric room where normoxic and continuous hypoxic conditions would be imitated.

Normoxic Control (N_CON): No drug was administered to the rats (n=8) to be kept in normal room air for 28 days.

Normoxy Chloroquine (N_CLQ): Daily 50 mg/kg Chloroquine was administered intraperitoneally to rats (n=8) which were kept in normal room air for 28 days.

Moderate Chronic Hypoxia Control (mcHX_CON): No medication was applied to rats (n=8) that were exposed to 10% O_2 for 28 days in a normobaric room.

Moderate Chronic Hypoxia + Chloroquine (mcHX_CON + CLQ): Daily 50 mg/kg Chloroquine was administered intraperitoneally to rats (n=8) which were exposed to 10% O_2 for 28 days in a normobaric room.

Tissue Preparation

Tissue samples taken from sacrified rats were followed up for light microscopic and immunohistochemical examinations at

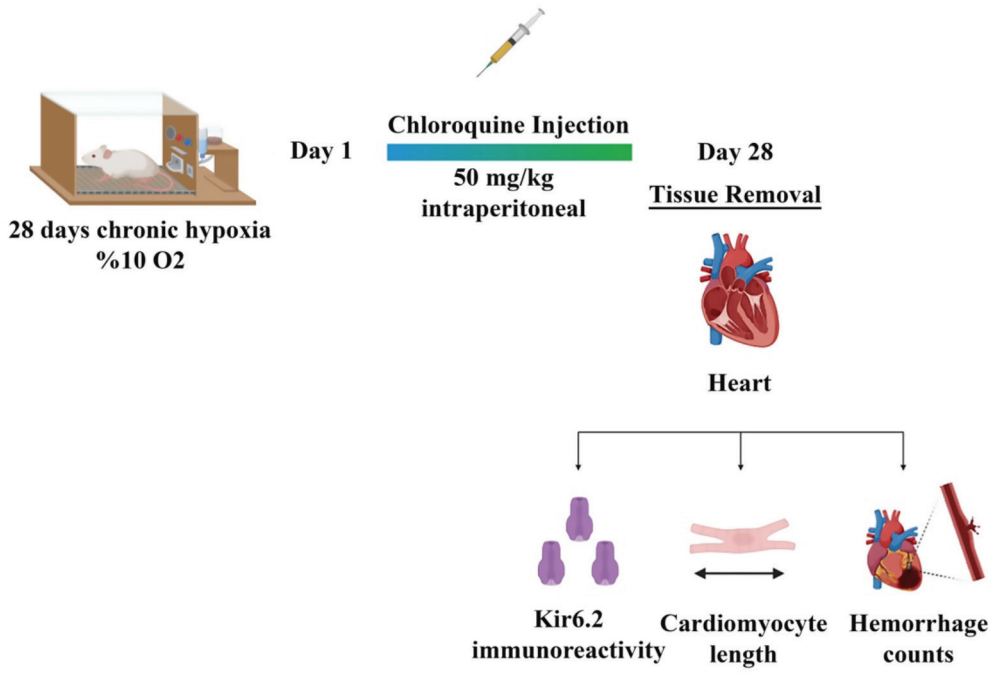

Figure 1. A pictorial representation of the experimental protocol

Yozgat Bozok University, Faculty of Medicine, Department of Histology and Embryology. Tissue samples taken were fixed for 1 day in 10% buffered neutral formaldehyde prepared in phosphate buffer (PBS). Detected tissue samples were dehydrated by passing through graded alcohols according to the routine light microscope tissue tracking method. After being transparent in xylol, they were embedded in paraffin. A fixed vacuum tissue tracking device was used to ensure optimization during tissue tracking. Thick sections of 3- 4 µm were taken from the paraffin blocks with a rotary microtome on grinded slides covered with chrome alum gelatin. Heart sections of 5-6 µm from paraffin blocks were left in the oven for a certain period of time using histological methods, then paraffin was removed with xylene and passed through graduated alcohol series and diluted. To see the general histological structure, hematoxylin and eosin (HE) staining was performed. For hemorrhage, it was done counting under a microscope in 25 different regions. The length of 30 cells was measured with the Image J program.

Hemotoxylin-Eosin Staining

Paraffin sections with a thickness of 3-4 µm taken on grinded slides were deparaffinised in the oven for 1 night and then staining was performed in the following order.

- Incubated 3 times 15 minutes in xylol.
- Rehydrated for 10 minutes in 96%, 96% and 80% alcohols, respectively.
- Washed in H_2O for 10 minutes.
- Incubated in filtered Hematoxylin for 15 minutes.
- Washed in H_2O for 10 minutes.
- Dipped in acid alcohol and removed.
- Washed in H_2O for 10 minutes
- Immersed in ammonia for 2 times.
- Incubated in eosin for 60 seconds.
- Passed through 80%, 96%, 96% alcohols with glaze.

After drying and incubating in xylol for 45 minutes, the slides were covered with Canadian balsam. Kir6.2 expression was detected immunohistochemically using by streptavidin-biotin-peroxidase technique. The sections were deparaffinized in xylene, rehydrated through graded alcohols and washed in deionized water. Antigen retrieval was performed by microwave treatment in 0.01 M sodium citrate buffer, pH 6.0, at 270 °C for 5 min. The slides were cooled and held at room temperature for 10 min. Sections were washed with PBS. Endogenous peroxide activity was inhibited by immersion in 3% (w/v) H_2O_2 for 12 min. Lab Vision™ UltraVision™ Large Volume Detection System (TP-125-HL; Thermo Fisher Scientific, Waltham, MA) was used. All sections were washed with distilled water, then Ultra V block was applied for 10 min at room temperature to block background staining. Sections then were incubated overnight at 4 °C with a Kir6.2 (alomone labs: APC-020), and after washing with PBS, sections were incubated with biotinylated goat anti-Polyvalent secondary antibodies (TP-125-BN) (TP-125-HL; Thermo Fisher Scientific. The immunoreaction was amplified using the streptavidin-avidin-peroxidase complex and visualized using 3,3' p-diaminobenzidine tetrahydrochloride (TA-060-HDX; Thermo Fisher Scientific). After counterstained with Gill's

hematoxylin, sections were washed 3 times with deionized water. Then, the sections were dehydrated through rising alcohols, cleared in xylene, and mounted with Entellan. Images were taken using a light microscope (Olympus BX51; Olympus). In total, 25 different fields were evaluated for each department. At least 3 randomly chosen fields in each slide were counted at the original 20 magnification.

Statistical Analysis

The Kolmogorov-Smirnov test was used to identify the normal distribution of the data. One-way analysis of variance and post-hoc Tukey test were used to determine differences between groups. Results were presented as mean ± standard deviation. The SPSS/PC program (Version 20.0; SPSS, Chicago, IL) and Graph pad Prism 8.0 software were used for statistical analysis. A p value <0.05 was considered as statistically significant.

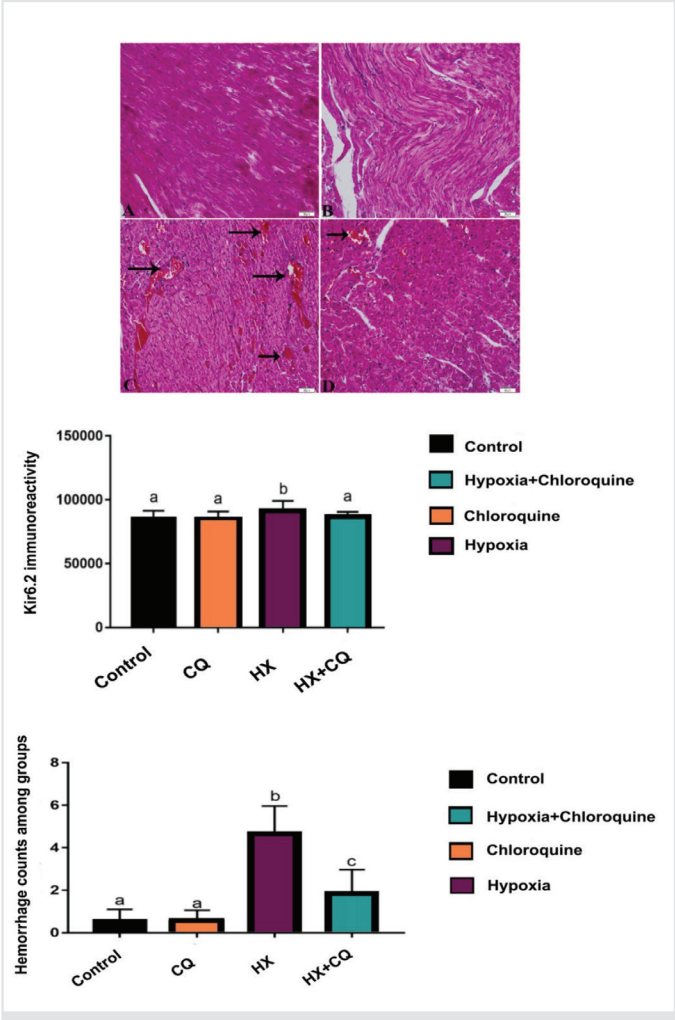

Figure 2. Cardiomyocyte length measurement charts with heart tissue HE images and hemorrhagic area measurements. A; control group, B; CLQ group, C; HX group, D; HX+CLQ group. Hemorrhagic areas were shown with black arrows. CLQ: Chloroquine group, HX: Hypoxia group, HX+CLQ: Chloroquine injected hypoxia group

Results

The image of the heart section HE is shown in Figure 2. In the hypoxia (HX) group, increased hemorrhagic areas were detected in the heart tissue compared to the control group ($p<0.001$). It was observed that hemorrhage was significantly decreased in the HX+CLQ group compared to the HX group ($p<0.001$). At the same time, cardiomyocyte length was significantly decreased in the HX group compared to the control group ($p<0.001$).

Heart tissue Kir6.2 immunohistochemistry results and staining images are shown in Figure 3. Kir6.2 immunoreactivity showed a significant increase in HX group compared to the control group ($p<0.05$). Kir6.2 immunoreactivity in the HX+CLQ group showed a significant decrease compared to the HX group ($p<005$).

Cardiomyocyte length was significantly decreased in the HX group compared to the control group ($p<0.001$). However, there was no significant difference between the HX+CLQ group and the HX group. Cardiomyocyte length was significantly decreased in the CLQ and HX+CLQ groups compared to the control group ($p<0.001$, figure 3).

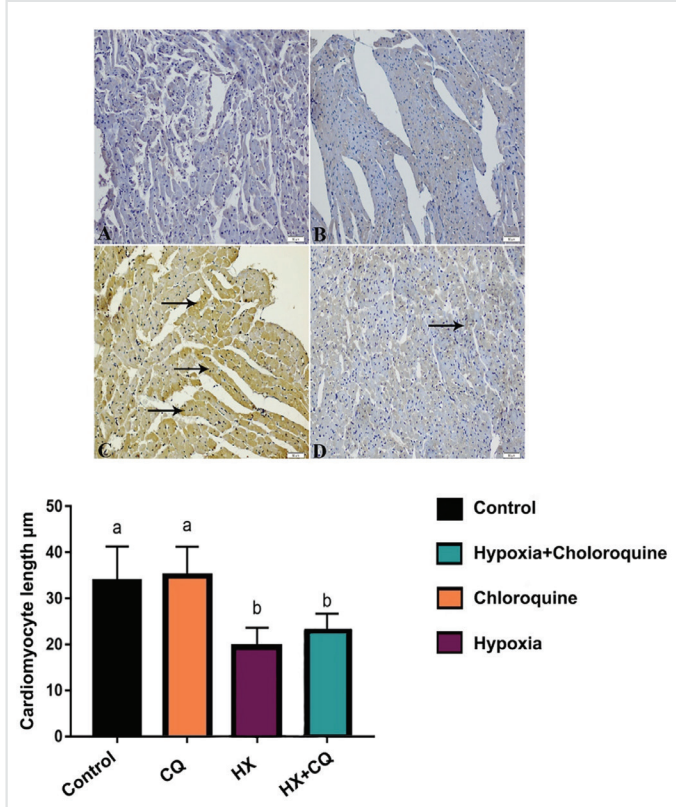

Figure 3. Heart tissue Kir6.2 immunohistochemistry staining image and immunoreactivity result plot. A; control group, B; CLQ group, C; HX group, D; HX+CLQ group. The immunoreactive areas were shown with black arrows. CLQ: Chloroquine group, HX: Hypoxia group, HX+CLQ: Chloroquine injected hypoxia group

Discussion

The findings showed that the chloroquine applied to the hypoxic heart significantly reduced the Kir6.2 immunoreactivity and hemorrhagic areas.

Chloroquine was reported to have a vasodilatory effect on coronary arteries (16). This agent also acts as a vasodilator in the pulmonary arteries in hypertension caused by hypoxia (17). Accordingly, in our study, reduced hemorrhage due to chloroquine administration in the hypoxic heart may be described as an adaptation or protection mechanism in terms of cardiac tissue and metabolism.

Atrial natriuretic peptide (ANP), an important cardiac peptide for cardiovascular homeostasis, significantly modulates the activity of ventricular K_{ATP} channels by an intracellular signaling mechanism consisting of NPR-A, PKG, ROS, ERK1/2, CaMKII and RyR2. This novel mechanism may regulate cardiac excitability and contribute cytoprotecting by partially opening myocardial K_{ATP} channels (18). Similarly, examining the effect of chloroquine on Kir6.2 channels in terms of possible ion current change and signal mechanisms involved in channel functioning may be shown as a target for future studies. On the other hand, our findings may indicate that the chloroquine administration, especially in hypoxic condition leads to decreased immunoreactivity of Kir6.2 channel. Data may need to be clarified in terms of hypoxia markers. This may be accepted as a limitation of the study. In order to understand the effects of changed Kir6.2 immunoreactivity and hemorrhagic areas on hypoxia adaptation mechanisms in more detail, functional and molecular studies can be done in the future. Investigation of potentially changing Kir6.2 protein levels and ion current measurements in tissue is essential in elucidating the mechanism.

The protective effect of phencyclidine hydrochloride substance against myocardial ischemia/reperfusion injury was investigated. Accordingly, the protective effects of phencyclidine hydrochloride against ischemic preconditioning and ischemia/reperfusion injury may be related to mitochondrial K_{ATP} channels and Akt/GSK-3β and Akt/mTOR signaling pathways (19). In this perspective, evaluation of chloroquine to reduce Kir6.2 immunoreactivity and hemorrhagic areas in hypoxic heart tissue in terms of cellular signaling pathways appears as a novel target. In addition, the therapeutically potential effect of chloroquine administration by providing inhibition of CD154-induced IL-6 production in acute kidney injury with hypoxia was shown (20). By investigating the effect of chloroquine on proinflammatory cytokines, especially under hypoxic conditions, more new therapeutic approaches can be developed.

Conclusion

Chloroquine may cause proarrhythmic or antiarrhythmic effects, particularly by affecting Na^+, Ca^{2+} and voltage-gated K^+ channels. Furthermore, ischemia/reperfusion damage by inducing autophagy pathways may be increased (21). The findings of our study show that chloroquine has a significant effect on the adaptation of the heart in hypoxic condition and ischemia/reperfusion damage by affecting Kir6.2 channels. The effect should continue to be investigated in future studies with signal pathways and functional measurements.

Our findings suggest that chloroquine may be a potential target in terms of adaptation mechanisms to hypoxia and the development of possible therapeutic targets.

Ethics

Ethics Committee Approval: The experimental protocol of this study was approved by the Animal Ethics Committee of Kayseri Erciyes University (ethics committee decision number: 2018/027).

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K.Ü., E.K., Concept: Z.D., E.A., S.Ö., B.Y., Design: Z.D., E.A., S.Ö., B.Y., Data Collection or Processing: A.K.Ü., E.K., Analysis or Interpretation: A.K.Ü., E.K., Z.D., E.A., Literature Search: A.K.Ü., E.K., Writing: A.K.Ü., E.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by the grant from The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number: 1919B011901981).

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Original Article

DOI: 10.14235/bas.galenos.2021.5788
Bezmialem Science 2022;10(2):174-83

Anatomical Evaluation of The Major Vessels with Ultrasound in Children Undergoing Cardiac Surgery

Kardiyak Cerrahi Geçiren Çocuklarda Majör Damarların Ultrason ile Anatomik Olarak Değerlendirilmesi

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ABSTRACT

Objective: The aim of this study was to evaluate anatomically bilateral internal jugular veins (IJVs), carotid arteries (CAs), femoral veins (FVs) and femoral arteries (FAs) in pediatric patients undergoing cardiac surgery using ultrasonography (USG) guidance.

Methods: A retrospective study was conducted involving 78 patients. The patients were divided into five different categories (0-1, 1-6, 6-12, 12-24 months and >24 months). The diameter (Dm) and depth (Dp) of the bilateral IJVs, CAs, FVs and FAs were measured with USG. The anatomical positions of the IJV, CA, FV and FA and variations were evaluated. The correlations of the Dm and Dp of the major vessels with age, height, weight, body surface area (BSA), and body mass index were analyzed.

Results: The Dms and Dps of IJV, CA, FV and FA were significantly different between the groups. The rate of anatomical variation was 23.1% in the right IJV, 28.2% in the left IJV, 19.2% in the right FV, and 28.2% in the left FV. The Dms and Dps of the major central vessels were significantly correlated with age, height, weight, and BSA. The regression coefficient indicated that the 1 year age increase was associated with an increase of 0.414 mm in the right IJV (RIV) Dm. And each 1 kg increase in weight was associated with an increase of 0.169 mm in the RIV Dm.

Conclusion: We found a high correlation between vascular Dms and age, weight, height, and BSA in children with congenital

ÖZ

Amaç: Bu çalışmada kalp cerrahisi geçiren pediatrik hastalarda ultrasonografi (USG) rehberliği ile bilateral internal juguler ven (IJV), karotis arter (CA), femoral ven (FV) ve femoral arterin (FA) anatomik olarak değerlendirilmesi amaçlanmıştır.

Yöntemler: Yetmiş sekiz hastayı içeren retrospektif bir çalışma dizayn edildi. Hastalar beş farklı yaş kategorisine ayrıldı (0-1 ay, 1-6 ay, 6-12 ay, 12-24 ay ve >24 ay). USG ile bilateral IJV, CA, FV ve FA çap (Dm) ve derinlikleri (Dp) ölçüldü. IJV, CA, FV ve FA'nın anatomik pozisyonları ve varyasyonları değerlendirildi. Majör damarların Dm ve Dp'si ile yaş, boy, ağırlık, vücut yüzey alanı (VYA) ve vücut kitle indeksi arasındaki korelasyonu analiz edildi.

Bulgular: İJV, CA, FV ve FA Dm'si ve Dp'si gruplar arasında anlamlı farklılık gösterdi. Anatomik varyasyon sağ İJV'de %23,1, sol İJV'de %28,2, sağ FV'de %19,2 ve sol FV'de %28,2 idi. Majör damarların Dm'si ve Dp'si yaş, boy, ağırlık ve VYA ile anlamlı derecede korele idi. Regresyon katsayısı, 1 yıllık yaş artışının sağ İJV Dm'sindeki 0,414 mm'lik bir artışla ve ağırlıktaki her 1 kg'lik artışın sağ İJV Dm'sindeki 0,169 mm'lik bir artışla ilişkili olduğunu gösterdi.

Sonuç: Konjenital kalp hastalığı olan çocuklarda vasküler Dm'ler ile yaş, ağırlık, boy ve VYA arasında yüksek bir korelasyon bulduk. Kardiyak cerrahi geçirecek pediyatrik hastalarda kateter girişimlerinden önce uygun damar ve kateter boyutunu belirlemek için majör damarların USG ile değerlendirilmelidir.

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Cite this article as: Salık F, Bıçak M, Erkul A, Aktiz Bıçak E, Akelma H, Kaya S, Doyurgan O, Kaçar CK. Anatomical Evaluation of The Major Vessels with Ultrasound in Children Undergoing Cardiac Surgery. Bezmialem Science 2022;10(2):174-83

Received: 22.12.2020
Accepted: 16.02.2021

heart disease. Major vessels should be evaluated by using USG to determine the appropriate vessel and catheter size before catheter interventions in pediatric patients undergoing cardiac surgery.

Keywords: İnternal jugular vein, femoral vein, femoral artery, correlation, ultrasound

Anahtar Sözcükler: İnternal juguler ven, femoral ven, femoral arter, korelasyon, ultrason

Introduction

In pediatric cardiac surgery, central venous cannulation (CVC) is mandatory for fluid-blood replacement, inotropic drug support, drug administration, and central venous pressure monitoring, just as arterial cannulation (AC) is also mandatory for continuous arterial pressure monitoring and frequent arterial blood sampling (1). Although the bilateral internal jugular veins (IJVs), subclavian veins (SVs), and femoral veins (FVs) are used for CVC, the radial artery and femoral artery (FA) are more frequently used for AC (2-4).

In most clinics, cannulation attempts are still performed using the anatomical landmark method. However, in pediatric patients, especially those with congenital heart disease, cannulation is difficult because of the diameter (Dm) of the small vessels, difficulty in finding the puncture vessel, and anatomical variations (3). With the increase in the use of ultrasound (USG), the success of CVC and AC administrations has increased and processing time and hospital costs have decreased (4-7). Another advantage of using USG is that it allows for anatomical examination before the intervention to determine the Dm and variations of the major vessels, to select the appropriate size catheter, and to apply cannulation in the correct localization (8). Some studies have also emphasized the correlation between vascular Dm and catheter size in terms of development of thrombosis, and researchers have emphasized that it is important to determine the appropriate catheter size for the vessel Dm using USG for anatomical evaluation before the intervention (9). In addition, USG reduces complication rates such as hematoma, arterial puncture, and pneumothorax in pediatric CVS, which is one of the most risky surgeries, by allowing fewer interventions and optimal vessel selection (10-12). It is a known fact that as the age of children decreases, the Dm of the vessels also shrinks. Thus, the area of the operation is narrower, and the cannulation procedures become more difficult, resulting in an increase in complication rates (13).

The Dm of the central arteries/veins and the anatomical relationships of these vessels may vary in pediatric patients undergoing cardiac surgery. This situation makes catheter administrations more difficult than in healthy patients and requires more experience and effort during administration. Therefore, the use of USG during CVC and/or AC administrations has become more important because of the possibility of anatomical variation, thrombosis, and bleeding in pediatric cardiac surgery patients, who are already at high risk.

In this study, we aim to evaluate anatomically the bilateral IJVs, carotid artery (CAs), FVs, and FAs in pediatric patients undergoing cardiac surgery using USG guidance and to determine the benefit of the findings in establishing the puncture vessel, needle length, and catheter Dm. Simultaneously, this study examines the correlation of the Dm and depth (Dp) of the major vessels with age, height, weight, body surface area (BSA), and body mass index (BMI).

Methods

Patients

This retrospective, observational, and descriptive study was conducted at local ethics committee of the hospital. The study was conducted after obtaining approval of the local ethics committee and in accordance with the principles of the Helsinki Declaration. Seventy-eight pediatric patients who underwent cardiac surgery between 01 January 2019 and 01 January 2020 and who underwent central venous catheter and/or FA catheter with USG were included in the study. Patients were divided into five different age categories (0-1 months, 1-6 months, 6-12 months, 12-24 months, and >24 months) to obtain measurements representing the general population. Written consent was obtained from the parents of the patients. Emergency patients, those with a history of operation or cannulation in the jugular or femoral region, and those allergic to the USG gel were excluded from the study. In addition, one patient whose right FA could not be visualized was excluded.

The demographic and clinical characteristics of patients, including age, gender, height, body weight, diagnosis, and presence of additional diseases, were recorded from medical records and observation forms. BSA was calculated in square meters (m^2) using the Mosteller formula: BSA: [height (cm) x weight (kg)/3,600] ^{½} (14). The BMI values of the patients were also calculated.

Measurements

In our clinic, most catheter administrations in pediatric cardiac surgery are performed using anatomical evaluation of large veins and arteries accompanied by USG and selection of the appropriate vessels after detection of any variations. For this purpose, in our clinic, the bilateral IJVs, CAs, FVs, and FAs of each patient are routinely evaluated. Patients' USG data and other parameters are recorded in the files.

After a 4 or 6-hour fasting period, patients were taken to the operation room, and standard anesthesia monitoring was performed. During the preoperative fasting period, the patients were given maintenance intravenous fluid according to the Holiday and Segar formula. The standard anesthesia regimen

consisted of general anesthesia. For induction, 0.1 mg/kg midazolam, 2-3 mg/kg propofol, 1-2 µg/kg fentanyl, and 0.6 mg/kg rocuronium were applied. After orotracheal intubation, mechanical ventilation was initiated without the use of positive end-expiratory pressure.

For all measurements, we used a USG device (Esaote, MyLab Six, the Netherlands) with a linear probe (5-12 MHz). IJV and CA measurements were obtained with patients in the supine position and after the head was turned about 15° to the opposite side. The practitioner placed the linear probe at the level of the cricoid cartilage with minimal compression to the neck. We performed the scan by visualizing the IJV on the short axis in the middle of the neck as suggested by the RaCeVA protocol (8). The Dm of the anteroposterior vessels and Dp of the IJV were measured on the short axis and recorded (Figure 1). The anatomical positions of the IJV and CA relative to each other (eight quadrants: anterior, posterior, medial, lateral, anteromedial, anterolateral, posteromedial, and posterolateral) and variations were evaluated and recorded bilaterally (Figure 2).

The FA-FV Dm and FA Dp were measured with the patients in the supine position and the thigh abducted at approximately 30°. The linear probe was placed with minimal compression on the FA pulsation 1 cm below the inguinal ligament. While the practitioner was on the side of the patient, the Dm of the anteroposterior vessels and FA Dp were measured on the short axis and recorded (Figure 3). The anatomical positions of the FA and FV relative to each other (possible eight quadrants: anterior, posterior, medial, lateral, anteromedial, anterolateral, posteromedial, and posterolateral) and variations were evaluated bilaterally and recorded (Figure 4).

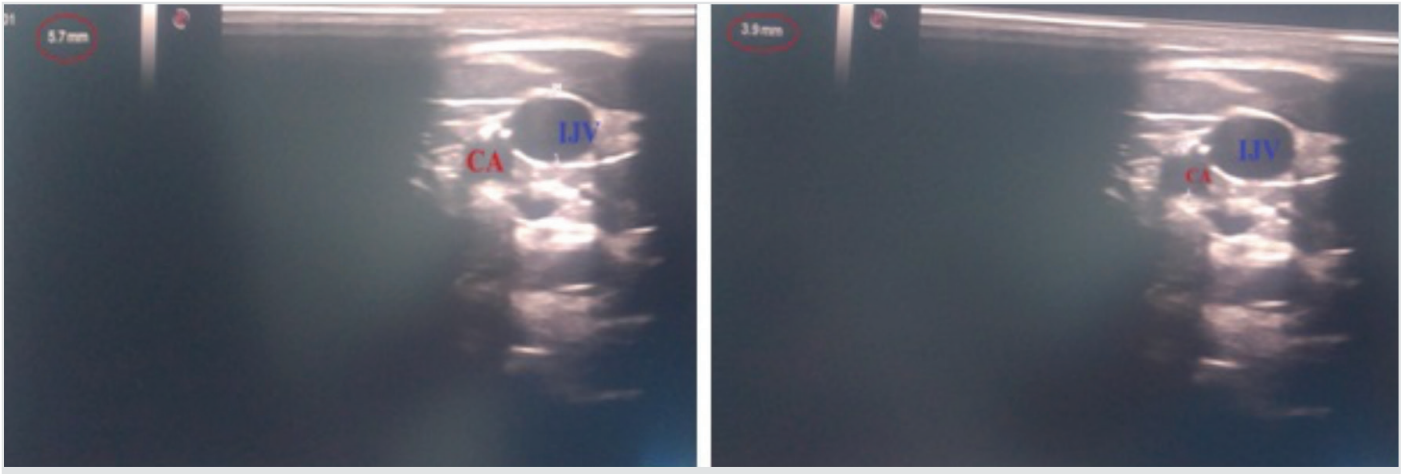

Figure 1. Vascular diameter measurements of the right IJV and CA in a 7-month-old boy
IJV: Right internal jugular vein, CA: Carotid artery

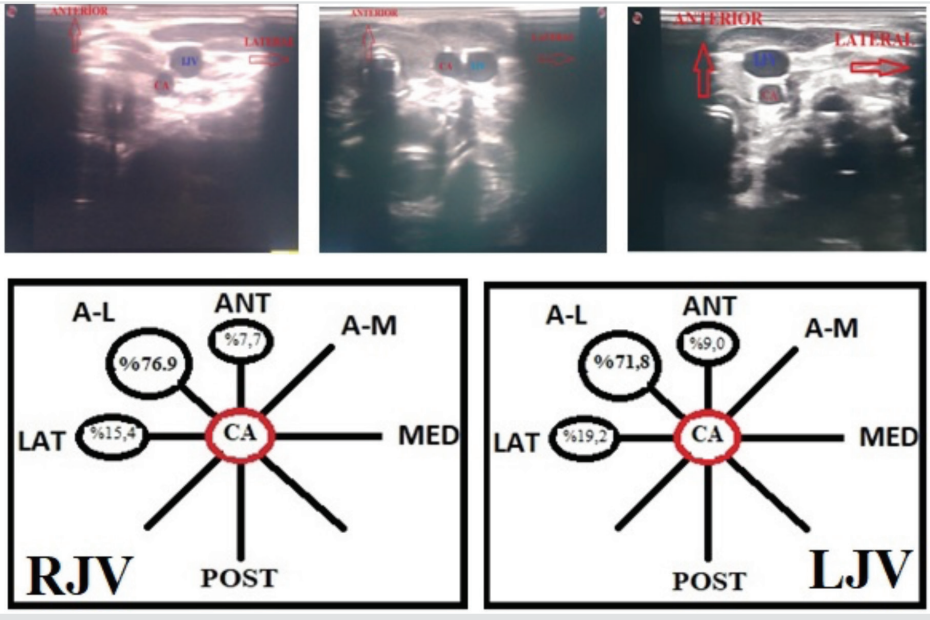

Figure 2. Anatomical variations of the IJV and CA
IJV: Right internal jugular vein, CA: Carotid artery

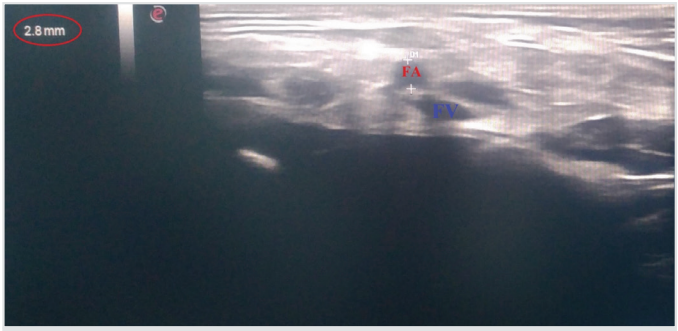

Figure 3. Vascular diameter measurements of the right FA and FV in a 17-month-old boy
FA: Femoral artery, FV: Femoral vein

The inner Dm of all vessels were measured anteroposteriorly using a caliper. For Dp, the distance from the skin surface to the anterior wall of the vessel was measured in millimeters. All measurements were performed by two anesthesiologists with USG training and at least 5 years of experience in USG use for interventional procedures and with continuous experience in pediatric CVS. After sterilization conditions were confirmed, the central venous catheter and/or FA catheter was inserted with USG by determining the appropriate vessel. The dimensions of the administered catheters were recorded.

Statistical Analysis

The SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All measurements were expressed in millimeters. Statistical data were expressed as mean and standard deviation, and categorical data were expressed as frequency and percentage. Categorical data in the groups were compared using chi-square test; the results were given as percentage (n). Pearson chi-square test was used to compare the difference between right and left sides. Kolmogorov-Smirnov test was used to determine whether the numerical data fit the normal distribution. Although the data matching the normality distribution were evaluated using one-way analysis of variance, the Kruskal-Wallis test was used to compare the data that did not conform to the normal distribution. In all comparisons, $p<0.05$ was considered significant. The relationship between the Dms of bilateral IJVs and FAs, the most frequently used central vessels, and the age was tested with separate, simple linear regressions. Correlation evaluations of normally distributed numerical data were performed by Pearson correlation test, whereas numerical data that did not fit the normal distribution were evaluated by Spearman correlation test. Correlation coefficient (r) results were interpreted as follows:

- R<0.2, weak correlation or no correlation
- R=0.2-0.4, weak correlation
- R=0.4-0.6, moderate correlation
- R=0.6-0.8, high correlation
- R>0.8, very high correlation

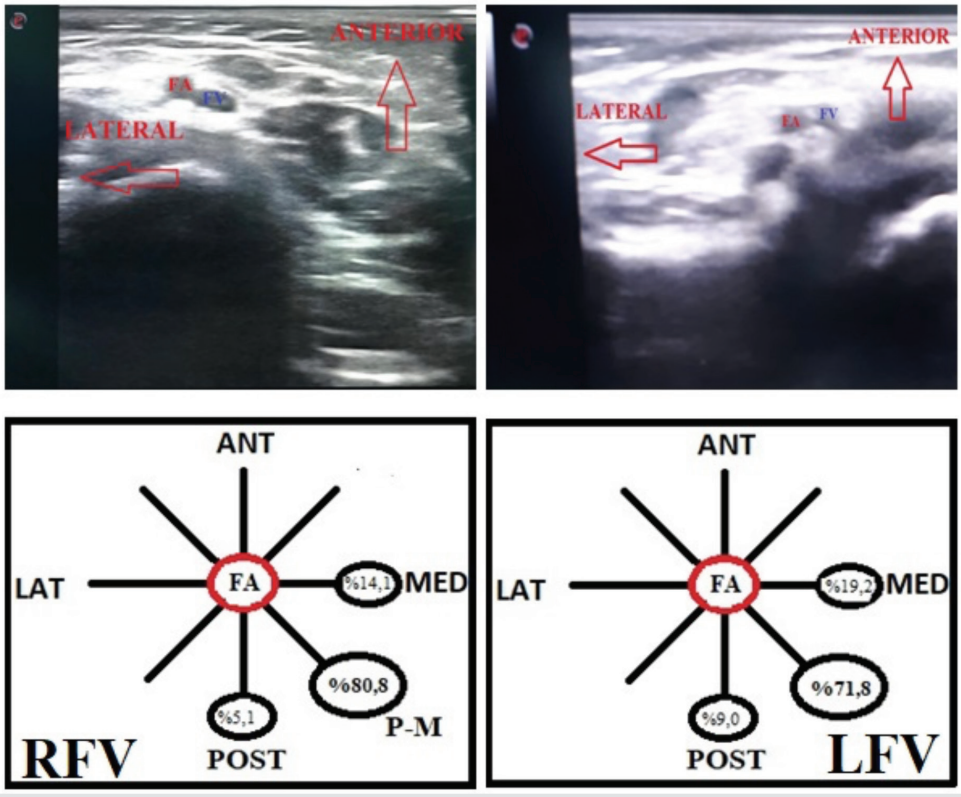

Figure 4. Anatomical variations of the FA and FV
FA: Femoral artery, FV: Femoral vein

Results

Seventy-eight patients were included in the study. The patients were divided into five groups based on age: 0-1 months (n=15), 1-6 months (n=14), 6-12 months (n=22), 12-24 months (n=13), and >24 months (n=14). Table 1 shows the demographic and clinical features of the patients.

Table 2 displays the internal Dm and distance to the skin of the vessels examined anatomically by USG. A statistically significant difference was found between the groups in all measurements. By including all patients (n=78), when the right and left measurements were compared, there was no statistically significant difference between the mean Dm (p values were 0.751 for IJV, 0.480 for CA, 0.793 for FV, and 0.720 for FA).

In the analysis that included all measurements, the rate of anatomical variation observed in patients was 25.6% in IJV and 23.7% in FV. These rates were 23.1% in the right IJV, 28.2% in the left IJV, 19.2% in the right FV, and 28.2% in the left FV. Table 3 displays the anatomical variations of the vessels examined with USG. When all measurements were included (n=78), there was no statistically significant difference in the right and left anatomic positions (p=0.461 for IJV-CA, p=0.207 for FV-FA). In addition, the right FA could not be visualized in one patient, who was excluded from the study.

Correlation

After demographic data and measurements were analyzed, correlations were determined to estimate the Dm and Dp of the vessels. The Dm and Dp of the major central vessels were significantly associated with age, height, weight, and BSA ($p<0.001$). There was no statistically significant relationship between the right and left FA Dp and BMI (p=0.078, 0.102, respectively).

A high or very high correlation was detected between the vessel Dm and age, height, weight, and BSA. Moderate correlations were detected between vascular Dp, age, height, weight, and BSA. A moderate correlation was detected between the Dm of the vessels and the BMI, and a weak correlation was found between the Dp of the vessels and BMI. The highest correlation coefficient values were between the vessel Dm and height. Pearson *R* correlation coefficients are shown in Table 4.

Regression

Separate simple linear regressions were performed to evaluate the relationships between the IJV, CA, FV, and FA Dm and Dp, and age, height, weight, BSA, and BMI. When we examined the results for the right IJV (RIJV) Dm, the regression coefficient indicated that the 1 year age increase was associated with an increase in the RIJV Dm of 0.414 mm. It was shown that each 1 kg increase in weyight was associated with an increase of 0.169 mm in RIJV Dm. Our results also showed that the DM of RIJV increased by 0.073 mm for every 1 cm increase in height (Table 5).

Multiple linear regressions of the RIJVs-left IJVs, the most used vessels for central venous catheter administration, and right-left FAs, which were frequently used in arterial catheterization, were performed.

Table 1. Demographic data and clinical features

	0-1 months (n=15) Mean ± SD	1-6 months (n=14) Mean ± SD	6-12 months (n=22) Mean ± SD	12-24 months (n=13) Mean ± SD	>24 months (n=14) Mean ± SD	p
Age (month)	0.38±0.16	2.78±1.26	7.27±1.31	17.07±3.49	81.97±68.65	≤0.01*
Height (cm)	51.5±1.8	58.5±5.5	67.1±4.5	76.5±6.4	109.0±28.7	≤0.01*
Body weight (kg)	3.35±0.37	4.32±0.9	6.25±1.1	8.61±1.0	20.9±14.3	≤0.01*
BMI	12.64±1.28	12.64±2.15	13.84±1.97	14.84±2.25	15.90±2.17	≤0.01*
Gender (male/female)	13/2	12/2	11/11	6/7	4/10	0.004*
ASA (2-3-4)	0-3-12	1-10-3	0-18-4	1-11-1	0-13-1	≤0.01*
Diagnosis						
Hypoplastic left heart	7	2	2	2	-	
TGA	6	1	-	-	-	
Pulmonary atresia/stenosis	2	-	-	-	1	
Aortic coarctation	-	3	2	-	-	
VSD	-	5	10	4	2	
ASD	-	-	-	-	7	
AVSD	-	-	5	1	1	
TOF	-	1	2	6	1	
Other	-	2	1	-	2	

SD: Standard deviation, BSA: Body surface area, BMI: Body mass index, ASA: American Society of Anesthesiologists, TGA: Transposition of the great arteries, VSD: Ventricular septal defect, ASD: Atrial septal defect, AVSD: Atrioventricular septal defect, TOF: Tetralogy of Fallot
*Statistically significant

Table 2. Major vein/artery diameters and distance to the skin by group

	0-1 months (n=15) Mean (SD)	1-6 months (n=14) Mean (SD)	6-12 months (n=22) Mean (SD)	12-24 months (n=13) Mean (SD)	>24 months (n=14) Mean (SD)	P
RIJV Dm (mm)	4.03 (0.55)	3.95 (0.84)	5.81 (0.97)	5.93 (1.28)	9.00 (1.47)	>.001*
RCA Dm (mm)	2.78 (0.37)	3.16 (0.60)	3.85 (0.57)	4.57 (0.86)	6.10 (1.21)	>.001*
RIJV Dp (mm)	5.10 (1.10)	5.70 (1.63)	6.61 (0.96)	6.73 (1.48)	8.35 (1.56)	>.001*
LIJV Dm (mm)	3.46 (0.58)	4.13 (1.01)	5.85 (0.95)	6.16 (1.82)	8.50 (1.57)	>.001*
LCA Dm (mm)	2.78 (0.64)	2.90 (0.71)	3.63 (0.49)	4.43 (0.87)	6.00 (1.08)	>.001*
LIJV Dp (mm)	5.00 (1.60)	5.42 (1.41)	6.66 (1.06)	6.03 (1.71)	8.17 (1.75)	>.001*
RFV Dm (mm)	2.40 (0.44)	2.74 (0.79)	3.06 (0.47)	3.54 (0.93)	5.58 (0.85)	>.001*
RFA Dm (mm)	1.82 (0.34)	2.00 (0.26)	2.07 (0.26)	2.90 (0.60)	4.73 (1.07)	>.001*
RFA Dp (mm)	3.90 (1.69)	4.92 (2.22)	4.90 (2.59)	4.67 (1.36)	7.54 (2.77)	.001*
LFV Dm (mm)	2.55 (0.64)	2.45 (0.73)	3.05 (0.53)	3.66 (0.93)	5.47 (0.86)	>.001*
LFA Dm (mm)	1.71 (0.22)	1.94 (0.36)	2.13 (0.34)	2.70 (0.51)	4.72 (0.99)	>.001*
LFA Dp (mm)	3.42 (1.33)	4.80 (2.74)	4.88 (2.47)	4.66 (1.03)	7.48 (2.72)	>.001*

SD: Standard deviation, RIJV: Right internal jugular vein, RCA: Right carotid artery, LIJV: Left internal jugular vein, LCA: Left carotid artery, RFV: Right femoral vein, RFA: Right femoral artery, LFV: Left femoral vein, LFA: Left femoral artery, Dm: Diameter, Dp: Depth
*Statistically significant

Table 3. Anatomical variations between groups

	0-1 months (n=15)	1-6 months (n=14)	6-12 months (n=22)	12-24 months (n=13)	>24 months n=14)	Total n=78	p
Right IJV-CA							0.832
Lateral	2 (13.3%)	3 (21.4%)	3 (13.6%)	2 (15.4%)	2 (14.3%)	12 (15.4%)	
Anterolateral	12 (80.0%)	9 (64.3%)	18 (81.8%)	10 (76.9%)	11 (78.6%)	60 (76.9%)	
Anterior	1 (6.7%)	2 (14.3%)	1 (4.54%)	1 (7.7%)	1 (7.1%)	6 (7.7%)	
Left IJV-CA							0.573
Lateral	3 (20.0%)	5 (35.8%)	3 (13.6%)	3 (23.1%)	1 (7.1%)	15 (19.2%)	
Anterolateral	10 (66.7%)	8 (57.1%)	17 (77.3%)	10 (76.9%)	11 (78.6%)	56 (71.8%)	
Anterior	2 (13.3%)	1 (7.1%)	2 (9.1%)	0 (0%)	2 (14.3%)	7 (9.0%)	
Right FA-FV							0.563
Lateral	0 (0%)	2 (14.3%)	4 (18.2%)	2 (15.4%)	3 (21.4%)	11 (14.1%)	
Anterolateral	14 (93.3%)	12 (85.7%)	17 (77.3%)	10 (76.9%)	10 (71.4%)	63 (80.8%)	
Anterior	1 (6.7%)	0 (0%)	1 (4.54%)	1 (7.7%)	1 (7.1%)	4 (5.1%)	
Left FA-FV							0.325
Lateral	3 (20.0%)	5 (35.7%)	4 (18.2%)	2 (15.4%)	1 (7.1%)	15 (19.2%)	
Anterolateral	11 (73.3%)	7 (50.0%)	16 (72.7%)	11 (84.6%)	11 (78.6%)	56 (71.8%)	
Anterior	1 (6.7%)	2 (14.3%)	2 (9.1%)	0 (0%)	2 (14.3%)	7 (9.0%)	

IJV: Internal jugular vein, CA: Carotid artery, FV: Femoral vein, FA: Femoral artery

Multiple linear regression RIJV Dm:

y=0.159 x BMI + 0.01 x age + 2,920

R^2=0.490; p [BMI]= 0.066; p [age] ≤0.01

Multiple linear regression LIJV Dm:

y=0.178 x BMI + 0.01 x age + 2,627

R^2=0.372; p [BMI]= 0.068; p [age] ≤0.01

Multiple linear regression RFA Dm:

y=0.040 x BMI + 0.01 x age + 1,620

R^2=0.657; p [BMI]=0.326; p [age] ≤0.01

Multiple linear regression LFA Dm:

y=0.061 x BMI + 0.01 x age + 1,286

R^2=0.667; p [BMI]= 0.127; p [age] ≤0.01

Discussion

The results of this study provide important information for the treatment of pediatric CVS patients who are likely to have various vascular anatomical variations, as they provide

Table 4. Correlations between RIJV, LIJV, RCA, LCA, RFV, RFA, LFV, and LFA diameters, and depths and age, weight, height, BSA, and BMI (r)

	Age (year)	Height (cm)	Weight (kg)	BSA (m^2)	BMI
RIJV Dm (mm)	0.683*	0.823*	0.708*	0.775*	0.478*
RCA Dm (mm)	0.738*	0.860*	0.755*	0.815*	0.464*
RIJV Dp (mm)	0.546*	0.655*	0.575*	0.622*	0.338*
LIJV Dm (mm)	0.585*	0.724*	0.592*	0.667*	0.442*
LCA Dm (mm)	0.715*	0.813*	0.724*	0.780*	0.501*
LIJV Dp (mm)	0.503*	0.563*	0.506*	0.546*	0.365*
RFV Dm (mm)	0.718*	0.810*	0.727*	0.783*	0.479*
RFA Dm (mm)	0.808*	0.881*	0.797*	0.850*	0.466*
RFA Dp (mm)	0.420*	0.448*	0.385*	0.424*	0.201
LFV Dm (mm)	0.702*	0.803*	0.715*	0.770*	0.453*
LFA Dm (mm)	0.810*	0.897*	0.819*	0.870*	0.498*
LFA Dp (mm)	0.453*	0.504*	0.425*	0.469*	0.187

BSA: Body surface area, BMI: Body mass index, RIJV: Right internal jugular vein, RCA: Right carotid artery, LIJV: Left internal jugular vein, LCA: Left carotid artery: RFV: Right femoral vein, RFA: Right femoral artery, LFV: Left femoral vein, LFA: Left femoral artery, Dm: Diameter, Dp: Depth
*Statistically significant

clinical practice guidance for the anatomical examination of the vessels to be performed before CVC and/or FA catheter administration with USG. At the same time, this study examines the correlation-regression relationship between age, height, weight, BSA, and BMI and major vessels and provides an idea to clinicians on appropriate vessel selection and catheter size during catheterization.

Internal Jugular Vein-carotid Artery

Barone et al. (9) measured IJVs of 100 newborns undergoing noncardiac surgery and found a strong correlation between the IJV Dm and body weight. Alderson et al. (15) evaluated 50 children younger than 6 years undergoing cardiac surgery and found a strong correlation between IJV Dp and age and body weight; they reported a weak correlation between IJV Dm and age and body weight. Sayin et al. (16), in a study involving 135 patients who had undergone noncardiac surgery, reported a weak correlation of IJV Dm and Dp with age, height, and weight. In our study, we determined that the mean Dm of the IJV varied between 3.46 and 9.00 mm and its Dp between 5.10 and 8.35 mm; the Dm of the CA was found to vary between 2.78 and 6.10 mm. A high or very high correlation was detected between the Dms of bilateral IJVs and CAs and age, height, weight, and BSA, whereas a moderate correlation was detected with BMI. The strongest correlation was found between vessel Dm and height. There was a moderate or high correlation between IJV Dp and age, height, weight, and BSA and a weak correlation with BMI. Therefore, we realized that the calculation of vessel Dm and Dp with BMI in children with congenital heart disease might not provide accurate results.

There was no significant difference between the Dms of right and left IJVs and between the Dms of right and left CAs in our study. This result was parallel with the findings of Karazincir et al. (13), who found similar results between the Dms of right and left IJVs in healthy children aged 0-6 years. In their study evaluating healthy children aged 7-12 years, Yildirim et al. (17) reported that the RIJV Dm was larger than the LIJV Dm.

Vascular malformations and variations may be more common in children with congenital heart disease. This can lead to accidental risk of arterial puncture and many complications. In the healthy population, IJV is the most common anterolateral

Table 5. Linear regression models

	BMI		Age		Height		Weight		BSA	
	R2	Coefficient	R2	Coefficient	R2	Coefficient	R2	Coefficient	R2	Coefficient
RIJV Dm (mm)	0.466	0.414	0.678	0.073	0.501	0.169	0.601	6.410	0.228	0.426
RCA Dm (mm)	0.545	0.297	0.740	0.050	0.570	0.119	0.665	4.473	0.215	0.275
RIJV Dp (mm)	0.299	0.273	0.428	0.048	0.330	0.113	0.386	4.238	0.114	0.249
LIJV Dm (mm)	0.343	0.361	0.524	0.065	0.350	0.143	0.445	5.607	0.196	0.401
LCA Dm (mm)	0.511	0.289	0.661	0.048	0.524	0.115	0.608	4.296	0.251	0.298
LIJV Dp (mm)	0.253	0.270	0.317	0.044	0.256	0.106	0.298	3.992	0.133	0.288
RFV Dm (mm)	0.516	0.274	0.656	0.045	0.529	0.109	0.613	4.068	0.229	0.268
RFA Dm (mm)	0.652	0.285	0.777	0.045	0.636	0.110	0.723	4.093	0.217	0.242
RFA Dp (mm)	0.176	0.311	0.201	0.048	0.148	0.112	0.180	4.276	0.040	0.219
LFV Dm (mm)	0.492	0.267	0.645	0.045	0.511	0.107	0.594	4.002	0.205	0.254
LFA Dm (mm)	0.657	0.284	0.805	0.046	0.670	0.113	0.756	4.159	0.248	0.257
LFA Dp (mm)	0.205	0.339	0.254	0.055	0.181	0.125	0.220	4.780	0.035	0.206

BSA: Body surface area, BMI: Body mass index, RIJV: Right internal jugular vein, RCA: Right carotid artery, LIJV: Left internal jugular vein, LCA: Left carotid artery, RFV: Right femoral vein, RFA: right femoral artery, LFV: Left femoral vein, LFA: Left femoral artery, Dm: Diameter, Dp: Depth

location compared with CA (18). However, variations that will complicate catheter interventions may be more frequent in children with congenital heart disease. It has been reported in the literature that IJV is in the anterolateral position at a rate of 54.4-87.8% compared to CA (18,19). Uzumcugil et al. (20) found that in 84% of neonatal patients weighing less than 4000 g, the IJV was in the lateral position compared with CA. Souza et al. (21) found that there was an anatomical variation between the IJV and CA in 7.7% of 142 patients they examined. They reported that the highest variation rate was in children between the age of 1 month and 2 years (21). We determined the position of the IJV relative to the CA on the right side as 76.9% anterolateral. This rate was 71.8% on the left. In our study, the rate of anatomical variation between the left-side IJV and CA was higher than that of the right side (28.2% and 23.1%). The anatomical variation rate on the left side in the newborn group and in the 1- to 6-month age group was more pronounced than the right side. We found that the most common variation type was IJV located lateral to CA.

Femoral Artery/Vein

Keiler et al. (22) found that the FV Dm in adults was strongly correlated with gender, age, weight, and BMI but did not correlate with height. López Álvarez et al. (23) found that there was a significant correlation between FA and FV Dm and age, height, weight, and BSA in pediatric patients followed up for non-cardiac reasons, and the highest correlation was found with height. In addition, they reported that there was a significant correlation between FA and FV Dp and age, height, weight, and BSA, but the highest correlation was with weight. In our study, the average FV Dm was between 2.40 and 5.58 mm; we determined that the average FA Dm ranged between 1.71 and 4.73 mm, and the average FA Dp ranged between 3.42 and 7.54 mm. A high or very high correlation was found between the Dms of bilateral FVs and FAs and age, height, weight, and BSA, and a moderate correlation with BMI was detected. The strongest correlation was seen between vessel Dm and height. There was a moderate correlation between the Dp of FA and age, height, weight, and BSA and a weak correlation with BMI. Our correlation results between FV and FA Dm and age, height, weight, and BSA were in line with previous studies. Therefore, we concluded that the calculation of the FV-FA Dm and FA Dp with BMI might not provide accurate results in children with congenital heart disease.

In our study, we found no significant difference between the Dm of right and left FVs or between the Dm of right and left FAs. This result was similar to the study of Keiler et al. (22), who detected significant bilateral asymmetry between the Dm of right and left FVs. However, in our study, the FV Dm was larger on both sides than the FA Dm in all age groups.

In their study of pediatric patients, Souza et al. (21) stated that there was an anatomic variation of 9.8% between FA and FV and this was most common in children between 1 month and 2 years of age (24.4%). They also reported that the most common variation type was FA with anterolateral location to FV (21). Warkentine et al. (2) stated that FA was 88% laterally located compared with FV. We determined the anterolateral position of FA according to FV on the right side in 80.8% of the patients. This rate was 71.8% on the left. The variation between the FA and FV on the left was greater than on the right (28.2% and 19.2%). We determined that the most common variation type was FV located medial to FA.

Effects on Clinical Practice

The relationship between vessel Dm and catheter size in terms of development of thrombosis is well known. Therefore, studies have reported that the ideal catheter Dm is approximately one-third of the vessel Dm (9,20). Based on the data from our study, for IJV cannulation, 3 French (1 mm) catheters can be used in newborns, 3-4 French (1 mm) in children aged 1-6 months, 4-5 French (1.3-1.6 mm) for children 6-12 months, 5-6 French (1.6-2.0 mm) for children 12-24 months, and 5-7 (1.6-2.4 mm) French for children >24 months. For FA cannulation, 2 French catheters can be used for newborns, 2 French for children aged 1-6 months, 2 French for children 6-12 months, 2-3 French for children 12-24 months, and 3 French for children >24 months. However, instead of estimating, it would be more accurate to determine the catheter Dm by measuring with USG by experienced people.

Study Limitations

1. Because USG evaluation is subjective and depends on clinical experience, measurements using computed tomography or angiography can provide more accurate results in children with congenital heart disease. To minimize this error, two anesthesiologists with at least 5 years of clinical USG experience and experience in pediatric cardiac surgery performed all measurements.

2. We did not measure the SV used in central venous catheter administrations.

3. Only the anterior and posterior Dms of the vessels were measured. The transverse Dm or cross-sectional area of the vessel was not measured. Long-axis anatomical evaluations of the vessels were absent in our study.

4. The investigation was made with the patient only in the supine position. No measurement was made in the Trendelenburg position, which could lead to a change in vessel Dm.

5. The Dms and anatomic variations of femoral vessels were not evaluated according to some leg positions.

6. Measurements were made with patients on mechanical ventilation. Therefore, the extent to which mechanical ventilation affected vessel Dm could not be evaluated.

7. Due to the low number of patients, we could not make a separate formulation that would determine the vessel Dm in all age groups. This may be the subject of larger series studies.

Conclusion

In this study, we found a high correlation between vascular Dms and age, weight, height, and BSA in children with congenital heart disease. However, we believe that determining the vessel Dm with BMI in children with congenital heart disease may not lead to the correct result. We found an anatomical variation rate of 28.2% on the left and 23.1% on the right between IJV and CA. We found that the most common variation type was IJV located lateral to CA. We established that the rate of anatomical variation between FA and FV was 28.2% on the left and 19.2% on the right.

Our study provides important data for clinicians in terms of USG evaluation for appropriate vessel selection, determination of anatomical variations, and determination of catheter size and needle length before catheterization in pediatric patients undergoing cardiac surgery, which is a special patient population. Therefore, the routine use of USG in venous catheter and/or arterial catheter interventions in pediatric patients undergoing cardiac surgery will benefit in terms of increased interference success and reduced complications.

Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee University of Health Sciences Turkey Gazi Yaşargil Training and Research Hospital (approval date and number: 20.12.2019-402).

Informed Consent: Retrospective study

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: F.S., Design: F.S., M.B., E.A.B., C.K.K., O.D., Analysis or Interpretation: H.A., S.K., A.E., Writing: F.S., M.B., E.A.B., C.K.K., O.D., H.A., S.K., A.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5534
Bezmialem Science 2022;10(2):184-93

The Relationship of Students' Coping Styles with Stress and E-Learning in the COVID-19 Pandemic

COVID-19 Pandemisinde Öğrencilerin Stresle Başa Çıkma Tarzlarının E-Öğrenme İle İlişkisi

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ABSTRACT

Objective: This research was planned to evaluate the relationship between coping styles with stress and e-learning of health services vocational school students during the distance education process in the Coronavirus diease-19 (COVID-19) pandemic.

Methods: The sample of this descriptive and cross-sectional research consisted of 425 students who agreed to participate in the research. The data were collected using the student information form created by the researchers, "The Coping with Stress scale" and "Attitude scale Towards E-learning".

Results: It was determined that 72.2% of the students were worried about COVID-19 pandemic process. In the helpless approach sub-dimension, a significant difference was found between the students with low socioeconomic level and those with middle socioeconomic level ($p<0.05$). Helpless approach score averages of students with low socioeconomic level were higher than other groups. In the self-confident approach sub-dimension, there was a significant difference between the students with high socioeconomic level and those with low ($p<0.05$). Students with a high socioeconomic level were found to have high median scores of self-confident approach sub-dimension. In addition, a difference was found between students with internet availability and those without internet availability in terms of the mean score of the e-learning attitude scale ($p<0.05$). A low positive correlation was found between "The Coping with Stress scale" and "Attitude scale Towards E-learning" ($p<0.01$).

ÖZ

Amaç: Bu araştırma; Koronavirüs hastalığı-19 (COVID-19) pandemisinde uzaktan eğitim sürecinde sağlık hizmetleri meslek yüksekokulu öğrencilerinin stresle başa çıkma tarzlarının e-öğrenme ile ilişkisini değerlendirmek amacı ile planlandı.

Yöntemler: Tanımlayıcı ve kesitsel nitelikte olan araştırmanın örneklemini çalışmaya katılmayı kabul eden 425 öğrenci oluşturdu. Veriler araştırmacılar tarafından oluşturulan öğrenci bilgi formu, "Stresle Başa Çıkma Tarzları ölçeği" ve "E-öğrenmeye Yönelik Tutum ölçeği" kullanılarak toplandı.

Bulgular: Öğrencilerin %72,2'sinin pandemi süreciyle ilgili olarak endişe duyduğu belirlendi. Çaresiz yaklaşım alt boyutunda, sosyoekonomik düzeyi düşük ve orta düzey olan öğrenciler arasında anlamlı fark bulundu ($p<0,05$). Sosyoekonomik düzeyi düşük öğrencilerin çaresiz yaklaşım puan ortalaması diğer gruplara göre daha yüksekti. Kendine güvenli yaklaşım alt boyutunda sosyoekonomik düzeyi yüksek olan öğrencilerle düşük olan öğrenciler arasında anlamlı fark saptandı ($p<0,05$). Sosyoekonomik düzeyi yüksek olan öğrencilerin kendine güvenli yaklaşım alt boyut ortanca puanı yüksek saptandı. Ayrıca internet erişimi olan ve olmayan öğrenciler arasında e-öğrenmeye yönelik tutum ölçeği ortalama puanı açısından fark bulundu ($p<0,05$). "Stresle Başa Çıkma ölçeği" ile "E-öğrenmeye Yönelik Tutum ölçeği" arasında düşük pozitif korelasyon saptandı ($p<0,01$).

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Received: 03.11.2020
Accepted: 14.02.2021

Cite this article as: Akbal Y, Pekmezci H, Çuvalcı B. The Relationship of Students' Coping Styles with Stress and E-Learning in the COVID-19 Pandemic. Bezmialem Science 2022;10(2):184-93

Conclusion: There is a positive relationship between the ways of coping with stress and the attitude towards e-learning in healthcare students during the pandemic process.

Keywords: COVID-19, e-learning, students, health, stress

Sonuç: Sağlık hizmeti öğrencilerinde pandemi sürecinde stresle başa çıkma yolları ile e-öğrenmeye yönelik tutumlar arasında pozitif bir ilişki vardır.

Anahtar Sözcükler: COVID-19, e-öğrenme, öğrenci, sağlık, stres

Introduction

The World Health Organization declared Coronavirus disease-19 (COVID-19) as an emergency high-risk pandemic on January 30 2020 (1). COVID-19 is a dangerous viral disease that involves a wide range of symptoms from mild to severe, causing pneumonia by affecting the respiratory tract and spreading globally (2). General symptoms include fever, cough, shortness of breath, weakness, muscle and body aches, headache, loss of taste, sore throat, runny nose, nausea, and diarrhea (3). The number of people losing their lives due to the COVID-19 pandemic is increasing day by day, and this number reaches millions (4). In line with the COVID-19 pandemic data, which is updated every day, every hour, countries have to constantly renew themselves in stopping and preventing the pandemic. Although there are uncertainties about the spread of the COVID-19 pandemic, it is known that it passes from person to person by respiration. The Center for Disease Control and Prevention has reported that COVID-19 can be transmitted to a healthy person in close contact with respiratory droplets produced by the coughing, sneezing or speech of infected or asymptomatic carriers (3). Management of the disease includes measures such as using a mask, providing hand hygiene, not traveling to high-risk areas, and avoiding people with suspected COVID-19 and crowded environments (5).

Isolation applied in order to reduce the spread and manage the process in the COVID-19 pandemic has affected social, economic and educational life. This process has led to the creation of new models in education. E-learning, which takes part in the teaching process in the distance education model, has quickly gained a place in the modern education approach (6). E-learning can also be defined as online learning, disorganized learning, web learning, and virtual learning (7). In e-learning, students have a role as well as educators. Students should have a high level of readiness in e-learning. This can be achieved by increasing technical and training support (8).

In this process, the rapid transformation of education and training into an e-learning model negatively affects especially students in associate degree programs who have a large number of applied courses such as first and emergency aid, anesthesia, physiotherapy, elderly care, medical laboratory techniques. Students are experiencing a lot of stress with both the rapidly changing education process and the threat of COVID-19 on health (9). In a research conducted with students studying in the field of health, it was observed that students experienced stress in both clinical and educational settings (10). Stress causes many negative conditions in people such as acute and chronic diseases, depression, exhaustion, anxiety and insomnia, increased smoking and alcohol use and aggressive behavior. Stress is an individual situation and there is no common solution for coping with stress. Each individual must manage his/her own stress, find a method of coping with stress that is appropriate for his/her personality structure and view of life, and cope effectively with stress (11).

Ways of coping with stress can set the stage for positive developments in university students towards learning by mobilizing them (11). Taking necessary precautions to manage stress on time is extremely important for the efficiency of education (12). Based on this information, we aimed to determine the relationship between e-learning and the ways of coping with stress of health services vocational school students during the COVID-19 pandemic process.

Method

Research Type

This research is planned as descriptive and cross-sectional.

Research Sample

The population of this research consisted of 714 students receiving education in emergency and first aid, anesthesia, medical laboratory techniques, aged care and physiotherapy program in the 2019-2020 spring semester at a health services vocational school in Turkey. Sample selection was not made in the research, it was tried to reach the population. The research included students who were educated and trained in the health services vocational school of the relevant institution, who used the e-learning method (to continue their education with technology tools such as telephone, computer, etc.) and who agreed to participate in the research. The data of the research were collected between May 28 and June 28 2020. The research was completed with 425 students. The return rate of students was calculated as 59%.

Ethical Approval

Ethics committee approval was obtained from the Non-Invasive Clinical Research Ethics Committee of the institution where the research was conducted with the decision numbered 40465587-102.01-105.

Research Variables and Data Collection Tools

After obtaining Ethics committee approval we reached the head of health services vocational school through corporate communication network. The research objectives were presented for ethical permission, while assuring that the research was voluntary and all data would be treated confidentially throughout the research phase. And finally, head of the health services vocational school agreed to share the e-mail address of their students. The data were collected electronically with the

form created with Google e-form. In the introduction part of this form, a voluntary consent was received from the students. The form of the students who did not voluntarily accept to participate in the research was terminated after this consent question.

The form consisted of three parts. In the first part, a student information form with 14 questions was used to determine the sociodemographic variables and information about e-learning of students. In the second part, "The Coping with Stress scale", which included 30 questions to determine the ways of coping with stress was used. In the third part, "The Attitude scale Towards E-learning" consisting of 23 questions was used to determine the effect of distance education. In the data; sociodemographic variables constituted the independent variables, and "The Coping with Stress scale" and "Attitude scale Towards E-learning" constituted the dependent variables.

Student Information Form: In this section, there were a total of 14 questions including nine questions about sociodemographic variables (age, program, school year, gender, marital status, place of residence, family type, socioeconomic level and working status in a health institution) and five questions about determining e-learning variables (having android phone, having personal computer, the internet availability in the place of residence, technological device used in education and training, technological device used in education and training, worrying about the COVID-19 pandemic) (7,9,13).

The Coping with Stress Scale: The scale was developed by Folkman and Lazarus (14) with 66 items, and validity and reliability study in Turkey was made by Siva (1991), and then, by making psychometric evaluations by Şahin and Durak (15), the short form with 30 items and 5 sub-dimensions was constituted. The scale is a self-assessment. The items are scored on a 4-point likert-type scale, anchored by 0=never and 3=always. The scale consists of self-confident approach (8,10,14,16,20,23,26), optimistic approach (2,4,6,12,18), helpless approach (3,7,11,19,22,25,27,28), submissive approach (5,13,15,17,21,24), seeking of social support approach sub-dimensions (1,9,29,30). Cronbach alpha values of sub-dimensions; range from 0.49-0.68 in the optimistic approach, 0.62-0.80 in the self-confident approach, 0.64-0.73 in the helpless approach, and 0.47-0.72 in the submissive approach (14,15). In this research, cronbach alpha value for self-confident approach sub-dimension was 0.80, for optimistic approach sub-dimension 0.71, for helpless approach sub-dimension 0.74, for submissive approach sub-dimension 0.61, and for seeking of social support approach sub-dimension 0.47.

Attitude Scale Towards E-Learning: The scale developed by Kisanga (16) to determine under what conditions and in what direction the attitudes of university students towards e-learning differ, consists of 23 items and 4 sub-dimensions. Validity and reliability study of the scale was made by Biçer and Korucu (17) in Turkey. The sub-dimensions of the scale are; tendency to use technology (1-6), satisfaction (7-11), motivation (12-17), usability (18-23) and the total score varies between 23 and 115. The higher score in the scale, the higher attitude towards e-learning. The Cronbach's alpha value of the scale is 0.78 (16,17). In this research, Cronbach's alpha value was found to be 0.69.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 21.0 program was used to evaluate the data of the research. Comparisons were made between independent variables and sociodemographic and e-learning variables, and between dependent variables and the scale coping with stress and attitude scale towards e-learning. Results were at 95% confidence interval and significance level was $p<0.05$. The compliance of the data to normal distribution was evaluated by using Kolmogorov-Smirnov/Shapiro-Wilk tests. Since the variances for post-hoc were homogeneous in the one-way ANOVA test, Tukey test was used. Bonferroni corrected Mann-Whitney U test was used for significant differences between groups in the test of Kruskal-Wallis H-test. Pearson's analysis was used for correlation.

Results

Of the students 90.4% were between the ages of 18-21, 24% were in the first and emergency aid program, 53.2% were first grade students, 82.1% were female, 98.6% were single, 39.8% were residing in the rural-urban, 83.3% of them lived with nuclear family, 89.2% had middle socioeconomic level. According to findings, 96.5% of the students currently did not work in a health institution, 97.6% had an android phone, 61.2% did not have a personal computer, 77.6% used a telephone in education and training, 78.1% had internet access in their place of residence and 72.2% were worrying about COVID-19 pandemic (Table 1).

Self-confident approach sub-dimension score distributions varied significantly according to the socioeconomic levels of the students ($p<0.05$). As a result of Post-hoc binary comparisons made to determine which group caused the difference; it was found that there was a significant difference between groups with low and high socioeconomic levels, and those with high socioeconomic levels had higher self-confident approach sub-dimension score. In the helpless approach sub-dimension, there was a significant difference between low and middle socioeconomic level students ($p<0.05$). Helpless approach sub-dimension scores of students with low socioeconomic status were higher than students with middle socioeconomic status. In the same sub-dimension, there was a significant difference between the students who were worried about the COVID-19 pandemic and the students who were not worried about the COVID-19 pandemic ($p<0.05$). The median score of those who were worried about the pandemic was higher than the students who were not worried (Table 2).

A significant difference was found in the sub-dimension of submissive approach according to family type ($p<0.05$). Submissive approach sub-dimension median score of students living in extended families was higher than students living in nuclear families (Table 2).

A significant difference was found in the optimistic approach sub-dimension according to the school year ($p<0.05$). The median

Table 1. Sociodemographic and e-learning variables of students (n=425)

Sociodemographic variables		n (%)
Age (year)	18-21	384 (90.4)
	22-25	35 (8.2)
	≥26	6 (1.4)
Program	First and emergency aid	102 (24.0)
	Anesthesia	101 (23.8)
	Medical laboratory techniques	87 (20.5)
	Physiotherapy	84 (19.8)
	Elderly care	51 (12.0)
School year	1st year	226 (53.2)
	2nd year	199 (46.8)
Gender	Female	349 (82.1)
	Male	76 (17.9)
Marital status	Single	419 (98.6)
	Married	6 (1.4)
Place of residence	Urban	155 (36.5)
	Rural-urban	169 (39.8)
Family type	Nuclear family	354 (83.3)
	Extended family	71 (16.7)
Socioeconomic level	Low	37 (8.7)
	Middle	379 (89.2)
	High	9 (2.1)
Working status in a health institution	Yes	15 (3.5)
	No	410 (96.5)
E-learning variables		
Having android phone	Yes	415 (97.6)
	No	10 (2.4)
Internet availability in the place of residence	Yes	332 (78.1)
	No	93 (21.9)
Technological device used in education and training	Phone	330 (77.6)
	Personal computer	88 (20.7)
	Tablet etc.	7 (1.6)
Worrying about the COVID-19 pandemic	Yes	307 (72.2)
	No	118(7.8)

COVID-19: Coronavirus disease-19

score of the optimistic approach sub-dimension of the second-year students was higher than the first-year students. There was a significant difference between the socioeconomic level groups of students in the same sub-dimension ($p<0.05$). A significant difference was found between students with low and middle socioeconomic levels, and low and high socioeconomic levels. Students with middle socioeconomic levels had higher optimistic approach scores than students with low socioeconomic level. Students with a high socioeconomic level had a higher optimistic approach score than those with a low socioeconomic level. Again, in the optimistic approach sub-dimension, there was a significant difference between the groups that were worried and that were not worried about the COVID-19 pandemic ($p<0.05$). The optimistic sub-dimension median score of students who were not worried about the pandemic was higher than the students who were worried about the pandemic (Table 2).

There was a statistically significant difference between seeking of social support sub-dimension and students' family type ($p<0.05$). Mean scores of seeking of social support sub-dimension of students living in nuclear families were higher than students living in extended families. There was a significant difference between the socioeconomic level groups of students and the same sub-dimension ($p<0.05$). In post-hoc paired comparisons, it was found that there was a significant difference between students with middle socioeconomic status and those with low socioeconomic level, and the median score of the seeking of social support sub-dimension was higher in students with middle socioeconomic level (Table 2).

There was a significant difference between the total score of the attitude scale towards e-learning and the worry about the pandemic of the students ($p<0.05$). The total median score of the attitude scale towards e-learning of students who were worried about the pandemic was higher than the students who were not worried about the pandemic. There was a significant difference between the groups according to the internet availability in the place of residence in the same sub-dimension ($p<0.05$). The total mean score of towards e-learning scale of students who did not have internet access in their place of residence was higher than students with internet availability (Table 3).

In the research, it was found that a positive significant correlation between the ways of coping with stress and the attitude scale towards e-learning ($r=0.194$, $p<0.01$). As the total score of the scale coping with stress of the students increased, their scores of attitude towards e-learning also increased. When looking at the relationship between the scale coping with stress and the sub-dimensions of attitude scale towards e-learning, a significant positive relationship was found between the scale coping with stress and usability ($r=0.109$, $p<0.05$) and tendency to use technology ($r=0138$, $p<0.01$). As the total score of the scale coping with stres increased, the usability and tendency to use technology for e-learning increased (Table 4).

Discussion

The COVID-19 is a disease that causes anxiety, fear and stress in people due to its rapid transmission through droplets and the lack of effective treatment or vaccine yet. The COVID-19 pandemic process, which affects the health of people in every field, has caused stress in students in academic life (18). Studies conducted during the pandemic period have also determined that students experience problems such as anxiety and worry (19,20). In the current research, a great majority of the students (72.2%), stated that they were worried about the COVID-19 pandemic. In another research conducted with nurse students in Turkey, it was found that students experienced moderate stress

Table 2. Comparison of students' the coping with stress scale sub-dimension scores according to sociodemographic variables

Sociodemographic variables	Self-confident approach	Helpless approach	Submissive approach	Optimistic approach	Seeking of social support approach
	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)
Age (year)					
18-21	21 (8-28)	19 (8-32)	13 (6-24)	14.5 (5-20)	13 (6-18)
22-25	22 (15-28)	18 (9-29)	13 (6-19)	15 (6-20)	13 (9-18)
≥26	21.5 (19-27)	17(15-20)	13.5 (12-16)	16 (13-16)	13.5 (12-14)
	p=0.696*	p=0.287**	p=0.770*	p=0.470**	p=0.308*
Program					
First and emergency aid	21.5 (8-28)	19 (8-29)	13 (6-17)	14 (6-20)	13 (6-18)
Medical laboratory techniques	21 (11-28)	20 (13-30)	13 (7-22)	14 (5-19)	13 (8-17)
Physiotherapy	21 (13-28)	19 (8-30)	13 (6-20)	15 (5-19)	13 (6-17)
Elderly care	21 (11-28)	19 (11-32)	12 (8-23)	15 (10-20)	13 (9-18)
Anesthesia	21 (15-28)	19 (9-29)	13 (8-24)	15 (6-20)	13 (8-18)
	p=0.061**	p=0.115*	p=0.148**	p=0.325**	p=0.110**
School year					
1st year	21 (8-28)	19 (8-32)	13 (6-24)	14 (5-20)	13 (6-18)
2nd year	21 (14-28)	19 (9-30)	13 (6-24)	15 (5-20)	13 (8-18)
	p=0.374***	p=0.925***	p=0.217***	p=0.007***	p=0.385***
Gender					
Female	21 (8-28)	19 (8-30)	13 (6-24)	15 (6-20)	13 (6-18)
Male	21 (11-28)	19 (10-32)	13 (6-24)	14 (5-20)	13 (8-18)
	p=0.638***	p=0.116***	p=0.265***	p=0.606***	p=0.080***
Marital status					
Married	21.5 (17-28)	17 (9-24)	13.5 (6-15)	15.5 (11-18)	14 (12-17)
Single	21 (8-28)	19 (8-32)	13 (6-24)	15 (5-20)	13 (6-18)
	p=0.723****	p=0.209****	p=0.902****	p=0.490****	p=0.116****
Place of residence					
Urban	21 (8-28)	19 (8-29)	13 (6-24)	15 (5-20)	13 (8-18)
Rural-urban	21 (11-28)	19 (8-32)	13 (7-22)	15 (6-20)	13 (6-18)
Rural	21 (13-28)	19 (11-30)	13 (6-22)	14 (5-19)	13 (6-18)
	p=0.407**	p=0.910*	p=0.082**	p=0.302**	p=0.659**
Family type					
Nuclear family	21 (8-28)	19 (8-32)	13 (6-24)	15 (5-20)	13 (6-18)
Extended family	21 (14-27)	20 (9-30)	14 (8-20)	14 (6-18)	13 (8-15)
	p=0.077***	p=0.382****	p=0.044***	p=0.076***	p=0.009***
Socioeconomic level					
Low[a]	20 (13-28)	22 (8-29)	13 (6-19)	13 (5-20)	12 (6-17)
Middle[b]	21 (8-28)	19 (8-32)	13 (6-24)	15 (6-20)	13 (8-18)
High[c]	24 (20-28)	17 (11-28)	12 (8-20)	16 (13-20)	13 (12-15)
	p=0.002* p^{a-b}=0.126 p^{a-c}=0.002 p^{b-c}=0.018	p=0.027* p^{a-b}=0.012 p^{a-c}=0.145 p^{b-c}=0.348	p=0.870*	p=0.000* p^{a-b}=0.003 p^{a-c}=0.000 p^{b-c}=0.019	p=0.008* p^{a-b}=0.008 p^{a-c}=0.155 p^{b-c}=1.000

Table 2. Continued

Sociodemographic variables	Self-confident approach	Helpless approach	Submissive approach	Optimistic approach	Seeking of social support approach
	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)
Working status in a health institution					
Yes	21 (16-26)	17 (8-24)	14 (10-18)	14 (7-15)	14 (9-17)
No	21 (8-28)	19 (8-32)	13 (6-24)	15 (5-20)	13 (6-18)
	p=0.985****	p=0.103****	p=0.348****	p=0.091***	p=0.373****
Worrying about the COVID-19 pandemic					
Yes	21 (8-28)	20 (8-30)	13 (7-24)	14 (5-20)	13 (6-18)
No	22 (13-28)	18 (8-32)	13 (6-24)	15 (5-20)	13 (9-17)
	p=0.000***	p=0.000***	p=0.434***	p=0.000***	p=0.424****

*Kruskal-Wallis H test, **One-way ANOVA, ***Independent t- test, ****Mann-Whitney U test, min: Minimum, max: Maximum, Med: Median, COVID-19: Coronavirus disease-19, a-c: There is no difference between similar groups

during the COVID-19 pandemic process (18). Similarly, it was found that students experienced high levels of stress in the severe acute respiratory syndrome epidemic experienced in the past years (21). In another research conducted with undergraduate students at the Lebanese University, the change in the education and training process in the COVID-19 pandemic caused anxiety, depression and stress in students (22). On the other hand, the stress experienced by students during the pandemic process also affected their academic self-efficacy (23).

When the research findings were examined, it was seen that students with a high socioeconomic level and who were not worried about the pandemic used self-confident approach in coping with stress. This research was conducted between May 28 and June 28 2020 when the number of patients with COVID-19 was very high and in the period in which the whole education system in Turkey was transformed into distance education rapidly. In this process, students studying in the field of health, who had a large number of face-to-face applied undergraduate courses, faced both the risk of disease and the change in the form of education. Therefore, high socioeconomic level facilitates easy access to technological devices and internet in the e-learning education process and the sustainability of education. It was also determined that students who were not worried about the COVID-19 pandemic used self-confident approach as their way of coping with stress. Researches show that people who use negative coping with stress styles focus on the emotions triggered by stress, and those who use positive coping with stress styles create structural support for themselves (24,25). In this context, the fact that students who are not worried about the pandemic adopt self-confident approach style is supported by the literature.

In the research, it was determined that students with a high socioeconomic level used self-confident approach to cope with stress, while students with a low socioeconomic level used the helpless approach. Researches conducted during the pandemic process showed that income during the pandemic process played a key role in accessing educational opportunities for students, and students with low income experienced more stress (26,27). This study determined that income level affected the approaches adopted by students, and showed that students with low income used the helpless approach while students with high income used self-confident approach. Another finding that supported this result was that students with a high socioeconomic level adopted optimistic approach as a way of coping with stress. The high socioeconomic level indicates the ease of access to computers and internet for students in the distance education process. Its is thought that students experience less stress or better manage stress during the pandemic period thanks to ease of access to computers and internet, which are the most important components of distance education. Students with medium socioeconomic level adopted the style of seeking social support more than students with low socioeconomic level. In the research of the Savcı and Aysan (28), it was emphasized that students with middle socioeconomic level used avoidance in coping with stress. Students with low socioeconomic level used ineffective coping methods to cope with stress. These results of our research are consistent with each other.

While the students living in the extended family adopted the submissive approach as a way of coping with stress during the pandemic period, the students living in the nuclear family adopted the style of seeking social support. In the Turkish family structure, especially in extended families, importance is given to the submission of children to their elders. If children conflict with family values, families take all necessary measures to ensure that their children behave submissively (29). For this reason, it is an expected result that our students living in extended families use a submissive approach to cope with stress. In addition, the concept of family is one of the most important sources of social support in reducing or completely eliminating stress. Therefore, it is thought that students living in nuclear families adopt the approach of seeking social support.

On the other hand, it was determined that 2nd grade students and the students not worrying about the pandemic used optimistic approach in their way of coping with stress. In a research conducted with nurse students, older age was associated with better knowledge and clinical experience. For this reason, it was emphasized that nurse students used coping styles with stress more effectively (30). In another research conducted with medical students, it was found that first-year students adopted

the helpless approach to coping with stress more (31). It was thought that our second-year students used optimistic approach to cope with stress because of the fact that they received longer period education during the face-to-face education process compared to the first-year students, they could reach lecturers more easily when necessary and they were more accustomed to the education system.

Table 3. Comparison of students' sociodemographic and e-learning variables with attitude scale towards e-learning

Sociodemographic variables	Med (min-max)	P
Age (year)		
18-21	56 (34-92)	0.865*
22-25	56 (40-75)	
≥26	56 (52-65)	
Program		
First and emergency aid	55 (40-69)	0.142*
Medical laboratory techniques	57 (34-75)	
Physiotherapy	56 (42-66)	
Elderly care	57 (43-75)	
Anesthesia	56 (43-92)	
School year		
1st year	56 (40-75)	0.804**
2nd year	56 (34-92)	
Gender		
Female	56 (34-92)	0.509**
Male	56 (40-75)	
Marital status		
Single	56 (49-73)	0.753**
Married	56 (34-97)	
Place of residence		
Urban	55 (34-75)	0.685*
Rural-urban	56 (40-92)	
Rural	56 (41-75)	
Family type		
Nuclear family	56(34-92)	0.456**
Extended family	56(42-75)	
Socioeconomic level		
Low	57(34-67)	0.787*
Middle	56(40-92)	
High	55(52-74)	
Working status in a health institution		
Yes	54(40-73)	0.375**
No	56(34-92)	
Worrying about the COVID-19 pandemic		
Yes	56 (34-92)	0.050**
No	55 (40-74)	
Internet availability in the place of residence		
Yes	55 (40-92)	0.011**
No	57 (34-75)	

*Kruskal-Wallis H test, **Mann-Whitney U test, min: Minimum, max: Maximum, Med: Median, COVID-19: Coronavirus disease-19

The average score of the students who were worried about the pandemic on the attitude scale towards e-learning was high. The pandemic caused the transition from formal education to distance education all over the world (32), interestingly, it was observed that students quickly adapted to this change during the pandemic period (33). Similar toour results, there are other studies in which students' attitudes towards e-learning in distance education are positive (34,35). There are also researches revealing that, contrary to our research results, students have a negative view on the distance education process. In a research conducted by Coşkun et al. (36) with students studying in the field of health in Turkey before the pandemic, it was emphasized that 86.2% of the students did not want to use the e-learning system. In another research conducted with medical and nursing students in Uganda, it was found that students' awareness of e-learning was high, but their attitudes were negative (37). However, this pandemic process has increased professional awareness, especially among students studying in the field of health. It was observed that desire of students for vocational education increased and students adapted to this period quickly for providing healthcare and helping people as soon as possible.

In current research students who did not have internet access at their place of residence had higher desire for e-learning. In the research conducted by Duraku and Hoxha (38), which supported our research results, it was observed that the stress experienced by the students during the pandemic process positively affected their learning. In addition, it was determined that students' attention distracted from the pandemic process with participation in e-learning. It is thought that this situation may be due to the motivation of the students by the educators, the fact that the pandemic process is closely related to the healthcare professionals and therefore the professional awareness of the students has increased.

As the total score of the scale coping with stress of students during the pandemic period, the mean score of the attitude scale towards e-learning also increased. In the studies conducted during the COVID-19 pandemic process, it was determined that students were afraid of losing their social lives and part-time jobs as well as their academic career dreams and they felt financial and

Table 4. Relationship between the scale coping with stress and attitude scale towards e-learning

	The coping with stress scale	
	r	p
Attitude scale towards e-learning	0.194*	0.000
Satisfaction	0.008	0.867
Motivation	0.068	0.160
Usability	0.109**	0.002
Tendency to use technology	0.138*	0.004

*Correlation is significant at 0.01 level, **Correlation is significant at 0.05 level

economic anxiety and were worried about the risk of infection (39,40). Also another research showed that the experienced academic stress directed students to creative activities and e-learning types, thus they could manage the pandemic process more effectively by getting away from depressive thoughts (41). Due to the threat of human health, during the pandemic process, professional sensitivity and dynamics have increased even more, especially among students who receive health services education. Ways of coping with stress regulate negative emotions, offer alternative solutions to individuals and add positive functions. In the current research, it was determined that the e-learning requests of the students increased, and this situation was thought to be due to the increase of awareness in students because the pandemic process greatly affected the health field.

A positive significant relationship was found between usability and tendency to use technology, which were sub-dimensions of the scale of attitude towards e-learning, and the scale coping with stress. It is evident that in recent years, young people use web-based applications in all areas of life (42). In addition, it has been reported that stress, which is effective in students during the pandemic period, has positive reflections on learning (38). Further, e-learning in health education is among the current issues in recent years (13,43,44). In a research, it was determined that 50.5% of the students were willing to continue the lessons with distance education during the pandemic process (45). In another research conducted with students studying in the field of health, it was found that students wanted to continue formal education in the post-pandemic period. In the same research, it was stated that applied undergraduate courses were inefficient with e-learning (46). Although different studies conducted with students before and during the pandemic process showed that there was variability in attitudes towards e-learning, this research showed that the coping with stress scale during the pandemic process increased the use of technology in students and e-learning was a useful method in this process (40,45,46).

Study Limitations

The most important limitation of the research was the fact that the research was conducted with only the students studying at the health services vocational school. For this reason, the results of the research could not be generalized to students studying in different departments. Another limitation of the research was that the research was conducted electronically due to the pandemic and that students with limitations on mobile phones, computers and internet could not be reached.

Conclusion

Since the day it was defined, the COVID-19 pandemic became a major public health problem for the whole world. Millions of people are still trying to keep themselves safe by applying full or partial quarantine. For this reason, thousands of educational institutions have replaced from formal education to distance education, which is an alternative solution, in order to continue education during the pandemic period. In the current research, it was found that students experienced worry during the pandemic process and their way of coping with stress positively affected their attitudes towards e-learning. One of the aims of coping with stress is to increase the success of students in education. This research is the basis for showing the positive effects of effective stress coping ways on education.

Note: On March 16, 2020 in the Republic of Turkey education was suspended temporarily due to the COVID-19 pandemic and it began again with distance education on March 23, 2020. Data were collected till the first wave of the lockdown, that was, 28 June 2020.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Non-Invasive Clinical Research Ethics Committee of the institution where the research was conducted with the decision numbered 40465587-102.01-105.

Informed Consent: In the introduction part of this form, a voluntary consent was received from the students.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: Y.A., H.P., B.Ç., Design: Y.A., H.P., B.Ç., Data Collection or Processing: Y.A., H.P., B.Ç., Analysis or Interpretation: Y.A., H.P., B.Ç., Literature Search: Y.A., H.P., B.Ç., Writing: Y.A., H.P., B.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5853
Bezmialem Science 2022;10(2):194-8

Effect of Trichloroacetic Acid on the Bond Strength of Calcium Silicate-Based Cements: A Modified Push-Out Test

Trikloroasetik Asidin Kalsiyum Silikat Esaslı Simanların Bağlanma Dayanımı Üzerindeki Etkisi: Modifiye İtme Testi

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ABSTRACT

Objective: This study aimed to investigate the effect of trichloroacetic acid (TCA) on the bond strength of calcium silicate-based cements to dentin.

Methods: Ten single-rooted bovine teeth were sectioned longitudinally into slices 2 mm thick. Six holes were drilled with a 1.2 mm diamond bur in each dentin slice (totally 60 holes). Cotton pellets with TCA were applied to three holes of each slice for 1 min, whereas no acid was applied to the other three. The TCA and non-TCA groups were divided into three subgroups according to the material used: ProRoot mineral trioxide aggregate [(MTA); n=10], Harvard MTA (n=10), and Biodentine (n=10). After seven days, the dislodgement resistance of the materials was calculated using a universal testing machine. The types of bond failure were examined under a stereomicroscope.

Results: The TCA had no statistically significant effect on the bonding strength of the tested materials (p>0.05). The Harvard MTA subgroup had the lowest mean bond strength values (2.25±0.79 MPa), while the Biodentine subgroup had the highest (10.49±3.32 MPa). The most common bond failure types were mixed in the ProRoot MTA subgroup (60%) and cohesive in the Harvard MTA (60%) and Biodentine (70%) subgroups.

Conclusion: The bond strength of Biodentine is greater than those of ProRoot and Harvard MTA. TCA does not affect the push-out bond strength of MTA or Biodentine.

Keywords: Biodentine, calcium silicate-based cements, mineral trioxide aggregate, push-out bond strength, trichloroacetic acid

ÖZ

Amaç: Bu çalışma, trikloroasetik asidin (TCA) kalsiyum silikat esaslı simanların dentine bağlanma dayanımına etkisini araştırmak amacı ile yapılmıştır.

Yöntemler: Toplam 10 adet tek köklü sığır dişi, 2 mm kalınlığında dilimler halinde uzunlamasına kesitlere ayrıldı. Bu 10 dişten elde edilen her dentin diliminde 1,2 mm'lik elmas frez ile 6 adet delik açıldı (toplam 60 delik). TCA içeren pamuk peletler, her dentin dilimde bulunan 3 deliğe 1 dakika süreyle uygulanırken, diğer 3 deliğe asit uygulanmadı. TCA uygulanan ve TCA uygulanmayan gruplar, kullanılan malzemeye göre tekrar üç alt gruba ayrıldı: ProRoot mineral trioksit agregat [(MTA); n=10], Harvard MTA (n=10) ve Biodentine (n=10). Toplam 7 gün sonra, malzemelerin yerinden çıkma direnci evrensel bir test makinesi kullanılarak hesaplandı. Bağlanma başarısızlık türleri bir stereomikroskop altında incelendi.

Bulgular: TCA, test edilen malzemelerin bağlanma dayanımı üzerinde istatistiksel olarak önemli bir etkiye sahip değildi (p>,05). Harvard MTA alt grubu en düşük ortalama bağlanma gücüne sahipken (2,25±0,79 MPa), Biodentine alt grubu en yüksek değere (10,49±3,32 MPa) sahipti. En yaygın bağlanma hatası tipleri, ProRoot MTA alt grubunda (%60) karışık, Harvard MTA (%60) ve Biodentine (%70) alt gruplarında ise koheziv bağlanma türünde bulundu.

Sonuç: Biodentine'in bağlanma gücü ProRoot ve Harvard MTA'nınkinden daha fazla bulunmuştur. TCA, MTA veya Biodentine'in itme bağlanma dayanımını etkilememektedir.

Anahtar Sözcükler: Biodentin, kalsiyum silikat esaslı siman, mineral trioksit agregat, itme bağlanma gücü, trikloroasetik asit

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Received: 01.01.2021
Accepted: 14.02.2021

Cite this article as: Uysal BA, Sarıalioğlu Güngör A. Effect of Trichloroacetic Acid on the Bond Strength of Calcium Silicate-Based Cements: A Modified Push-Out Test. Bezmialem Science 2022;10(2):194-8

Introduction

Loss of dental hard tissue and root resorption may occur as a result of osteoclastic action (1). Root resorption may be associated with many causes, such as cystic lesions, trauma, impacted teeth, and endocrine disturbances (2,3). External cervical resorption (ECR) is a type of resorption that affects the periodontal ligament, cementum, and dentin most commonly localized at or below the cervical margin (4). When resorption is correctly diagnosed and treated with a biocompatible material with adequate sealing ability, the prognosis can be favorable (5).

Mineral trioxide aggregate (MTA) has many desirable properties, including bactericidal effects, biocompatibility, radiopacity, and good sealing ability (6). However, it also has certain drawbacks, such as a high price, a long setting time, and potential tooth discoloration (7). To overcome these drawbacks, new calcium silicate-based materials have been produced. Biodentine (Septodont, Saint-Maur-des-Fossés, France), a silicate-based cement, exhibits several favorable properties, such as a short setting time, high mechanical strength, and superior handling characteristics (8). It is an effective repair material due to its excellent sealing ability, short setting time, high compressive strength, and biomineralization properties (8-10).

The use of MTA or Biodentine as repair materials is recommended after the elimination of granulomatous tissue in the perforation area (11). In external cervical resorption, topical application of 90% aqueous trichloroacetic acid (TCA) solution to granulomatous tissue is recommended (12). TCA induces coagulation necrosis, and tissue in the resorption cavity loses its vascularization features. TCA inactivates potentially resorptive cells and penetrates into smaller resorptive areas that may not be cleaned with mechanical instrumentation (13,14).

Several studies have investigated the push-out bond strength of calcium silicate-based cements, such as MTA and Biodentine, to dentin (15-21). However, to our knowledge, no study has examined whether TCA used in ECR has an effect on the push-out bond strength of these materials. Therefore, this study aimed to investigate the effect of TCA on the bond strength of ProRoot MTA (Dentsply Sirona), Harvard MTA (Harvard Dental International, Hoppegarten, Germany), and Biodentine. The null hypotheses were that TCA would not significantly affect push-out bond strength of calcium silicate-based cements and there would be no significant difference in bond strength between the tested materials.

Methods

Sample Preparation

A total of 10 freshly extracted bovine teeth with single root canals were used to get 60 holes in this study. Animals were slaughtered for feeding purposes, and the teeth were donated to this study. This investigation was not classified as an animal study because our work had no influence on the premortal fate of the animals or the slaughtering process. After the teeth were decoronated, the apical and middle thirds of the roots were removed. Then, sections at a distance of 8 mm from the coronal thirds were used. The root pieces were embedded in acrylic blocks parallel to the root canal. The root dentin was sectioned longitudinally into slices 2 mm thick using a cutting machine (Mecatome T180; Presi, Eybens, France) with a disc (Metkon Instruments Inc., Bursa, Turkey) under water cooling. Similar to the study of Orhan et al. (18), six holes were drilled in each dentin slice, three on the right and three on the left side of the root canal to standardize all samples. A size 1.2 mm diameter diamond bur and a high-speed dental handpiece were used (Figure 1). The holes were drilled midway between the external cementum and the root canal wall. The samples were divided into two groups: TCA (n=30) and non-TCA applied (n=30). Small amounts of 90% aqueous TCA were absorbed into small cotton pellets and then dampened on gauze. The cotton pellets were applied to three holes of each dentin slice for 1 min (13), whereas no acid was applied to the other three. The TCA and non-TCA groups were divided into three subgroups according to the material used: ProRoot (MTA; n=10), Harvard MTA (n=10), and Biodentine (n=10).

For standardization purposes, ProRoot MTA, Harvard MTA, and Biodentine were incrementally placed in two holes each, one with and one without TCA, in each slice. All materials were prepared according to the manufacturers' instructions. The same procedure was repeated for all 10 samples. Residual material was removed from the dentin surfaces with a scalpel. The dentin slices were subsequently placed in wet gauze and then placed in an incubator at 37 °C with 100% humidity for seven days.

Push-out Test

A universal testing machine (Instron Universal; Elista, Istanbul, Turkey) was used to calculate the push-out bond strength of the materials. The samples were placed on a metal plate with a gap in the middle. A 1 mm plunger tip was positioned on the materials. Pressure was applied at the center of the material in each hole with the Instron probe moving at a speed of 0.5 mm/minimum. The greatest force applied to the materials at the time of dislodgement was recorded in newtons. The push-out bond strength was calculated in megapascals according to the formula

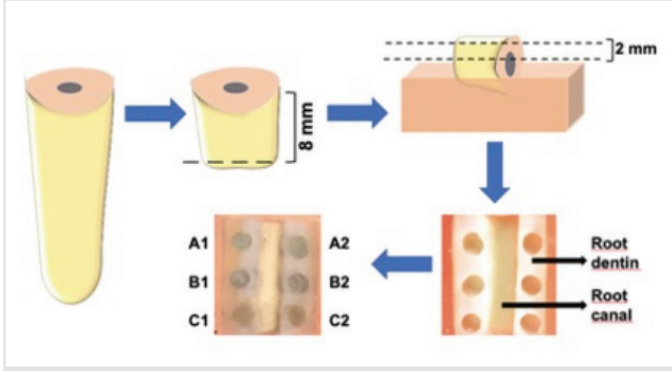

Figure 1. Preparation of the samples for the push-out test. (A1) ProRoot MTA, (A2) ProRoot MTA + TCA, (B1) Harvard MTA, (B2) Harvard MTA + TCA, (C1) Biodentine. (C2) Biodentine + TCA

After the measurements, the types of failures were examined under a stereomicroscope at 8X magnification (Olympus Corporation, Taichung, Taiwan). Bond failures were classified as adhesive, cohesive, or mixed.

Statistical Analysis

The normality of data distribution was examined using the Shapiro-Wilk test. As the data were normally distributed, parametric tests were used. The independent t-test was used for comparisons between the groups. The statistical analysis was performed using IBM SPSS Statistics version 24 (IBM, Armonk, NY, USA) for Windows. The level of statistical significance was set to 5%.

Results

Table 1 shows the mean push-out bond strength values obtained from the displacement of the materials in the TCA and non-TCA groups and the ProRoot MTA, Harvard MTA, and Biodentine subgroups. TCA had no statistically significant effect on the bonding strength of the tested materials (P>0.05). The Harvard MTA subgroup had the lowest mean bond strength values (2.25±0.79 MPa), whereas the Biodentine subgroup had the highest (10.49±3.32 MPa). Figure 2 presents the results in a graph.

The most common bond failure types were mixed in the ProRoot MTA subgroup (60%) and cohesive in the Harvard MTA (60%) and Biodentine (70%) subgroups (Table 2). Stereomicroscopic images of samples are shown in Figure 3.

Discussion

The TCA is manufactured by the chlorination of acetic acid, and its aqueous solution is highly acidic, yielding a pH of 1.0.

Table 1. Mean and standard deviation of the push-out bond strength values (MPa) for the displacement of tested materials from the samples

	Mean ± SD	P
ProRoot MTA	4.08±2.75[b]	0.985
ProRoot MTA + TCA	4.06±2.28[b]	
Harvard MTA	2.25±0.79[a]	0.863
Harvard MTA + TCA	2.32±0.82[a]	
Biodentine	10.49±3.32[c]	0.230
Biodentine + TCA	8.79±2.78[c]	
P.	0.042	

SD: Standart deviation, TCA: Trichloroacetic acid, p<.05

Table 2. Percentage of failure types in each tested group

	Adhesive/cohesive/mixed (%)
ProRoot MTA	10/30/60
Harvard MTA	10/60/30
Biodentine	0/70/30

When applying TCA to a ECR area to exert its caustic effect, it may make contact with dental hard tissues (22). Khoroushi and Tavasoli (23) reported that the contact of TCA with enamel had a positive effect on the shear bond strength of resin composite to enamel. Although the effects of TCA on resin composite have been investigated, to the best knowledge, no study has examined whether TCA has an effect on the push-out bond strength of MTA and Biodentine. TCA did reduce the push-out bond strength, especially of Biodentine. However, this decrease did not significantly affect the push-out bond strength of the tested materials. Therefore, our first null hypothesis was accepted. This result is not in line with previous studies reporting that acidic environments significantly affect the physical and chemical properties of MTA and Biodentine (21). This inconsistency might be due to the fact that in this study, TCA was applied for 1 min before the materials were placed in the holes, whereas previous studies exposed the tested materials to large amounts of acetic acid (24,25), EDTA (17,21), or citric acid (17) for more than 1 min, thus significantly affecting their push-out bond strength.

In our study, ProRoot MTA showed predominantly mixed failure, whereas Harvard MTA and Biodentine displayed mostly cohesive failure. A previous study found higher Ca^{+2} ion release with Harvard MTA than with ProRoot MTA after seven days and lower solubility of the former than the latter (26). Ion release results in a porous surface structure, which weakens the material's structure and reduces its cohesion (27). It is thus possible that Harvard MTA may be broken up by itself, resulting in cohesive rather than adhesive bond failure. The high calcium ion release observed in Biodentine is associated with a higher proportion of tricalcium silicate than in MTA (28) and may result in the presence of cohesive failure in Biodentine, as in Harvard MTA. Additionally, previous studies reported that MTA-dentin bond failures were usually adhesive (10,16), but in our study, it might be possible to observe mixed or cohesive bond failures depending on the use of TCA.

In a previous study, roots filled with Biodentine displayed significantly greater push-out bond strength than Harvard and ProRoot MTA, while there was no statistically significant difference between the latter two (20). In our study, Biodentine also exhibited the greatest push-out bond strength, but there was also a statistically significant difference between ProRoot MTA and Harvard MTA, with the latter exhibiting significantly less push-out bond strength. According to these results, our second null hypothesis was rejected.

Harvard MTA is composed of 75 wt% calcium magnesium orthosilicate (merwinite), while ProRoot MTA contains 90% tricalcium silicon pentaoxide (hatrurite) (26). Merwinite exhibits low solubility and good mechanical properties (29). On the other hand, the fact that Harvard MTA contains a high proportion of merwinite, unlike ProRoot MTA, may be responsible for the lower push-out bond strength of Harvard MTA. A previous study found that Biodentine and ProRoot MTA had comparable bond strength in apical dentin, but Biodentine exhibited significantly greater bond strength in coronal dentin (15).

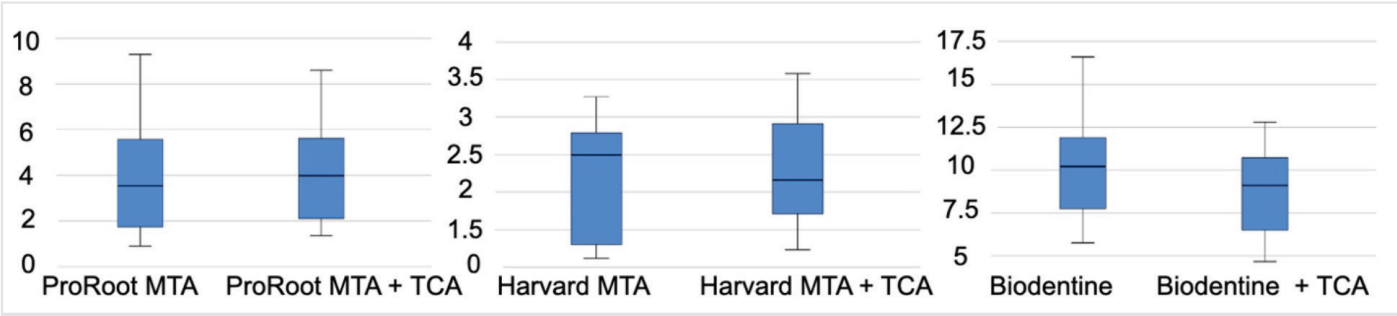

Figure 2. Box plot of the push-out bond strength (MPa) of the tested materials

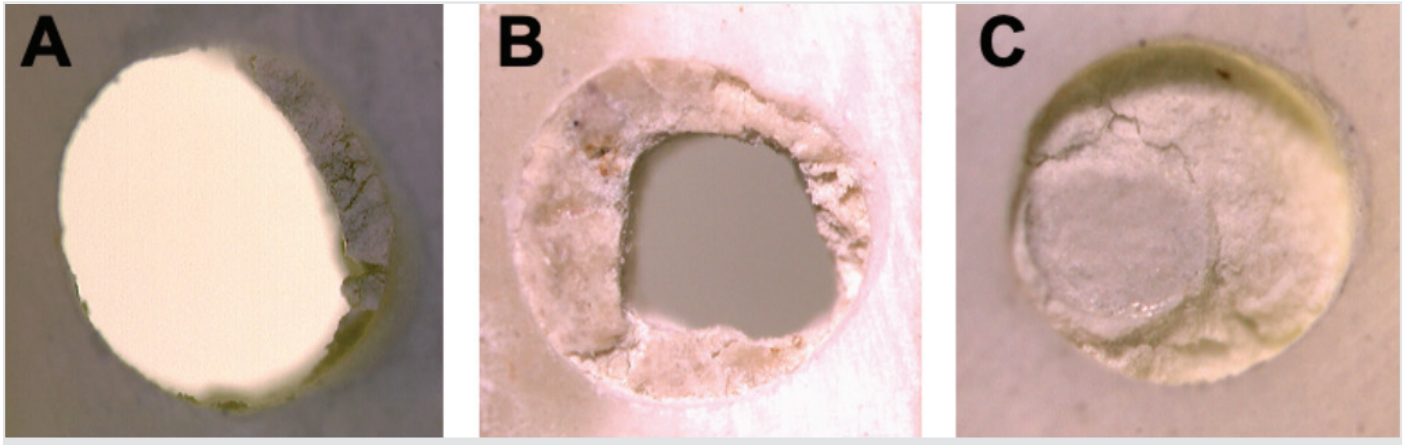

Figure 3. Representative failure types, (A) ProRoot MTA mixed failure, (B) Harvard MTA cohesive failure, (C) Biodentine cohesive failure

In our study, it may be possible to find this result depending on the use of the coronal part of the root. Biodentine is made of smaller particles than other cements, which allows deeper penetration into dentine tubules (30). This ability, combined with its short setting time and the presence of calcium chloride, might explain Biodentine's relatively greater push-out bond strength.

Study Limitations

The limitation of this study was that TCA-induced changes in the dentin walls were not scanned with a microscope such as the scanning electron microscope, which would provide more detailed examination. In further studies, it will be more appropriate to examine TCA-induced changes in the dentin walls in more detail.

Conclusion

The results of this study indicate that the bond strength of Biodentine is greater than that of ProRoot and Harvard MTA and that Harvard MTA exhibits the least bond strength. TCA does not significantly affect the push-out bond strength of MTA or Biodentine.

Ethics

Ethics Committee Approval: No human tissue was used.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: B.A.U., A.S.G., Design: B.A.U., Data Collection or Processing: B.A.U., Analysis or Interpretation: B.A.U., A.S.G., Literature Search: B.A.U., A.S.G., Writing: B.A.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5776
Bezmialem Science 2022;10(2):199-205

Comparisons of the Radiolucent Lines Between Cemented and Cementless Oxford Unicondylar Knee Arthroplasty: A Non-designer Group Report

Çimentolu ve Çimentosuz Oxford Unikondiler Diz Artroplastilerinde Radyolusent Hatların Karşılaştırılması: Tasarımcı Olmayan Bir Grup Raporu

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ABSTRACT

Objective: Aseptic loosening is one of the most important reasons for failure in unicondylar knee arthroplasty (UKA). The aim of this study was to compare early physiological and pathological radiolucent lines (RLL) between cementless and cemented UKA within a non-designer group cohort.

Methods: Two groups of patients who underwent 38 cemented UKA and 47 cementless UKA between 2012 and 2018 were compared retrospectively. In evaluating the clinical results of the patients, the Oxford Knee Score, EQ-5D-3L, EQ-VAS, and KOOS scoring were used. In the evaluation of the presence of RLLs, the tibial and femoral component interfaces were divided into regions and evaluated for RLLs.

Results: There was no statistically significant difference between the groups in terms of clinical results (p>0.05). No complete RLLs were observed in either the femoral or tibial component interfaces in any patient. Partial radiolucent regions at the tibial component interface were detected in 32 (11.3%) regions in cemented UKAs and 13 (5.7%) in cementless UKAs. The incidence of partial RLLs in the tibial component interface and the total number of radiolucent zones were higher in the cemented arthroplasties (p=0.040 and p=0.025).

Conclusion: It was determined that the excessive physiological RLLs observed in UKA had no effect on the clinical outcomes of

ÖZ

Amaç: Unikondiler diz artroplastisinde (UDA) aseptik gevşeme başarısızlığın en önemli nedenlerinden biridir. Bu çalışmanın amacı, tasarımcı olmayan bir grup kohortunda çimentosuz ve çimentolu UDA'lar arasındaki erken fizyolojik ve patolojik radyolusent hatları (RLH) karşılaştırmaktı.

Yöntemler: Bu çalışmada 2012-2018 yılları arasında 38 çimentolu UDA ve 47 çimentosuz UDA uygulanan iki hasta grubu retrospektif olarak karşılaştırıldı. Hastaların klinik sonuçlarının değerlendirilmesinde Oxford Diz Skoru, EQ-5D-3L, EQ-VAS ve KOOS skorlamaları kullanıldı. RLH'lerin varlığının değerlendirilmesinde, tibial ve femoral bileşen ara yüzleri bölgelere bölündü ve RLH'ler için değerlendirildi.

Bulgular: Klinik sonuçlar açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu (p>0,05). Hiçbir hastada femoral veya tibial bileşen ara yüzlerinde tam RLH gözlenmedi. Tibial bileşen ara yüzünde parsiyel radyolusent bölgeler çimentolu UDA'larda 32 (%11,3) ve çimentosuz UDA'larda 13 (%5,7) bölgede tespit edildi. Tibial bileşen arayüzünde kısmi RLH görülme sıklığı ve toplam radyolusent bölge sayısı çimentolu artroplastilerde daha yüksekti (p=0,040 ve p=0,025).

Sonuç: Unikondiler diz artroplastisindegözlenen aşırı fizyolojik RLH'lerin hastaların klinik sonuçları üzerinde etkisi olmadığı

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Cite this article as: Aslan H, Atilla HA, Büyükdoğan K, Akdoğan M, Çevik HB. Comparisons of the Radiolucent Lines Between Cemented and Cementless Oxford Unicondylar Knee Arthroplasty: A Non-designer Group Report. Bezmialem Science 2022;10(2):199-205

Received: 21.12.2020
Accepted: 16.02.2021

the patients. The rate of physiological RLLs was significantly lower in cementless UKA than in cemented UKA.

Keywords: Unicondylar knee arthroplasty, cementless, cemented, radiolucent lines, aseptic loosening

tespit edildi. Fizyolojik RLH oranı çimentosuz UDA'da çimentolu UDA'ya göre önemli ölçüde daha düşüktü.

Anahtar Sözcükler: Unikondiler diz artroplastisi, çimentosuz, çimentolu, radyolusent hatlar, aseptik gevşeme

Introduction

Unicompartmental knee arthroplasty (UKA), developed by Marmor (1) in the 1970s, is a successful method in the treatment of medial or lateral end-stage knee arthritis. Although unsuccessful results and high revision rates in the very first applications led to suspicion of UKA (2,3), it was again popularized following the improvements in prosthetic design and surgical techniques within the last two decades (4). However, aseptic loosening is still one of the most important reasons for failure in the modern designs (5). Radiolucent lines (RLLs) observed around the prosthesis are a strong indicator of loosening (6). However, Goodfellow et al. (7) reported that RLLs did not always indicate implant loosening and they could be classified in two groups as pathological and physiological in UKA. Pathological RLLs are generally thicker than 2 mm, increase in thickness over time, and indicate infection or loosening. Physiological RLLs are non-progressive, thinner than 2 mm, and do not indicate loosening. The differential diagnosis of pathological and physiological RLLs is difficult especially in the presence of pain. Tibial pain is usually observed in the early rehabilitation phase of patients who have undergone UKA. This condition, which is associated with increased stress in the proximal tibia, usually resolves spontaneously within the first year (8,9). This condition can be easily assessed as an aseptic loosening of the prosthesis if physiological RLLs are observed. Cementless UKA has a constructional advantage in this respect as physiological RLLs in such prostheses are considerably less than in cemented ones. A few studies have shown that cementless UKA reduces RLL incidence and revision rates (10,11). Nevertheless, the results reported by non-designer groups are quite limited (12,13).

The hypothesis of this study was that there would be no difference in clinical outcomes due to RLLs in cemented and uncemented UKA. The aim of this study was to compare early physiological and pathological RLLs in cementless UKA with cemented versions within a non-designer group cohort. With the comparison made, RLL in the groups cemented and cementless, and its effect on the clinical results of the patients were investigated.

Methods

The study was approved by the Local Ethics Committee and all procedures were applied in compliance with the Declaration of Helsinki. Written informed consent was obtained from all the study participants.

A retrospective evaluation of 37 consecutive patients (38 knees) who underwent cemented Oxford UKA for isolated anteromedial osteoarthritis between 1 November 2012 and 31 May 2018 and who had completed at least 2 years of clinical follow-up, and a cohort with cementless UKA of similar age, gender, and body mass index (41 patients, 47 knees) was made (Table 1). All the operations were performed by the senior surgeon (HA). For statistical evaluation, operation periods, clinical results, [Oxford Knee Score, Knee Injury and Osteoarthritis Outcome score (KOOS), EQ-5D-3L scores and range of motion] incidence of RLLs in tibial and femoral component interfaces and reoperation rates for any reason were evaluated and compared between the groups.

The inclusion criteria of the study were:

1. Patients were operated on according to the selection criteria of the Oxford group with a cemented or cementless Oxford UKA design (4).
2. Patients completed at least two years of follow-up of adequate radiographic and physical evaluation.

Two patients with progressive osteoarthritis in the lateral compartment in the cemented group, and one patient with an intraoperative tibial plateau fracture in the non-cemented group

Table 1. Demographic data of the patients

	Cementless (n=37)	Cemented (n=41)	p value
Age (year)	57.4 (47-74)	59.2 (47-72)	.277 (t-test)
Sex (F/M)	28:9	37:4	.156 (x^2)
Side (R/L) *	22:16	23:24	.157 (x^2)
Length (m)	1.62 (1.52-1.70)	1.63 (1.53-1.86)	.489 (t-test)
Weight (kg)	80.6 (55-112)	81.1(64-108)	.854 (t-test)
BMI (kg/m^2)	29.9 (23.3-34.5)	30.3 (23.1-40.6)	.719 (t-test)
Follow-up period (month)	25.9 (25-38)	38.1 (28-65)	<0.001(t-test)

Statistically significant values are in italic and bold
*Both knees were evaluated in patients who had undergone bilateral unicondylar arthroplasty.
F: Female, M: Male, R: Right, L: Left, BMI: Body mass index

were excluded from the study, as they were patients who missed regular follow-up appointments or whose follow-up radiographs were not of sufficient quality. Patients' age and weight, and the degenerative status of the patellofemoral joint were not accepted as contraindications.

Surgical Technique

Oxford phase 3 cemented and cementless UKA were used in all patients. Of the 38 knees in the cemented UKA group, 24 were operated under epidural anesthesia and 14 under general anesthesia, and of the 47 knees in the cementless UKA group, 32 were operated under epidural anesthesia and 15 under general anesthesia. All surgical procedures were performed under tourniquet control. The anteromedial minimally invasive technique was used as the surgical incision in all patients. Following the cleaning of osteophytes in the medial of the femoral condyle and intercondylar notch, tibia and femur cuts were made. The prostheses were placed after stability and mobility control with gap measurement and trial components, respectively. In deciding whether the prosthesis was to be applied with or without cement, the bone hardness test was applied as described by Stempin et al. (14). If there was a stability problem during the placement of the trial components following the tibial and femoral cuts, (ie, movement detected in the trial components during flexion-extension), then cemented application was preferred for such patients.

Full weight-bearing was allowed after the removal of drains (24 h after surgery). All patients wore compression stockings as thromboembolic prophylaxis.

Clinical and Radiological Outcome Assessments

In evaluating the clinical results of the patients, the Oxford Knee score (OKS), EQ-5D-3L, EQ-VAS, and KOOS scoring were used.

The OKS is a short patient-reported outcome measure to evaluate physical function and pain developed by Dawson et al. (15), and validated by Tuğay et al. (16) for Turkish patients. KOOS is a disease-specific questionnaire that can be used to evaluate physical function in patients with knee problems (17). EQ-5D-3L score is descriptive system for health-related quality of life states in adults, consisting of the following five domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression, and each domain has three severity levels (18).

To standardize the preoperative radiographs, the X-ray beam was directed from the posterior with the knees flexed at 20º-30º, as defined by Lyon-Schuss (19). Radiographs were repeated on the postoperative first day, at the end of the first month and of the following three months. In addition, axial radiography was taken in all patients preoperatively and in the postoperative 6th week. Annual follow-up examinations were made in the following period. To ensure minimal rotational variations, attention was paid to forward facing of the patella and that the tibial spines were located in the center relative to the intercondylar notch on the radiographs.

In the evaluation of the presence of RLLs, the tibial component interfaces were divided into 6 regions according to the method described by Gulati et al. (20) and these were evaluated for RLLs (Figure 1). Cemented femoral component interfaces were divided into 6 zones according to the technique described by Kalra et al. (21) (Figure 2). In the cementless arthroplasties, the same method was modified and the femoral component interface was divided into 6 regions and evaluated for RLLs.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS for Windows v. 12.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were tested using the Independent Samples t-test and Mann-Whitney U test. Pearson's χ2-test was used in the comparisons of categorical variables. The prevalence of radiolucency in the cemented and cementless radiographs was also examined using a χ2-test. A value of $p<0.05$ was accepted as statistically significant. To assess the interobserver reliability of categorical data, kappa coefficients were calculated using 95% confidence intervals.

Results

Evaluation was made of 37 patients treated with 38 non-cemented Oxford UKA and 41 patients with 47 cemented Oxford UKA. The mean follow-up period was 25.9 months (25-38) in the cementless group and 38.1 (28-65) months in the cemented group. The mean operation time was 39.2 minutes (36.92-41.48) in the cementless group and 51.5 minutes (47.31-53.29) in the cemented group, with a difference of 12 minutes ($p<0.001$). The mean age of the patients was 57.4 (47-74) years in the cementless group and 59.2 (47-72) years in the cemented group. The body mass index was 29.9 (23.3-34.5) in the cementless group and 30.3 (23.1-40.6) in the cemented group (Table 1).

In evaluating the clinical results of the patients; OKS, EQ-5D-3L, EQ-VAS, and KOOS scoring were used.

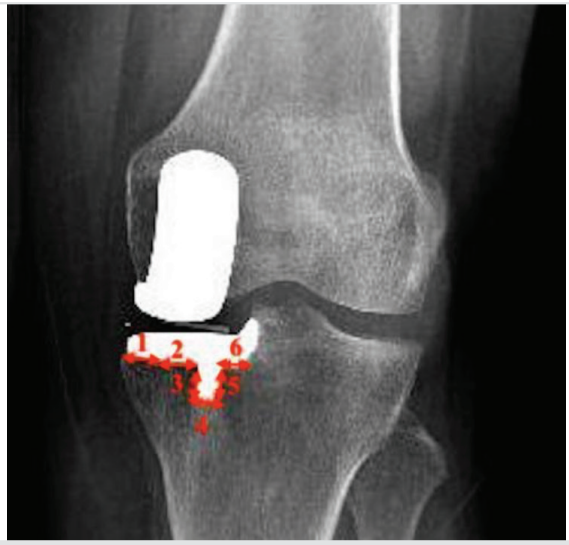

Figure 1. Tibial component interfaces evaluated according to the method described by Pandit et al. (22).

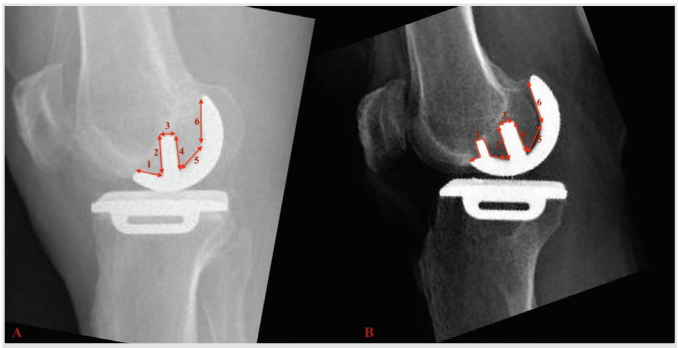

Figure 2. Femoral component interfaces evaluated according to the method described by Kalra et al. (21) (A). In cementless arthroplasties, the same method was adapted and used again (B)

There was no statistically significant difference between the groups in terms of clinical results (Table 2).

During the follow-up period, mobile bearing dislocation occurred in 2 patients in the cementless group in the 6th and 8th months, respectively. In the cemented group, two cases of mobile bearing dislocation occurred in the 5th and 22nd months, respectively. In all these cases, the bearings were exchanged for 1mm larger ones. In the cemented group, 2 patients underwent revised total knee arthroplasty in the 31st and 33rd months because of progressive lateral osteoarthritis. Total knee prosthesis was applied to a patient with unexplained medial tibial pain in the 28th postoperative month. No significant difference was determined between the two groups in respect of re-operation rates (p=0.461). In the survival analysis where reoperation for any reason was accepted as the last point, the cementless group had a survival rate of 94.7% at 25 months and the cemented group had a survival rate of 87% at 38 months and there was no significant difference between the groups (Figure 3).

No complete RLLs were observed in either the femoral or tibial component interfaces in any patient. The total number of partial radiolucent regions in cemented UKA was 32 (11.3%) and the most partial RLLs were found in 1.5 and 2 regions, respectively. In the cementless UKA group, the total number of partial

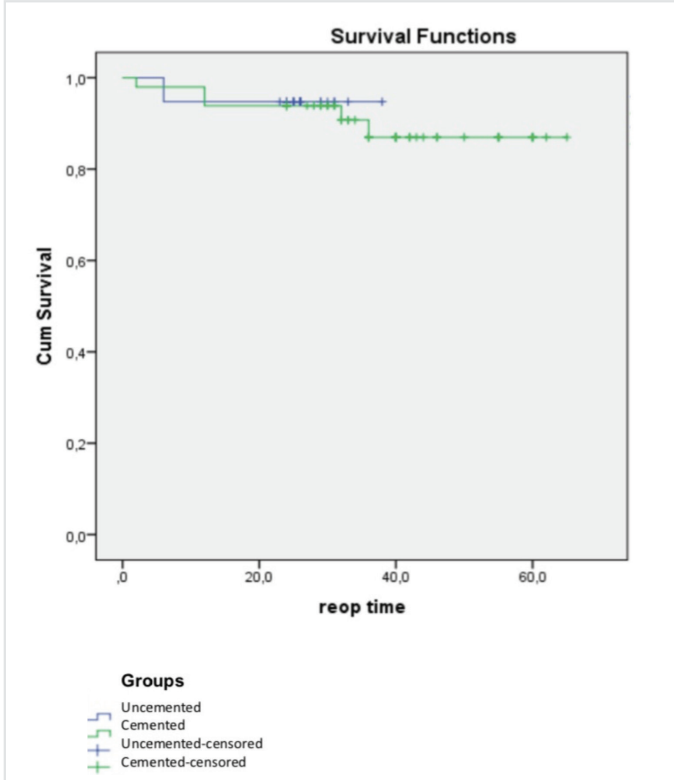

Figure 3. Kaplan-Meier curve of re-operation for any reason was determined as the last point

radiolucent regions in the tibial component interface was 13 (5.7%) and the maximum partial RLLs were observed inzones 2, 3 and 5. The incidence of partial RLLs in the tibial component interface and the total number of radiolucent zones were higher in the cemented arthroplasties (p=0.040 and p=0.025).

Considering the femoral radiolucent areas, none of the cemented and cementless UKA had complete radiolucent areas. Partial radiolucent areas were observed in 1 (2.6%) cementless UKA and in 2 (4.3%) cemented UKA cases. There was no significant difference between the groups in terms of the incidence of partial RLLs in the femoral components (Table 3).

Table 2. Clinical outcomes of the patients

	Cementless (n=37)	Cemented (n=41)	p value (MWU)
Oxford Knee score	41.1 (12-48)	40.6 (20-48)	.236
EQ-5D-3L	0.80 (0.59-1)	0.79 (0.49-1)	.625
EQ-VAS	82.8 (55-100)	82.9 (60-100)	.869
KOOS - pain	81.3 (1.67-100)	83.7 (33.3-100)	.289
KOOS - symtom	85.3 (42.8-100)	84.5 (42.8-100)	.512
KOOS - daily living	84.3 (14.7-100)	86.1 (30.8-100)	.785
KOOS - sport	67.0 (20-100)	68.7 (25.0-100)	.922
KOOS - quality of life	82.0 (25-100)	79.4 (25-100)	.245
Range of motion	112.7 (95-120)	111.4 (80-120)	.411
flexion (°)extension (°)	0.5 (0-10)	0.3 (0-10)	.793

KOOS: Knee Injury and Osteoarthritis Outcome score, EQ-5D-3L: Generic Quality of Life scale, EQ-VAS: Generic Quality of Life scale, MWU: Mann-Whitney U test

Table 3. Incidence of radiolucent lines

Tibial component	Cementless (n=38)	Cemented (n=47)	p value (x^2)
Total radiolucency	0 (0%)	0 (0%)	1.00
Partial RL (absent) Partial RL (present)	32 (84.2%) 6 (15.8%)	29 (61.7%) 18 (38.3%)	.040
Zone 1	1 (2.6%)	9 (19.1%)	.021
Zone 2	4 (10.5%)	5 (10.6%)	1.00
Zone 3	3 (7.9%)	2 (4.3%)	.652
Zone 4	2 (5.3%)	4 (8.5%)	.687
Zone 5	3 (7.9%)	7(14.9%)	.501
Zone 6	0 (0%)	2(4.3%)	.500
Sum of the zones	13 (5.7%)	32 (11.3%)	.025
Femoral component			
Total radiolucency	0 (0%)	0 (0%)	1.00
Partial RL (absent) Partial RL (present)	37 (97.4%) 1 (2.6%)	45 (95.7%) 2 (4.3%)	1.00
Zone 1	0 (0%)	0 (0%)	1.00
Zone 2	0 (0%)	0 (0%)	1.00
Zone 3	0 (0%)	0 (0%)	1.00
Zone 4	0 (0%)	1 (2.1%)	1.00
Zone 5	1 (2.6%)	0 (0%)	.447
Zone 6	0 (0%)	1 (2.1%)	1.00
Sum of the zones	1 (0.4%)	2 (0.7%)	.691

Statistically meaningful values are in italic and bold.
RL: Radiolucent lines

Discussion

The most important finding of this study was that physiological RLLs were statistically less common in the tibial component in cementless UKA compared to cemented UKA. In the literature, there is consensus that the incidence of femoral RLLs is very low in the cemented and cementless UKAs (22,23). In terms of tibial RLLs, there are different opinions in designer and non-designer user groups, and few publications include the results of non-designer user groups. Therefore, this report is of importance as it presents the results of a non-designer user group.

In the current study, the partial tibial radiolucency rate of 15.8% in cementless UKA was slightly higher than reported in the literature. In a designer user group, 5-year, prospective, randomized study, Pandit et al. (24) found no partial RLLs in the cementless femoral component and found a partial RLL rate of 11% in the cementless tibial component. In another study, which included a designer user group and a non-designer group, the survival of 1,000 cementless Oxford UKA was found to be 97.2% at 6 years of follow-up. No complete RLLs were observed at all, and the partial RLLs ratio on the bone-implant surface was found to be 8.9% (24). From the non-designer user group studies, Kerens et al. (13) compared cemented and cementless Oxford UKA and reported 7% partial and 0% complete RLLs in cemented UKA, and 21% partial and 0% complete RLLs in cementless UKA during a mean follow-up period of 34 months with no statistically significant difference between them. In the study of another independent center, no progressive RLLs were observed in cementless UKA, and the revision rate was found to be 0.23/100 at 5 years follow-up. Femoral RLLs were not observed at all, whereas in the tibial components of cementless UKA, RLLs were significantly fewer than in cemented UKA (22). In contrast, Bruni et al. (25) reported cementless implant survival as 74.3% at 6 years of follow-up and did not recommend the widespread use of cementless UKA because of the relationship between the high revision rates and the low bone quality. In this present study, it was seen that tibial radiolucency was more than the designer group and less than the other non-designer groups. We think that the success of UKA, which has a long and difficult learning curve, is related to the annual number of UKA case surgeries performed by the surgeon.

In the current study, the rate of partial RLLs of 38.3% determined in the cemented group was found to be in accordance with the literature. Gulati et al. (20) reported this rate as 32% at the end of a 5-year follow-up period and Pandit et al. (22) reported 43% after a 1-year follow-up. In this present study and the similar

studies about RLLs in UKA in the literature, the majority of patients who underwent cemented UKA were patients with insufficient bone quality. In our opinion, we see that cemented UKA applied to patients whose trabecular structure is not tight enough in the bone hardness test cannot complete the deficiency caused by insufficient bone quality.

When the distribution of the radiolucent line according to each zone was examined, physiological RLLs were mostly observed in zones 2 (10.5%), 3 (7.9%) and 5 (7.9%) in cementless tibial components, and in zones 1 (19.1%), 5 (14.9%) and 2 (10.6%) in the cemented tibial components, respectively. Similar to the current study, when Kleeblad et al. (26) analyzed RLLs according to zones, all tibial radiolucencies were observed in zones 1 and 5. In the current study, the total number of partial radiolucent regions was 13 (5.7%) in cementless UKAs and 32 (11.3%) in cemented UKAs. This difference was statistically significant (0.025). No complete RLLs were observed in any UKAs when both cemented and cementless prostheses were considered. In cemented UKAs, the incidence of partial RLLs and the total number of radiolucent regions in the tibial component interface were higher. However, the excess physiological line observed in cemented UKAs was not reflected in the clinical results of the patients, and no statistically significant difference was found between the groups when the postoperative OKS, EQ-5D-3L, EQ-VAS and KOOS scores were compared.

When the duration of surgery of the patients was evaluated, it was seen to be 12 minutes shorter in the cementless group. The time taken for the diagnostic arthroscopy in 32 patients was not included in the arthroplasty operative time. The shorter operative time in cementless UKA is expected when bone cement hardening time is taken into consideration in cemented UKA. The insert dislocation that is observed in a patient with a cemented UKA is because the excess cement has not been cleaned well at the posterior of the tibial component and the insert is dislocated in the third month postoperatively due to impingement. When a minimally invasive surgical technique is used, the view of the surgical field may be minimal. This can lead to problems in noticing and cleaning the cement residues at the posterior of the components, especially if a cemented femoral component is used and this may lead to problems such as pain, impingement, and insert dislocation in the postoperative period (27). Therefore, this can be stated as a further advantage of a cementless UKA.

Clarius et al. (28) reported that pulsed lavage significantly reduced the incidence of RLLs in cemented fixations. Panzram et al. (23) reported lower RLL incidence in cemented UKAs with this device but no significant difference was determined in comparison with the cementless UKAs. In the current study, jet lavage was applied to every patient and a significantly lower number of radiolucent regions was detected in the cementless group (15.8% vs 38.3%).

Study Limitations

This study had several limitations, primarily that it was a retrospective study. Second, this was a case series performed by a single surgeon with extensive surgical experience (more than 250 cases) and might not be reproducible in other centers. Another limitation was the relatively small number of cases and shorter follow-up time of the cementless group compared with the cemented group. Despite these limitations, the major strength of this study was that it was one of the few comparative studies that examined RLLs in cemented and non-cemented UKAs as a non-designer user group. The study also included the clinical results of two different fixation methods.

Conclusion

The most important finding of this study was that the rate of physiological RLLs was significantly lower in cementless UKAs than in cemented UKAs, and the presence of RLLs had no effect on clinical outcomes. This result supports the views of the designer group. This study is also important in terms of reflecting the results of a non-designer group as there are few studies in literature with comparative results of non-designer groups. Nevertheless, there is a need for larger patient series and longer follow-up results to examine the clinical safety and efficacy of cementless implants.

Ethics

Ethics Committee Approval: The study was approved by Ankara Dışkapı Yıldırım Beyazıt Research and Training Hospital Institutional Review Board (protocol no: 56/29, date: 12.11.2018). The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Informed Consent: Written informed consent obtained from all patients.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: H.A., Concept: H.A., H.A.A, M.A., K.B., H.B.Ç., Design: H.A., H.A.A, M.A., K.B., H.B.Ç., Data Collection or Processing: H.A., H.A.A, M.A., K.B., H.B.Ç., Analysis or Interpretation: H.A., H.A.A, M.A., K.B., H.B.Ç., Literature Search: H.A., H.B.Ç., H.A.A., Writing: H.A., H.B.Ç., H.A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5333
Bezmialem Science 2022;10(2):206-11

The Effect of Weight Loss on Osteoarthritis Symptoms in Obese Patients with Osteoarthritis

Osteoartriti Olan Obez Bireylerde Ağırlık Kaybının Osteoartrit Semptomlarına Etkisi

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ABSTRACT

Objective: Knee osteoarthritis (OA) is a very common joint disease and obesity is accepted as a modifiable risk factor for knee OA. This study aims to reduce OA symptoms with dietary intervention that will provide at least 10% body weight loss in volunteers diagnosed as having knee OA and obesity.

Methods: As an open, uncontrolled randomized study, it was conducted with patients admitted to İstanbul Fatih Sultan Mehmet Hospital. Fourty volunteers (mean of age: 58±10.9 years, 4 males, 36 females) having knee OA grade ≥2 according to Kellgren-Lawrence radiological classification and obesity [body mass index (BMI) ≥30 kg/m^2] were included in the study. Individuals were given a diet program containing 50-60% carbohydrate, 15-20% protein and 25-30% fat for 16 weeks, and they were planned to lose weight and followed up. WOMAC OA index was used for the evaluation of pain, stiffness and physical functions.

Results: After 16 weeks, individuals lost an average of 7.5% of their weight. A decrease of 2.75 kg/m^2 was observed in the BMI ($p<0.05$). A significant reduction was observed between the first and the last WOMAC pain scores ($p<0.05$). A significant relationship was also found between the decrease in BMI and the decrease in WOMAC score ($p<0.05$).

Conclusion: The decrease of 2.75 kg/m^2 in BMI and the weight loss of 7.5% provided decrease in WOMAC pain score. Weight

ÖZ

Amaç: Diz osteoartriti (OA), oldukça yaygın olarak görülen bir eklem hastalığıdır ve obezite diz OA'sı için değiştirilebilir bir risk faktörü olarak kabul edilmektedir. Bu araştırmanın amacı; diz OA ve obezite tanısı almış gönüllü bireylerde diyet müdahalesi ile en az %10 vücut ağırlığı kaybı gelişmesi ve OA semptomları arasındaki ilişkinin saptanmasıdır.

Yöntemler: Çalışma açık, kontrolsüz randomize bir araştırma olarak İstanbul Fatih Sultan Mehmet Eğitim ve Araştırma Hastanesi'ne başvuran hastalarla gerçekleştirildi. Çalışmaya Kellgren-Lawrence radyolojik sınıflamasına göre evre ≥2 diz OA'sına sahip, obezite [vücut kitle indeksi (VKİ) ≥30 kg/m^2] tanısı almış, yaş ortalaması 58±10,9 yıl olan, 4 erkek, 36 kadın olmak üzere 40 gönüllü birey dahil edildi. Bireylere 16 hafta boyunca %50-60 karbonhidrat, %15-20 protein ve %25-30 yağ içeren diyet programı verilerek hastaların ağırlık kaybetmeleri planlandı ve hastalar takip edildi. Ağrı, tutukluk ve fiziksel fonksiyonlarının değerlendirilmesinde WOMAC OA indeksi kullanıldı.

Bulgular: On altı hafta sonrasında bireyler ağırlıklarının ortalama %7,5'ini verdi. VKİ'de 2,75 kg/m^2 düzeyinde bir azalma saptandı ($p<0,05$). İlk ve son WOMAC ağrı skorları arasında anlamlı düşüş gözlendi ($p<0,05$). BKİ'nin azalması ile WOMAC puanının azalması arasında ise anlamlı bir ilişki bulundu ($p<0,05$).

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Received: 15.09.2020
Accepted: 16.02.2021

Cite this article as: Dülger N, Akman M, Yılmaz B. The Effect of Weight Loss on Osteoarthritis Symptoms in Obese Patients with Osteoarthritis. Bezmialem Science 2022;10(2):206-11

loss should be the optimal approach in the management of obese patients with knee OA.

Keywords: Obesity, osteoarthritis, BMI, diet, WOMAC

Sonuç: VKİ'de 2,75 kg/m^2 düzeyinde azalma ve %7,5 ağırlık kaybı WOMAC ağrı skorunun azalmasını sağlamaktadır. Ağırlık kaybı, diz OA'sı olan obez hastaların yönetiminde optimal yaklaşım olmalıdır.

Anahtar sözcükler: Obezite, osteoartrit, BKİ, diyet, WOMAC

Introduction

Today, obesity is one of the most common diseases affecting lifestyle. Obesity is the leading cause of death in countries where the majority of the world's population lives. Although the incidence of obesity is constantly increasing, obesity is a preventable disease (1). Obesity significantly increases the risk of development of multiple diseases, including osteoarthritis (OA), which is the most common cause of severe disability among older people in the UK and North America. This situation is similar in Turkey (2).

Numerous studies confirmed the strong association between obesity and knee OA, which was first documented in 1945 (3-5). Obesity is considered as a risk factor for knee OA and every 5 kg increase in body mass increases the risk of OA by 35% (6). Many studies have shown that with advancing age, the burden of chronic musculoskeletal disorders such as OA increases. OA is the most common type of arthritis that affects older individuals. It is a degenerative joint disease that can affect any joint in the body and cause chronic pain, functional restriction and emotional discomfort, and it adversely affects quality of life and causes disability (7). Both obesity and OA reduce mobility in individuals. With the decrease in mobility, energy expenditure decreases and as a result, body weight increases. Increased body weight causes the symptoms of OA to be aggravated and thus to a decrease in mobility. This vicious cycle is an important problem in the treatment of obese patients with OA. For this reason, reducing and controlling body weight have an important place in OA treatment among the steps of non-drug treatment and they are recommended in numerous treatment guidelines (8).

The purpose of this study was to examine the effects of providing at least 10% weight loss with healthy dietary intervention on OA symptoms in voluntary individuals over 40 years of age who were diagnosed as having knee OA (K-L ≥2) and obesity [body mass index (BMI) ≥30 kg/m^2].

Method

The research was an open, uncontrolled and randomized study. This study was conducted with the patients who were diagnosed as having knee OA and obesity in the Orthopedics and Traumatology Outpatient Clinic of İstanbul Fatih Sultan Mehmet Training and Research Hospital between June 2018 and March 2019 with the approval of Clinical Research Ethics Committee of İstanbul Fatih Sultan Mehmet Training and Research Hospital.

Individuals

Fourty voluntary individuals who were diagnosed as having knee OA and obesity and who were admitted to the clinic and approved the voluntary form were randomly included in the study.

Patients who were diagnosed as having OA according to the criteria of the American Association of Rheumatology (ACR), were over 40 years old, had a BMI of 30 kg/m^2 and above, had a diagnosis of OA for at least 6 months, and had at least Ggrade 2 OA according to the Kellgren-Lawrence (K-L) Evaluation in the radiographs taken over the last 6 months, were included in the study. Patients who had a operation for knee or hip, received physical therapy in the last 3 months, had intraarticular injection to the knee or hip joint in the last 3 months, suffered severe physical trauma in the last 3 months, or had severe psychological trauma in the last 3 months, were excluded from the study. Among the patients who were included in the study, patients who underwent a surgical operation, did not apply the diet program for 1 month, or had physiotherapy or intraarticular injection to the knee joint were excluded from the study. Firstly, 40 volunteers were questioned in terms of age, gender, height, BMI, chronic diseases, continuous medications, food consumption, cigarette and alcohol consumption. Body analysis and weight change follow-up of individuals were performed with TANITA MC 780ST model body analyzer.

WOMAC Osteoarthritis Index

The WOMAC OA Index was used to evaluate pain, stiffness and physical functions of the individuals. WOMAC is a valid, reliable criterion specific to OA and includes 24 questions in three subheadings: pain, stiffness and physical function. Each question is scored on the Likert scale from 0 to 4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). The score of each section is calculated within itself, and the total score ranges from 0 to 100. High scores indicate increased pain and stiffness and impaired physical function (9). The WOMAC OA index was applied twice to individuals as at the beginning of the study and after 16 weeks.

Diet Program

According to the results of the anthropometric measurements, a healthy diet program was administered by the dietician, with which individuals could lose at least 10% of their body weight for 16 weeks. In this diet program, a healthy weight loss program was prepared by calculating the daily energy needs according to the weight, Basal Metabolic Rate value, gender, age and comorbidities of OA patients. The energy distribution of this

diet program consisted of an average of 50-60% carbohydrates, 15-20% protein and 25-30% fat according to the needs of the individual (10). The individuals participating in the study were followed up with weekly and monthly controls and energy changes were made and weight loss of at least 10% in 16 weeks was targeted.

Radiological Evaluation

Lateral and anterior-posterior graphs of the right and left knees of the individuals included in the study were classified by the specialist radiologist according to the radiological classification of K-L. The OA grades are as follows:

- Grade 0: Normal,
- Grade 1: Suspected osteophytes, normal range of joint,
- Grade 2: Significant osteophyte, suspected narrowing of joint gap,
- Grade 3: Moderate osteophytes, moderate narrowing of the joint gap, mild sclerosis,
- Grade 4: Large osteophytes are identified as advanced narrowing of the joint gap, pronounced subchondral bone sclerosis, cysts.

The patients' weight, BMI, body fat and muscle mass percentage, WOMAC scores and K-L grades were recorded in the follow-up forms of monthly periods.

Statistical Analysis

Averages of continuous variables, standard deviation and median; the frequency and percentages of categorical variables were calculated. For data showing normal distribution; the Independent Sample t-test and the Dependent Sample t-test were used to compare the previous and subsequent measurements. For data not showing normal distribution; The Mann-Whitney U test was used to compare the previous and subsequent measurements, and the Wilcoxon test was used to compare the previous and subsequent measurements. Spearman Rank Differences Correlation analysis and McNemar test were used to examine the relationship between the data. The SPSS 21 (Statistical Package for the Social Sciences) package program was used for the data analysis. The analysis was tested for 95% confidence and 5% error margin. Statistically, the limit of significance was considered $p<0.05$.

Results

This study which was carried out to examine the effect of weight loss on disease symptoms in individuals diagnosed as having obesity and knee OA, was conducted with a total of 40 adult volunteers, including 36 women and 4 men, between June 2018 and March 2019. Individuals voluntarily attended to the 16-week weight loss program in a period between these dates. The ages of individuals ranged from 40 to 80 years, and their average age was 58±10.90 years.

Regarding the anthropometric measurements of the individuals, mean body weight was determined as 91.2±15.76 kg, mean BMI as 36.96±4.49 kg/m^2, average body fat percentage as 39.14±4.23%, and average muscle mass percentage as 57.85±4.05%. Considering the anthropometric measurements after 16 weeks, mean body weight was determined as 84.34±14.78 kg, mean BMI as 34.21±4.51 kg/m^2, mean body fat percentage as 36.93±5.46%, and mean muscle mass percentage as 59.88±5.23. Individuals lost an average of 7.52±3.5% of their body weight in 16 weeks. While the highest body weight loss percentage was 18.2%, it was determined that one of the individuals gained body weight of 1.7% without weight loss (Table 1).

In terms of the classifications by their BMI, 37.5% of the patients were obese in the first degree, 37.5% were obese in the second degree, and 25% were morbidly obese at the beginning of the study. According to K-L radiological evaluation, 60% of the individuals were diagnosed as having grade 2 and 40% as grade 3 OA.

When the initial WOMAC OA indexes of the individuals were evaluated, the mean value was found to be 45.25±21.94 points. When the WOMAC OA indexes were evaluated after 16 weeks, the mean value was found to be 28.44±18.93 points. When the difference between the first and the last WOMAC OA indexes of the individuals was examined, the average pain scores decreased by 16.81±11.02 points (Table 1). According to the results of the WOMAC test, which was administered at the beginning and after 16 weeks, in consideration of the individuals' pain scores; it was detected that 35% of the patients had a 1-10 scoring decrease, 30% had a 10-20 scoring decrease, 15% had a 20-30 scoring decrease, 17.5% had a 30-40 scoring decrease, and 2.5% had a 40-45 score decrease.

Table 1. Differences between the initial and final anthropometric measurements and WOMAC OA index scores

Weight (kg)	Mean	sd	Min	Max
Initial	91.2	15.76	69.9	151.9
Final	84.34	14.78	61.5	138.7
BMI (kg/m^2)				
Initial	36.96	4.49	30.4	48.4
Final	34.21	4.51	27.0	45.1
Body fat (%)				
Initial	39.14	4.23	27.9	47.7
Final	36.93	5.46	23.2	50.5
Muscle mass (%)				
Initial	57.85	4.05	49.7	69.0
Final	59.88	5.23	47.0	73.0
WOMAC score				
Initial	45.25	21.94	9.4	92.0
Final	28.44	18.93	3.0	84.0
WOMAC difference				
	16.81	11.02	2.0	40.0
Weight loss (%)				
	7.52	3.50	-1.7	18.2

Min: Minimum, Max: Maximum

According to the results of the Wilcoxon test performed to determine whether there was a difference between the initial score of the WOMAC OA index, BMI and muscle mass percentage, and their values after 16 weeks; a statistically significant difference was observed between two periods in terms of WOMAC OA index scores (Z = -5.51; p<0.05), BMI values (Z = -5.50; p<0.05) and muscle mass percentage (Z = 4.70; p<0.05) (Table 2).According to the result of the Dependent sample t-test conducted to determine whether there was a difference between the calculated body fat percentage (%) before and after the diet of individuals and the body fat percentage (%) after 16 weeks; a statistically significant difference was observed [t (39) = 6.21; p<0.05] (Table 2).

According to the result of Mann-Whitney U test conducted to investigate whether there was a difference between groups in terms of reduction in WOMAC OA index scores of individuals with grade 2 OA and grade 3 OA by means of K-L score, no statistically significant difference was observed (U= 137.00; p>0.05) (Table 2). Similar results were observed in the individuals with grade 2 OA and grade 3 OA.

According to correlation analysis, a statistically significant relationship between WOMAC OA index scores and weight loss in the 16-week weight loss process of individuals was not found (p>0.05). There was a statistically significant positive mid-level correlation between decreased WOMAC OA index scores of individuals and decreased BMI (p<0.05) (Table 3).

Statistically significant positive intermediate relationship between the age of individuals and the first WOMAC pain scores and recent WOMAC pain scores (p<0.05), and statistically significant positive intermediate relationship between the initial WOMAC pain scores of individuals and the initial weights were found (p<0.01) (Table 3).

According to the results of the McNemar test, it was observed that there was a statistically significant difference between the WOMAC pain scores of the individuals at baseline and after 16 weeks (p<0.01). The WOMAC pain score of 12 individuals was above 30 and after the diet, it decreased below 30 (Table 4).

Table 2. Changes in WOMAC, BMI, muscle mass percentage, body fat percentage and K-L grades after the diet was applied

	n	Z	p
WOMAC difference *			
	40	-5.51	.00
BMI difference *			
	39	-5.50	.00
Muscle mass percentage difference*			
	32	-4.70	.00
	n	t	p
Body fat percentage difference**			
	40	6.21	.00
	n	U	p
K-L degree ***			
Grade 2	24	137.00	0.13
Grade 3	16		

*Wilcoxon test, **Dependent Sample t-test, ***Mann-Whitney U test

Discussion

OA is the leading cause of chronic disability in elderly adults. Obesity and the age factor are important known risk factors for knee OA. These two factors cause an increase in disease symptoms, but obesity is a changeable risk factor (11). Decreased body mass through dietary intervention leads to significant improvements in knee OA, reduced degeneration, and improved quality of life. As a result of the study conducted by Aaboe et al. (12), it was found that the peak load on the knee decreased by 2.2 kg with every 1 kg reduction in the body.

A statistically significant positive moderate correlation was found between the age of individuals and the first WOMAC pain scores and the last WOMAC pain scores (p<0.05). As the age increases, it can be stated that individuals' WOMAC pain scores may increase. As mentioned before, age is the most important risk factor for knee OA. In a study conducted by Atamaz et al. (13), it was shown that obesity, aging, female gender and advanced radiological grade were factors associated with pain and disability.

Table 3. Correlation analysis

	n	r	p
BMI difference correlation with weight difference correlation*			
	40	0.205	0.205
BMI difference correlation with WOMAC difference*			
	40	0.307	**0.044**
Age correlation with first WOMAC*			
	40	0.362	**0.022**
Age correlation with last WOMAC*			
	40	0.461	**0.003**
Weight correlation with first WOMAC*			
	40	0.409	**0.009**

*Spearman Rank Correlation Coefficient Analysis was applied

Table 4. Comparison of the initial and subsequent WOMAC pain scores

		First WOMAC score *		Total	p
		<30 points	>30 points		
Last WOMAC score *	<30 points	11	12	25	**0.000**
		44.0%	56.0%	100.0%	
	>30 points	0	15	7	
		0.0%	100.0%	100.0%	
Total		11	29	40	
		100.0%	100.0%	100.0%	

*McNemar test was applied

When the disease conditions of individuals were examined, it was observed that 22.5% of the individuals had diabetes, 50% had hypertension, 27.5% hypercholesterolemia and 15% had hypothyroidism.

When individuals were classified according to BMI, 37.5% were found to be first degree obese, 37.5% as second degree obese and 25% as morbid obese. In a study by Reyes et al. (14), it was reported that being overweight increased the knee OA risk 2 times, being first degree obese 3.1 times, and being second degree obese 4.7 times. Similar results were obtained in this study, and it was observed that the WOMAC pain score increased due to the increase in body weight ($p<0.01$).

According to K-L radiological evaluation, 60% of the individuals were diagnosed as having grade 2 OA and 40% grade 3 OA. There was no statistically significant difference between individuals with grade 2 OA and grade 3 OA in terms of the WOMAC OA pain scores ($p>0.05$). Individuals diagnosed as having grade 2 OA and grade 3 OA had similar pain scores. This could be due to the fact that there were no patients diagnosed as having grade 1 OA and grade 4 OA in the study group.

Anthropometric measurements of the individuals were evaluated after 16 weeks. The average body weight loss of individuals was 6.86 kg and the average BMI loss was 2.75 kg/m^2 ($p<0.05$). Body fat was decreased by 2.22% ($p<0.05$), and muscle mass was increased by 2.03% ($p<0.05$) after 16 weeks. In a study by Bartels et al. (15), 13.6 kg loss was achieved by using diet intervention in obese patients with knee OA in 16 weeks. In another study, 12.8 kg loss was achieved in 16 weeks (16). It was found that individuals who participated in the study lost an average of 7.5% of their body weight with dietary intervention within a 16-week period. In the patient group with a BMI average of 36 kg/m^2, Aaboe et al. (12) achieved an average of 13.5% reduction in body weight with diet in 16 weeks. The aim of the study was to achieve at least 10% weight loss in individuals, but it might be considered that individuals were unable to physically engage due to knee OA and could not reach the target due to their comorbidities.

The average WOMAC pain scores before dietary intervention in individuals was 45.3 points. In Tütün et al.'s (17) study, the mean BMI of 50 knee OA patients was 33.2 kg/m^2 and the average WOMAC pain score was 55.7 points. In the study of Doğan et al. (18), the average WOMAC pain score of 83 individuals with an average age of 59.5 years was 56.3 points. These data are similar to the current study, and although the WOMAC test is a subjective test, it is considered to be reliable.

The individuals lost an average of 7.5% of their body weight during the study, and the difference between the first and last WOMAC pain scores decreased significantly ($p<0.05$). There was a positive moderately significant correlation between the reduction of BMI in the 16-week diet intervention process and the reduction of WOMAC pain scores ($p<0.05$). A reduction of 2.75 kg/m^2 in BMI may reduce WOMAC pain. In the case report presented by Sevinç (19) in 2014, significant decrease in WOMAC pain score and increase in physical activity were observed in the female individual whose BMI decreased from 40 kg/m^2 to 32.5 kg/m^2.

In many cohort studies, it has been shown that after bariatric surgery, body weight loss improves symptoms of knee OA, quality of life and daily activities (20-23). In addition, it was shown that the development of postoperative complications after total knee arthroplasty was less in morbid obese patients who underwent bariatric surgery than in morbid obese patients who did not undergo bariatric surgery (24).

As a result of the research, individuals achieved 7.5% weight loss showed a significant difference in WOMAC pain scores and individuals did not have to achieve at least 10% weight loss. In a study conducted by Messier et al. (25), it was reported that 5% weight loss reduced pain, improved function and increased activity. While at the beginning of this study, the WOMAC pain score of 12 individuals was above 30, it decreased to less than 30 after dietary intervention.

Study Limitations

Most of the individuals participating in the study verbally reported that their knee pain decreased, their physical activity increased, their eating habits changed, and their quality of life improved.

Conclusion

As a result; obesity is a risk factor for the progression of OA, increases the incidence of OA and adversely affects the outcome of the disease. Weight loss might relieve knee OA symptoms, improve functions and improve quality of life. Weight loss and exercise should be the optimal approach in managing obese patients with OA. Individuals were not required to lose at least 10% weight. A decrease in BMI of 2.75 kg/m^2 and a weight loss of 7.5% resulted in a statistically significant decrease in WOMAC pain score. For all obese patients with OA, it may be recommended to lose at least 5% of body weight. Typically, arthritis is treated when overweight or obese patients are admitted with symptomatic OA, but the underlying diseases are not treated.

Fighting against both obesity and OA as an ideal treatment approach and providing weight loss will be an important step in treating this globalizing problem. Treatment of patients with a multidisciplinary approach involving physicians, dieticians, physiotherapists and nurses may play a significant role in the coming years and is thought to improve the overall health of the patient and to help reduce the healthcare expenditure at the same time.

Ethics

Ethics Committee Approval: This study was conducted with the patients who were diagnosed as having knee OA and obesity in the Orthopedics and Traumatology Outpatient Clinic of Fatih Sultan Mehmet Training and Research Hospital between June 2018 and March 2019 with the approval of Clinical Research Ethics Committee of İstanbul Fatih Sultan Mehmet Training and Research Hospital.

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: N.D., M.A., Design: N.D., B.Y., Data Collection or Processing: N.D., Analysis or Interpretation: N.D., B.Y., Literature Search: N.D., Writing: M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.4751
Bezmialem Science 2022;10(2):212-8

Role of Positron Emission Tomography in Staging Lymph Nodes in Non-small Cell Lung Cancer

Pozitron Emisyon Tomografisinin Küçük Hücreli Dışı Akciğer Kanserinde Lenf Nodu Evrelemesindeki Rolü

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ABSTRACT

Objective: Lung cancer is in the cancer group with high mortality, and early diagnosis and accurate staging prolongs survival. Positron emission tomography (PET) is routinely in use for staging of non-small cell lung cancer. Since there were still controversies, we aimed to investigate the reliability of PET especially in mediastinal lymph node (MLN) staging.

Methods: A total of 141 surgical procedures were performed on 133 patients with non-small cell lung cancer clinically suitable for surgery. With PET, lymph nodes having a standart uptake value-SUV_{max} >2.5 were noted. With the results, the stations reached by mediastinal examination in 25 patients and direct thoracotomy in 8 patients were sampled. With the results, a total of 123 patients underwent resection, and 13 patients were referred to oncology clinics. In staging of MLN, tomography and PET were compared in terms of sensitivity and specificity, and also the relationship of PET with type, localization and size of tumor was investigated.

Results: For mediastinal staging, sensitivity of PET in squamous cell lung cancer was 100%, while specificity 88%, positive predictivity 50%, negative predictivity 100%, and accuracy 89.5%. Hilar, intrapulmonary and mediastinal lymph nodes were analyzed and the same parameters were 64.5%, 80%, 80%, 64.5% and 74.4%, respectively. PET-SUV_{max} values were found to be statistically significantly associated with tumor size, central location, epidermoid histology and especially lymph node 7. The accuracy of PET in evaluating this lymph node station seemed to be higher.

ÖZ

Amaç: Akciğer kanseri mortalitesi yüksek kanser grubunda olup erken tanı ve doğru evreleme sağkalımı uzatmaktadır. Pozitron emisyon tomografisi (PET) küçük hücreli dışı akciğer kanserinde tanı ve evreleme yöntemi olarak rutin kullanılmaktadır. Halen tartışmalı durumlar olduğundan özellikle mediyastinal lenf nodu (MLN) evrelemesinde PET'nin güvenilirliğini araştırmayı amaçladık.

Yöntemler: Klinik olarak operasyona uygun küçük hücreli dışı akciğer kanseri tanılı 133 hastaya 141 cerrahi işlem uygulandı. Bu yöntem ile metastaz olduğu belirtilen (standart uptake değeri-SUD_{maks} >2,5) lenf nodları kaydedildi. Sonuçlarla 25 hastada mediasten incelemesi, 8 hastada direkt torakotomi yapılarak ulaşılan istasyonlar örneklendi. Sonuçlarla toplamda 123 hastaya rezeksiyon uygulandı, 13 olgu onkoloji kliniklerine yönlendirildi. MLN tutulumu açısından tomografi ile PET'nin duyarlılık ve özgüllük sonuçları karşılaştırılarak, PET'nin tümör tipi, lokalizasyonu ve boyutu ile ilişkisiaraştırıldı.

Bulgular: Skuamöz hücreli karsinomun mediyastinal evrelemesinde PET'nin duyarlılığı %100, özgüllüğü %88, pozitif prediktivite değeri %50, negatif prediktivite değeri %100 ve doğruluğu %89,5 olarak bulundu. Hiler, intrapulmoner ve MLN istasyonları çalışmaya alınarak yapılan değerlendirmede ise aynı değerler sırası ile %64,5, %80 %80, %64,5 ve %74,4 olarak hesaplandı. PET-SUV_{maks} değerleri tümör büyüklüğü, santral yerleşim, epidermoid histoloji ve özellikle 7 nolu lenf nodu ile istatistiksel olarak ilişkili bulundu.

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Received: 04.06.2020
Accepted: 13.01.2021

Cite this article as: Akgül AG, Temel U. Role of Positron Emission Tomography in Staging Lymph Nodes in Non-small Cell Lung Cancer. Bezmialem Science 2022;10(2):212-8

Conclusion: PET gives statistically significant results when we evaluate the MLN stations one by one. In addition, PET results should be confirmed, especially in the centrally located adenocarcinoma subgroup. If PET can provide more reliable results in staging with advancing technology in medicine, it will perhaps take its place as the gold standard in lung cancer as a non-invasive technique.

Keywords: Lung cancer, mediastinal staging, PET

PET'nin özellikle bu lenf nodu istasyonunu değerlendirmedeki doğruluğu daha yüksek görünmektedir.

Sonuç: MLN istasyonlarını tek tek değerlendirdiğimizde, PET istatistiksel anlamlılık vermektedir. Bunun yanında, özellikle de santral yerleşimli adenokarsinom alt grubunda PET sonuçları doğrulanmalıdır. Tıpta ilerleyen teknoloji ile PET, evrelemede daha güvenilir sonuçlar verebilirse non-invazif teknik olarak akciğer kanserinde belki de altın standart olarak yerini alacaktır.

Anahtar Sözcükler: Akciğer kanseri, mediastinal evreleme, PET

Introduction

Lung cancer is one of the most common malignancies and its frequency is increasing all over the world. In the early diagnosis of the tumor, computerized tomography (CT), sputum cytology and bronchoscopy can increase the number of patients suitable for surgery. Mediastinal lymph node (MLN) staging is the most important factor in determining both the treatment method and the prognosis (1,2).

Staging is mainly based on imaging techniques. CT is the most commonly used method for non-invasive staging of lung cancer, especially for lymph nodes. Evaluation on CT for lymph nodes is based on shape and size. Usually, the diameter over 1 cm is in favor of the enlarged lymph node; however, in histological examinations, metastasis can be detected in lymph nodes below 1 cm, and some lymphadenopathies (LAP) may be due to reactive hyperplasia or other benign causes (3). For this reason, mediastinoscopy is required for the majority of patients for mediastinal staging and is still considered the gold standard. Today, positron emission tomography (PET), which shows the biological activities of tumor cells, has been shown to be superior to thorax CT in the mediastinal staging of patients diagnosed as having non-small cell lung cancer (NSCLC). Therefore, PET, which is the noninvasive method for detecting MLN metastasis, has been reported to replace invasive mediastinoscopy (4-7).

In this study, we aimed to compare the histopathological results of mediastinal lymph nodes with non-invasive methods such as thorax CT and CT integrated PET for patients diagnosed as having NSCLC in our clinic.

Method

Within 4 years, 141 surgical procedures were applied to 133 patients who were admitted to our clinic, diagnosed as having NSCLC by tissue biopsy, and were found to be eligible for surgical treatment. More than one surgical procedures were applied to 5 patients. All patients were evaluated with contrast thorax CT and PET examinations, which were performed within 30 days maximum before the operation, retrospectively and prospectively.

As laboratory tests, all patients underwent routine blood tests, arterial blood gas and related blood tests in those with additional diseases. Routinely, pulmonary function tests, electrocardiograms, echocardiography -to evaluate cardiac functions more detailed- and other related examinations were also performed. Radiological examinations including postero-anterior and lateral direct chest X-rays and thorax CT which were performed to evaluate the localization and size of the tumor, lymph nodes and resectability of the tumor, were applied in all patients. Thorax multislice CT imaging was performed by delivering nonionic iodized contrast agent intravenously with an automatic injector pump. All thorax was scanned from upper clavicle level to upper surge at both lower adrenal gland image area and the raw data obtained were reconstructed without gap. Five-mm-thick axial images in multislice CT were evaluated in mediastinal window. In defining localization, tumors related to mediastinal pleura were defined centrally and others peripherally in 1/3 of the hemithorax. In the evaluation of mediastinal lymph nodes by using thorax CT, the lymph nodes with a short diameter greater than 1 cm were suspiciously considered pathological with probability of metastasis.

For the histopathological diagnosis of the lesion, bronchoscopic biopsy, transbronchial needle aspiration biopsy, transthoracic needle aspiration or tru-cut biopsy was performed. In patients with non-diagnostic results, thoracoscopy, and finally thoracotomy and frozen-section tissue examination were performed.

PET imaging was performed following 4-6 hours of fasting and a good hydration. If the blood glucose level was measured within normal limits, 10-15 mCi 18-fluorodeoxyglucose (FDG) injection was administered in the patient, and after waiting for 60 minutes images were gathered with integrated PET/CT camera. Examination for mediastinal lymphatic metastasis was evaluated visually first. In visual evaluation, mediastinal foci with increased uptake compared to the back-ground and surrounding tissue activity with normal biodistributionwere interpreted as suspicious for malignancy, and in cases where measurement was possible, the maximum standard uptake value (SUV_{max}) higher than 2.5 was interpreted in favor of malignancy.

In the identification of MLN, in accordance with the Mountain (8) classification, the records were created. After imaging MLN with PET as areas with high SUV_{max}, standart cervical mediastinoscopy for lymph nodes 2, 4, 7, anterior mediastinotomy for 5 and 6, and VATS for 8 were performed in 15 days for verification. After examining the pathology of the samples, patients with metastases were re-evaluated and directed to oncology clinics for neoadjuvant or non-surgical treatment. Appropriate resection and systematic mediastinal lymphatic sampling with thoracotomy or VATS were

performed in patients in whom metastasis was not detected in lymph node examinations.

The histopathological diagnosis obtained as a result of interventions for lymph nodes and lesions has been accepted as the gold standard. It was aimed to compare histopathological diagnosis with imaging methods. Thorax CT, PET, mediastinoscopy, anterior mediastinotomy, VATS and thoracotomy results were recorded. In accordance with the definitions below, true positive, true negative, false positive, false negative rates were determined, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy rates were compared.

Statistical Analysis

Excel and SPSS (Statistical Package for Social Sciences) 16 for Windows statistics program were used. In comparison of parameters, Fisher verification test, chi-square test and Student's t-test were used. As a result of the analyzes, "p" values that were less than 0.05 were considered significant.

Results

Ninety-nine (74.4%) of the patients were male and 34 of them (25.6%) were female. Their ages ranged from 24 to 83, with the mean of 60.7. In the histopathological differentiation of malignant tumors, squamous cell carcinoma (SCC) was detected in 48 (34%), adenocarcinoma in 45 (32%), sarcoma in 8 (5.7%), and adenosquamous carcinoma in 5 (3.5%) patients. Other histologies were metastatic carcinoma (n=4), large cell carcinoma (n=2), lymphoma (n=2), and the rest were pleomorphic carcinoma, carcinoid tumor, mesenchymal tumor and anaplastic carcinoma.

Localization of 141 tumors in 133 patients was right lung in 75 (53.2%) patients and left in 66 (46.8%) patients. Seventy four (52.5%) tumor lesions were located peripherally and 67 (47.5%) centrally. Enlarged lymph node was observed with 33 (23.4%) tumors in thorax CT evaluation. After surgery, 17 (51.5%) of the patients were not detected as having malignant tumor, while 6 (6%) of 108 patients that were not suspicious for malignancy in CT, were detected as having malignant tumor (Table 1).

Mediastinal LN involvement was detected in 33 (23.4%) patients on PET. Lymph nodes were evaluated by applying mediastinoscopy, mediastinotomy, VATS and thoracotomy. Eight patients were taken directly to thoracotomy, 2 of them were diagnosed as having lymphoma with wedge resections, N2 was detected in 2 patients (positive lymph nodes were 5 and 6 in one patient, and a different node from PET; no=9, was positive in other patient). Four patients were taken directly to thoracotomy after neoadjuvant. In 12 patients who were performed preoperative invasive lymph node evaluation, malignancy was found, while 8 patients had no tumor metastasis, and 5 patients had benign disease. Thirteen patients (with 1 patient added from PET negative group, but mediastinoscopy was applied because of the brain metastasis and it was found positive) with LNM were referred to oncology clinic for the purpose of neoadjuvant or adjuvant therapy. Mediastinal LN dissection was conducted on patients who underwent thoracotomy with a negative lymph node. In all patient groups, lobectomy and bilobectomy were performed in 80 (56.7%) patients, wedge resection in 32 (22.7%), pneumonectomy in 11 (7.8%), and mediastinal staging in 18 (12.8%). The tumors were localized at upper lobes in 84 (68.3%) of the resections, at lower lobes in 33 (27%), and hilar in 6 (4.7%).

In 1 patient, who underwent preoperative invasive evaluation (mediastinoscopy for no=7 positivity) and was not detected as having mediastinal metastasis, was found as having N2 (no=8 lymph node) positivity (16.6%). In this patient, the histopathology of the tumor was adenocarcinoma and it was located in the right lower lobe. N2 or N3 lymph nodes were found positive in 12 of 25 patients who underwent preoperative invasive staging with mediastinoscopy after detecting positivity with PET. Sensitivity and specificity could not be calculated because thoracotomy was performed only in patients with N2 negative results in mediastinoscopy.

From 108 patients who did not have metastases in MLN, 1 patient with solitary brain metastasis was examined, and histopathological evaluation revealed N2 (no=6) despite PET negativity and referred also to oncology. And N2 was detected in 5 patients also, who were directly resected and dissected with thoracotomy.

Table 1. PET and CT evaluation between each-other and for various parameters

	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
CT	67	83	42	93	80.3
PET	70	85	45	94	82.5
PET SCC mediasten	100	88	50	100	89.5
PET adenocarcinoma mediasten	54	81.5	58	78.5	72.5
PET central	81	78	54	93	79
PET peripheric	40	90	25	95	86
PET hilar LN	23	88	39	78	72.4
PET paranchimal LN	-	98	-	89	87.4
PET all LN	64.5	80	64.5	80	74.4

PPV: Positive predictive value, NPV: Negative predictive value, CT: Computed tomography, PET: Positron emission tomography, SCC: Squamous cell carcinoma, LN: Lymph node

Of the patients with false positive results, 29% (n=5) had adenocarcinoma, 65% (n=11) squamous cell carcinoma, and 1 (6%) pleomorphic carcinoma. In 9 (53%) patients, the tumor was localized in the right lung and in 13 (76.4%) in the upper lobe. Tissue diagnosis was adenocarcinoma in all 6 patients with false negative results with PET. Four (66.6%) of the tumors localized in the upper lobe, were in the left lung and 2 (33.4%) in the right.

In measuring the accuracy of mediastinal lymph nodes evaluated by using PET, according to histopathological subtypes, statistical evaluation was made and the rates in SCC and adenocarcinoma were examined (Table 1). As a result of comparing the two histopathological types of tumors, the likelihood-ratio, which was found to be 8.9 in SCC, was significantly higher than the value of 2.9 in adenocarcinoma. When we looked at the differences in sensitivity, a significant difference was observed again. With these data, a statistically significant difference was observed between the two histopathologies in terms of the reliability of PET in MLN staging. In addition, all lymph node stations were included in the study and PET results were evaluated (Table 1).

The sensitivity of PET results in terms of MLN in centrally located tumors was found to be significantly high (Table 1). The relationship between the high value of SUV_{max} measured for the MLN and the central location was statistically significant.

In the examination of hilar lymph nodes (no=10 and 11), metastasis was detected in 18 patients by using PET examination. While positivity was found at 7 (38.8%) of 18 stations evaluated by surgical staging, metastasis was not observed in 11 (61.2%). In the examination of 105 patients without any involvement in the hilar region, metastatic lymph node was detected in 23 (22%) patients after surgical staging (Table 1).

In the statistical evaluation, the high value of SUV_{max} for hilar lymph nodes and no=9 mediastinal node was found to be related with tumor size (>1.5 cm) and gender (male). In the examination of the parenchymal lymph nodes (no=12-14) with PET screening, metastasis was detected in 2 patients. While metastasis was not detected in these 2 patients after staging with surgery, 14 (11.2%) positivities were detected among 125 stations which were indicated to have no metastasis in PET. Since both patients with lymph nodes detected in the parenchyma were found to be negative in pathological evaluation, the sensitivity could not be determined (Table 1).

The SUV_{max} values measured for MLN were compared with pathology results of lymph node stations and examined individually. In the statistical evaluation, a significant relationship was observed between a high SUV_{max} value of meanly 12.4 (7.3-19), and the positive pathology results of the stations numbered 4, 7, 8 and 9.

In the evaluation of lymph nodes considering the final stages of the patients with the histopathological results determined after all surgical interventions, 83 (63%) patients had N0, 27 (20.6%) N1, and 21 (16.4%) N2. Ten patients were exceptional of staging because of having lymphoma, sarcoma, metastatic tumors, and carsinoids. According to these results, N1 was found in 15 of the patients and N2 in 4 which were found as N0 in PET. N2 was found in 2 of 14 patients, and N0 in 9 which were found as N1 in PET. N1 in 9 and N0 in 8 were detected which were found as N2 in PET (Table 2).

When a comparison was made between the two groups, the N2 rate was 4.3% higher in patients who had N0 with PET examination, and the N2 rate was 14.3% higher after histopathological diagnosis in patients who were detected as having N1 with PET. These results should be taken into consideration in clinical evaluation, as it showed that the probability of N2 was higher in patients with N1 detected with PET compared to those with N0.

Clinical staging with PET performed by evaluating MLN was compared to histopathological staging achieved after surgical methods. While compatibility with the initial stage was found in 88 (62.4%) patients, a regression in the stage was observed in 31 (22%), and a progress in stage was observed in 22 (15.6%).

Comparing the final stages determined according to histopathological diagnoses and clinical staging, it was found that the stage was confirmed in 61% of 1A group, 60% of 1B group, 0% of 2A group, 60% of 2B group, 50% of 3A group, and 100% of 3B and 4 groups. When statistical analysis was performed, no significant difference was found between tumor diameter and histology and the first- last stage change, but it was determined that left sided and centrally localized tumors had a statistically significant. It was also determined that the "p" value calculated in relation to the SUV_{max} of the mass was at the limit (p=0.05). This may give an idea that tumors of left lung and centrally localized tumors with tumor size of greater than 2 cm and having high SUV_{max} have a greater risk of malignancy correlance.

Of 17 false positive results obtained withMLN, 1 was detected as reactive hyperplasia, 2 as granulomatous reaction, 2 as sarcoidosis, 2 as sinus histiocytosis, 3 as anthracosis, and 7 as tuberculosiswith PET. The SUV_{max} calculated for lymph nodes ranged from 2.7 to 10.8. Nine of them had also positivity in the form of lymphadenopathy in thorax CT.

Tumor was localized in the left lung in 8, right lung in 9 in the upper lobes in 13, and centrally in 11 patients. Of the lymph nodes with false positive results with PET, 10 were lower right paratracheal (no=4R), 5 subcarinal (no=7), 3 right upper paratracheal (no=2R), 2 left lower paratracheal (no=4L), 2 subaortic (no=5), 1 wasleft upper paratracheal (no=2L), 1 wasparaaortic (no=6), and 1 was right paraesophageal (no=8R) lymph nodes.

Table 2. Comparison of PET and histopathology for mediastinal staging

Histopathology				
		N0	N1	N2
PET	N0	66	15	4
	N1	9	3	2
	N2	8	9	15

PET: Positron emission tomography

Seven of the mediastinal lymph nodes evaluated by using PET were identified as false negative. Histopathological diagnosis was adenocarcinoma in all of them. Three were identified as LAP in CT. Tumor was located in upper lobes in all of them and it was localized in the left lung in 4 patients, and centrally in 3. Of false negative results in PET, 3 were detected in lymph station 5, 2 in lymph node station 6, and 2 in lymph node station 4R.

Discussion

In a patient with diagnosis of lung cancer, the research steps include clinical evaluation, risk factors, and CT image in the first step. Possible diagnosis, cell type and stage are determined. In the second step, diagnosis and staging are confirmed by using advanced radiological and invasive techniques. In the last step, the treatment option is determined. The choice of treatment and prognosis in a patient with lung cancer are closely related to the stage at the time of diagnosis. The main purpose of the intrathoracic staging of NSCLC is to investigate the involvement of MLN. It is divided into imaging and sampling magnetic resonance and PET are used in staging together with thorax CT. The most important indication of screening is to decide surgery or chemotherapy and/or radiotherapy by separating potentially resectable early stage cancers from nonresectable advanced ones (5,9).

Pathological sampling can be performed via transthoracic fine needle aspiration, transbronchial fine needle aspiration, endoscopic ultrasound, Endobronchial ultrasound-guided, mediastinoscopy, mediastinotomy, thoracoscopy and thoracotomy. Thorax CT detects a pathologic lymph node, but is unable to distinguish benign disease-related enlargements from metastasis. In studies, it has been reported that metastases can not be detected in 40% mediastinal lymphadenopaties at thorax CT, while metastases are detected in 15-20% of lymph nodes that do not reach the pathological size. In MLN staging, the sensitivity of CT with approximate values is reported as 65%, specificity as 76%, and accuracy as 73% (9-12). While the results we obtained in our study were found to be within the range specified in the literature, it was decided that CT would not be sufficient for mediastinal staging alone.

None of today's methods seems ideal in mediastinal staging alone for NSCLC. Various combinations of these methods are used for reliable mediastinal staging. Given all this, PET has been introduced as a promising method. Comparative studies of CT-PET efficacy including early periods showed that PET was superior in MLN staging (5,13,14). PET is thought to be a non-invasive method that can replace mediastinoscopy with high accuracy rates. However, the studies in the following years were not as satisfactory as the positive results we achieved in the early period. In the study of Gonzalez-Stawinski et al. (15) including 202 patients, PET sensitivity was calculated as 64%, specificity 77%, PPV 44%, and NPV 88% in MLN staging.

Toloza et al. (5) reported sensitivity, specificity, PPV and NPV of mediastinoscopy as 81%, 100%, 100%, and 91%, respectively in the study, which included 14 series involving 5,687 patients. In an invasive guide-study of Detterbeck et al. (16), sensitivity and specificity of mediastinoscopy were found as 78% and 100%, respectively in the examination of the results of 19 series containing a total of 6505 patients. The disadvantage of the procedure is being an invasive method that requires general anesthesia and having risks of morbidity and mortality.

By some authors, it is argued that mediastinoscopy can be neglected in patients with a peripherally localized tumor in small diameter without suspicion of N2 with screening while some others believe that it is mandatory in every patient (6,13,16,17). In our study, mediastinoscopy was not performed in patients who were not considered to be N2 clinically and radiologically. Twenty five patients with high FDG uptake in MLN according to PET, underwent mediastinal staging and 8 patients without metastasis underwent thoracotomy. When the last pathologies of the lymph nodes were examined, 7 patients had no lymph node involvement in the mediastinum, while 1 (16.6%) patient had a false negative result. With this result, the accuracy rate of mediastinoscopy was calculated as 83.4%. However, mediastinoscopy was performed for lymph node 7, which was indicated as positive in PET, and pathology result was learned as negative. After the thoracotomy, it was the lymph node station number 8, which was found to be positive. Therefore it may not be correct to define the result of mediastinoscopy as false negativity. In addition, the fact that there may be problems in the correct definition of anatomical structures with PET examination should not be ignored in this patient.

In determining mediastinal metastases, many studies were conducted to compare PET and other conventional imaging methods, and sensitivity, specificity, PPV and NPV were calculated. In the report of Birim et al. (18) in 2005, CT and PET were compared by evaluating 17 series. Consecutive CT and PET sensitivities were 59% and 83%, and their specificities were 78% and 92%, respectively. In more comprehensive and detailed studies, PET was not found to be a 100% reliable method (10,19). In the study published by Ebihara et al. (20), it was stated that besides detecting N1, PET could be superior for especially upper mediastinal lymph nodes. The accuracy of PET was also investigated in different series including high patient numbers and it was stated that PET was not significant in diagnosis, staging and prognosis evaluation in tumors smaller than 2 cm (9,10).

In the light of the statistical results we reached in our study, the sensitivity and specificity of CT and PET were compared in terms of MLN. The comparison of the SUV_{max} measured by the PET scan especially at no=7 station and number 4, 8 and 9 lymph node stations, was found statistically significant.

In some studies, FDG uptake in PET was found to be lower in adenocarcinoma, bronchoalveolary carcinoma and carcinoid tumors due to false negativity, whereas some did not support this (21,22). In our study, PET's sensitivity in MLN detection was found high for 48 patients diagnosed as having SCC. However, 45 patients diagnosed as having adenocarcinoma were found to have lower rates of detecting MLN (Table 1). When comparing

the two histopathological tumor types, the high difference between them in terms of PET sensitivity and specificity and accuracy rate was found statistically significant. A significant relationship was determined also between the high SUV_{max} of the mass and the SCC.

The most important problem in PET is that FDG uptake may increase in some benign pathologies other than tumor as well as malignant tumor tissue. Granulomatous lesions such as tuberculosis- seen frequently in our country- sarcoidosis, aspergillosis, coccidioidomycosis, histoplasmosis and reactive hyperplasias that may develop secondary to pneumonia can give misleading results between 16-55% as stated in the literature (23,24).

In our study, statistical analyses were made for the lymph nodes, besides, false positive results were obtained in 15 (10.6%) patients after histopathological examination for lung lesions also. Final diagnoses after surgical procedures were tuberculosis in 7 (46.6%) patients, suture granuloma in 3 (20%), Bronchiolitis obliterans organized pneumonia in 2 (13.3%), and aspergilloma in 1, sarcoidosis in 1, and sequestration in 1 (6.7%). SUV_{max} had a median value of 4.82 (2.5-15). When the tumor diameter (>2 cm) and the SUV_{max} of the tumor were compared, a significant relationship was determined.

Study Limitations

It is reported by some authors that PET is not sufficient to detect small-size metastatic lymph nodes (3,25). In our study, 14 (42.4%) of 33 patients with MLN detected in CT, were also confirmed pathologically. In 3 (21.4%) of the same 14 patients, there was no involvement in the MLN with PET. This result suggests that in patients with a lymph node detected with CT, it is necessary to verify it by using invasive methods even if PET is negative. In our study, relationship between central and peripheric tumor locations and PET accuracy was statistically significant. Similarly, a significant correlation was found between the SUV_{max} measured for mediastinal lymph nodes and central location. Likewise, the correlation of localization of the upper lobe with the SUV_{max} was also significant, but this significance was controversial due to the apparent numerical superiority of tumors localized to the upper lobe in our study.

Conclusion

The use of PET has an important place in the staging of intrathoracic malignancies today. Although the PET's efficacy is generally low in MLN staging compared to mediastinoscopy, the question of whether it may have the potential to replace mediastinoscopy in some specific patients based on our study with considering developing technology, is still open to debate.

As a result of our study;

PET seems to be sufficient in terms of MLN staging, especially in peripherally located squamous cell lung cancer.

The reliability of PET is low in adenocarcinoma, especially in those located centrally, so preoperative invasive staging should be done.

PET results only seem reliable at the subcarinal lymph node station (no=7), in cases of single-station positivity.

PET results are not reliable in lesions smaller than 1 cm. Clinical correlation should be considered as false negative results can be encountered.

Considering the rate of false negativity in patients with negative PET involvement who have mediastinal lymphadenopathy detected in thorax CT, it will be appropriate to perform invasive staging for mediasten in radiologically N2 patients.

In clinical evaluation, final results should be taken into consideration as it shows that the probability of N2 is higher in patients with N1 detected with PET compared to those with N0.

Ethics

Ethics Committee Approval: The ethical committee approval was obtained from Marmara University Medical Faculty, with approval number of 21.07.2006- B.30.2.MAR.0.01.00.02/ AEK-275

Informed Consent: An informed consent form was obtained from all participants.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: A.G.A., U.T., Concept: A.G.A., Design: A.G.A., U.T., Data Collection or Processing: A.G.A., U.T., Analysis or Interpretation: A.G.A., U.T., Literature Search: A.G.A., U.T., Writing: A.G.A., U.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5381
Bezmialem Science 2022;10(2):219-25

Impact of a Surface Sealant Application on the Color Stability of a Nano-hybrid Composite Resin

Yüzey Örtücü Uygulamasının Nano-hibrit Kompozit Rezinin Renk Stabilitesi Üzerindeki Etkisi

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ABSTRACT

Objective: Mouthwashes containing chlorhexidine can cause discoloration in restorative materials when used for the long-term. To prevent staining of a restorative material, several protective materials are used. The aim of this *in vitro* study was to evaluate the impact of a surface sealant on the color stability of a nano-hybrid composite resin material immersed in different mouthwashes.

Methods: A total of 42 composite resin discs (10x2 mm) were prepared with a nano-hybrid composite resin (Herculite XRV Ultra) using Teflon moulds. The composite specimens were first divided into two groups (with or without a surface sealant) then into three subgroups (n=7) according to the mouthwashes (Gengigel, Oderol, and Chlorhex). After SS application to 21 specimens, all the discs were subjected to baseline color measurements using a spectrophotometer according to the Commission Internationale de l'Eclairage L*a*b* system. The specimens underwent thermal cycling. Following immersion in different mouthwashes for 24 hours, the color measurements were repeated. The ΔE data were assessed using Kruskal-Wallis one-way ANOVA. Mann-Whitney U test was performed for different immersion mouthwashes ($p<0.05$).

Results: Statistically significant differences ($p<0.05$) were found between the unsealed composite specimens immersed in Chlorhex and the other mouthwashes in terms of ΔE values. The Chlorhex

ÖZ

Amaç: Klorheksidin içeren ağız gargaraları, uzun süreli kullanıldıklarında restoratif materyallerde renklenmelere neden olabilirler. Restorasyonların renklenmesini önlemek için çeşitli koruyucu materyaller kullanılmaktadır. Bu *in vitro* çalışmanın amacı, yüzey örtücü bir materyalin farklı ağız gargaralarında bekletilmiş bir nanohibrit kompozit rezinin renk stabilitesi üzerindeki etkisini değerlendirmektir.

Yöntemler: Bir nano hibrit kompozit rezin materyali (Herculite XRV Ultra) kullanılarak 42 adet disk şeklinde kompozit örnekleri (10x2 mm) hazırlandı. Örnekler önce iki gruba (yüzey örtücü uygulanan veya uygulanmayan) ardından daldırılan ağız gargaralarına (Gengigel, Oderol ve Chlorhex) göre üç alt gruba (n=7) ayrıldı. Yirmi bir kompozit örneğine yüzey örtücü uygulamasını takiben, tüm disklerin başlangıç renk ölçümleri bir spektrofotometre (Vita Easyshade) kullanılarak Uluslararası Aydınlatma Komisyonu L*a*b* sistemine göre yapıldı. Örneklere termal siklus uygulandı. Yirmi dört saat boyunca farklı ağız gargaralarında bekletilen örneklerin renk ölçümleri tekrarlandı. ΔE verileri Kruskal-Wallis tek yönlü one-way ANOVA kullanılarak değerlendirildi. Farklı gargaralar için Mann-Whitney U testi kullanıldı ($p<0,05$).

Bulgular: Yüzey örtücü uygulanmamış kompozit örneklerinde, Chlorhex ve diğer gargaralar arasında ΔE değerleri bakımından

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Cite this article as: Sarıalioğlu Güngör A, Dönmez N, Kahya DS, Hergüner Siso Ş. Impact of a Surface Sealant Application on the Color Stability of a Nano-hybrid Composite Resin. Bezmialem Science 2022;10(2):219-25

Received: 01.10.2020
Accepted: 10.01.2021

appeared to be associated with the most severe alteration in color (5.14±0.83), followed by Gengigel (2.21±1.46) and Oderol (1.94±1.75). There were no statistically significant differences (p>0.05) between the sealed and unsealed composite specimens in terms of the color changes.

Conclusion: The application of a low viscosity liquid surface sealant material did not show the expected effect on the color stability of a nano-hybrid composite resin in terms of three different mouthwashes.

Keywords: Color change, mouthwash, surface sealant, composite resin, thermal cycling

istatistiksel olarak anlamlı farklılıklar (p<0,05) bulundu. Chlorhex, en şiddetli renk değişikliğine neden olurken (5,14±0,83), Gengigel (2,21±1,46) ve Oderol (1,94±1,75) onu takip etti. Renk değişimleri açısından yüzey örtücü uygulanan ve uygulanmayan örnekler arasında istatistiksel olarak anlamlı bir fark (p>0,05) bulunmadı.

Sonuç: Düşük viskoziteli sıvı bir yüzey örtücü materyalin uygulanması, nano-hibrit bir kompozit rezinin renk stabilitesi üzerinde, üç farklı gargara açısından beklenen etkiyi göstermedi.

Anahtar Sözcükler: Renk değişimi, ağız gargarası, yüzey örtücü, kompozit rezin, termal döngü

Introduction

A coordinate interaction can occur between the ultimate aesthetic characteristics of dental restoration and the efficacy of the finishing and polishing strategies applied (1). The utilization of solvent polishing liquids with unique systems has been suggested alongside the immediate placement and finishing of resin-based restorative materials. Such an application has been recommended to overcome the various defects feasibly related to the physical characters of the materials as well as the potential faults of the operator (2). The postoperative sealing agents are generally described as rebonding/glazing/liquid-polishing agents or surface sealants (SSs) (2,3). These SSs are placed on the surface of the restoration and the neighbouring tooth structure, thereby boosting the marginal sealing, and also penetrating any surface micro defects and/or gaps formed during the material placement and polymerization (2,3).

The color and surface roughness of the teeth are critical qualities in terms of aesthetics when designing a smile (4). Since the development of composite materials in 1960, attempts have been made to boost the durability of composite restorations. However, as relatively little progress has been made to date, the visual characteristics of these materials still need to be upgraded (5). The color alteration experienced by composites is multifactorial, being related to the intrinsic staining and extrinsic discoloration of the material. The intrinsic factors are related to changes in the chemical balance of the resin matrix and the matrix/particle interface, while the extrinsic factors are associated with the consumption of coloring liquids from exogenous sources related to hygiene, nourishment, and smoking (6).

In recent decades, the number of individuals who use mouthwashes for anti-microbial control has grown, not only due to professional guidance but also due to the capability of such liquids to provide a cooling sensation and to diminish halitosis (7). Mouthwashes have diverse ingredients including alcohol, emulsifiers, organic acids, detergents, and dyes. It should be noted that composite resins exposed to ethanol display decreased microhardness values when compared with non-exposed materials (8). Alcohol appears to act as a plasticizer of the polymeric matrix, thereby making the resin material more ductile (9). Additionally, ethanol can reduce the adhesion between the resin matrix and inorganic fillers, which may increase the softening of the surface and leads to discoloration of the resin material (8).

Endeavors to quantitatively characterize the color alteration experienced by a tooth-colored restoration have been facilitated by the Commission Internationale de l'Eclairage (CIE), which has presented L*a*b* three-dimensional color estimation system whereby the L* axis assesses the luminosity or lightness (extending from 0 [black] to 100 [white]), the a* coordinate assesses the redness (a>0) or greenness (a<0), whereas the b* coordinate assesses the yellowness (b>0) or blueness (b<0) (10,11). The quantitative estimation of the entire alteration in color (ΔE) hence consolidates a unique equation for a qualitative application relating to both discernment and acceptance limits (10,11).

In dental practice, mouthwashes may need to be used for a long time if the patient is to benefit from both their wound-healing properties and their antibacterial properties. However, chlorhexidine (CHX) can result in a permanent color change following long-term use. Besides, reversible local side effects such as discoloration of teeth, and the tongue, impaired taste sensation (12), increased formation of supragingival calculus and mucous membrane irritation, and desquamation (13) are associated with the use of CHX mouth rinse in long-term use. Oderol mouthwash contains zinc lactate and CHX, although the manufacturer claims that it does not cause discoloration after long-term use. Gengigel, which is a hyaluronic acid mouthwash, is marketed offering a wound-healing potential. To the best of our knowledge, no studies have yet examined the discoloration associated with both Oderol and Gengigel in composite restorations following long-term use.

The first aim of this study was to evaluate the long-term use of three different types of mouthwash on the color stability of composite restorations. The second aim of this study was to assess the effect of SS application on the coloration resistance of a nanohybrid resin composite soaked in different types of mouthwash. The null hypothesis was that the application of a SS would not be able to protect the color stability of a nano-hybrid composite restoration after using different mouthwashes in the long-term.

Method

Sample Preparation

Forty-two disc-shaped specimens (10.0 mm inner diameter x2.0 mm thickness) were prepared in Teflon moulds utilizing the Herculite XRV Ultra (Kerr Corporation, Orange, CA, USA) shade A2 nanohybrid composite material. Each material was covered with transparent polyethylene matrix strips (Kerr Hawe Striproll, Kerr Corporation, Orange, CA, USA) on each side to ensure a uniform finish. The composite resins were compressed between two microscopic glass slides to expel any excess material. Photopolymerization was performed for 20 seconds using a VALO Cordless (Ultradent Products Inc., South Jordan, USA) light-emitting diode (LED) device with an irradiance of 1,800 mW/cm^2. The directly lighted surfaces were progressively polished using aluminium oxide polishing discs (Sof-Lex, 3M ESPE, St Paul, MN, USA). A single operator performed the polishing process with a low-speed handpiece at around 4,000-5,000 rpm. Each disc was used on a surface for 15 s. Then, the polished surfaces were water-rinsed for 60 s to expel any surface debris. Afterward, they were air-dried for 30 s. The thickness of each sample was checked using a digital calliper (Yamer, İzmir, Turkey). To guarantee the absence of any deformities, the surfaces were inspected under a metallographic microscope (ME 600 Eclipse, Nikon-Kogaku, Tokyo, Japan). Then, the specimens were submerged in artificial saliva (14) (1 L aqua, 0.1 g H_3BO_3, 2.4 g KCl, 1.7 g NaCl, 0.1 g $MgCl_2 \cdot 6H_2O$, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.2 g KSCN, 0.7 g KH_2PO4) and stored in an incubator (Memmert GmbH, Schwabach, Germany) at a temperature of 37±1 °C for 24 h to provide complete polymerization.

Twenty-one of the specimens were etched with 37% phosphoric acid (Etchant Gel; Prime Dent, Chicago, IL, USA) for 15 s, then washed with distilled water and air-dried. Later, the BisCover LV SS was applied directly using a micro brush for 15 s. The top surfaces of the specimens were tenderly air-thinned for 3 s and then light-cured for 30 s, as recommended in the manufacturer's instructions, using a LED light unit. The other twenty-one specimens remained unsealed. The compositions of all the materials used in this study were detailed in Table 1. The specimens were stored in artificial saliva at 37±1 °C for 24 h to provide complete polymerization of the SS. At that point, all the specimens were subjected to ageing through 1,500 thermal cycles (SD Mechatronik GmbH, Feldkirchen-Westerham, Germany) at temperatures rotating between 5 °C and 55 °C with a stay time of 30 s in each water bath (15).

After the thermocycling, the specimens were divided into three groups (n=14) based on the mouthwash in which they would be immersed (Table 1): Group 1 - Gengigel, Group 2 - Oderol, and Group 3 - Chlorhex. The specimens were stored in 20 mL of each mouthwash for 24 h, which was equivalent to 2 min/day of mouthwash use for two years (16). The specimens within the mouthwashes were kept at 37 °C throughout the experiment, and they were shaken every 3 h to ensure their homogeneity (17). At the end of the experiment, the specimens were evacuated and then submerged in distilled water.

Color Measurements

Following the application of the surface sealant to 21 composite specimens, the baseline color measurement of all the specimens was performed under a white background (Gardner Laboratory Inc., Bethesda, MD, USA) using a spectrophotometer (Vita Easyshade, Vita Zahnfabrik, Bad Säckingen, Germany). This device is specifically designed for colour measurement. Before the measurement, the top surface of each sample was dried using tissue paper and the contact guide of the spectrophotometer was situated at the center of the sample's surface. Three successive readings were taken for each sample and then averaged.

After thermal cycling and immersion of all 42 composite specimens in the mouthwashes for 24 h, the color measurements were repeated as detailed above. Based on the L*, a*, and b* values, the overall color change (ΔE) was calculated using the following equation:

$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (18).

ΔE values ≥3.3 were considered to be clinically improper (19).

Statistical Analysis

The distribution of the data was determined using the Shapiro-Wilk test.

Table 1. Materials used in the study

Material	Batch no.	Composition	Manufacturer
Herculite XRV Ultra (A2) (Nano-hybrid composite resin)	7069792	Bis-GMA, TEGDMA and Bis-EMA	Kerr, Orange, CA, USA
BisCover™ LV (Low viscosity liquid polish)	1400001759	20-50% Bis-EMA, 20-40% urethane acrylate ester, and 20-40% polyethylene glycol	BISCO Inc. Schaumburg, IL, USA
Gengigel (Hyaluronic acid mouthwash)	1912100	Hyaluronic acid (0.025%), sodium fluoride (0.02%), xylitol and excipients	Ricer Farma, Milan, Italy
Oderol (Zinc lactate mouthwash)	1943201	Zinc lactate (0.014 mg) and chlorhexidine gluconate (0.025 mg)	Helba, Bahçelievler, Turkey
Chlorhex (Chlorhexidine gluconate mouthwash)	19LO96211	0.2% chlorhexidine gluconate, propylene glycol and glycerine	Drogsan, Ankara, Turkey

Due to the non-normal distribution of the data, the differences in the color changes (ΔL*, Δa*, Δb* and ΔE) between the different mouthwash groups were analyzed using a Kruskal-Wallis one-way ANOVA. To assess the differences between the sealed and unsealed groups, a Mann-Whitney U test was performed. Data were submitted for statistical analysis at the p=0.05 level of significance. The statistical calculations were performed using SPSS for Windows 24.0 (SPSS Inc., Chicago, IL, USA).

Results

The mean, standard deviation, and median (minimum-maximum) of the ΔE, as well as of the ΔL, Δa, and Δb values, for the sealed and unsealed groups following immersion in different mouthwashes, are presented in Tables 2 and 3.

Statistically significant differences were found between ΔE values of the specimens without surface sealant but immersed in Chlorhex and the other mouthwashes tested. Chlorhex showed a greater degree of color alteration ($p<0.05$), followed by Gengigel and Oderol (Table 2).

Regarding the SS, statistically significant differences ($p<0.05$) were found between the groups in relation to the Δa values (sealed and unsealed), except for Oderol and Chlorhex groups (Table 3).

Concerning the tested mouthwash solutions, no statistically significant differences were found between the groups ($p>0.05$) (in relation to the ΔL, Δa, and Δb values), except for the ΔL values in the Chlorhex group ($p<0.05$) (Table 3).

Discussion

The null hypothesis was partially accepted in this study. Although there was a considerable increase in ΔE values after the SS application (except for Chlorhex group), no statistically significant difference in color was observed between the sealed and unsealed composite specimens ($p>0.05$). The preservation of the color throughout the functional period of dental restorations is one of the foremost aesthetic characteristic considerations concerning composite materials. However, this characteristic is not consistent among the different available restorative materials. In our study, we used a nano-hybrid composite material that could be used both in the anterior and posterior restorations, due to the superior polishability, better optical and aesthetic properties, and less polymerization shrinkage (20). Chairside polishing of composite resins with sealant agents should provide improved stain resistance. However, the success and longevity of these sealant agents on composite resin restorations are still unknown. Since one of our purposes in the study was to evaluate the effect of a surface sealant on the color stability, we preferred to choose only one resin composite material.

Table 2. ΔE median (min-max) and mean ± standard deviation of the experimental groups

	Without sealant		With sealant		p
	Mean ± SD	Median (min/max)	Mean ± SD	Median (min/max)	
Gengigel	2.21±1.46^{aA}	2.03 (0.7/4.5)	3.09±0.65^{aA}	2.9 (2.3/4.42)	0.445
Oderol	1.94±1.75^{aA}	1.58 (0.57/5.67)	3.28±0.66^{aA}	3.5 (1.9/3.9)	0.051
Chlorhex	5.14±0.83^{bA}	5.22 (4.27/6.73)	4.02±1.71^{aA}	3.6 (0.7/6.72)	0.234
p	0.005		0.815		

The different lowercase letters in the columns indicate statistically significant differences between the mouthwash groups ($p<0.05$), while the uppercase letters in the lines indicate statistically significant differences between the sealed and unsealed groups ($p<0.05$).
min: Minimum, max: Maximum, SD: Standard deviation

Table 3. ΔL, Δa, Δb median (min-max) and mean ± standard deviation of the experimental groups

		Without sealant	With sealant	p
		Median (min/max)	Median (min/max)	
Gengigel	ΔL	-0.7 (-0.4/0.1)^{Aa}	-2.9 (-4.4/-2.3)^{Ba}	0.016
	Δa	0.3 (-0.1/0.4)^{Aa}	-0.1 (-0.3/0.1)^{Ba}	0.005
	Δb	-0.8 (-3/-0.6)^{Aa}	0 (-0.7/0.3)^{Ba}	0.002
Oderol	ΔL	0.3 (-5.6/1)^{Aa}	-3.4 (-3.9/-1.9)^{Ba}	0.024
	Δa	0.1 (-1/0.5)^{Aa}	0 (-0.1/0.2)^{Aa}	0.748
	Δb	-0.9 (-2.3/0.2)^{Aa}	0.2 (-0.1/1.2)^{Ba}	0.004
Chlorhex	ΔL	-4.9 (-6.7/-4.2)^{Ab}	0.3 (-3.6/6.7)^{Bb}	0.001
	Δa	0.1 (-0.2/0.2)^{Aa}	-0.2 (-0.4/0.2)^{Aa}	0.086
	Δb	-0.8(-2.1/-0.2)^{Aa}	0 (-0.9/0.6)^{Ba}	0.035
p		<0.05	<0.05	

The different lowercase letters in the columns indicate statistically significant differences between the mouthwash groups ($p<0.05$), while the uppercase letters in the lines indicate statistically significant differences between the sealed and unsealed groups ($p<0.05$).
min: Minimum, max: Maximum

In most prior studies, a spectrophotometer was used in accordance with the CIE's L*a*b* framework to measure the ΔE values (21,22). This electronic device uses a normal light in a dark component, separated from the surrounding light, to measure the color and, thus, the results are convincing, definitive, and quotable. The ΔE value of composite resins that is greater than 3.3 indicates a clinically critical and unacceptable color change (18).

The color stability of aesthetic composite restorations is influenced by several extrinsic and intrinsic variables. Extrinsic discoloration results from factors such as poor oral hygiene, dietary, and smoking patterns. The use of mouthwash is another extrinsic variable that influences the color stability of aesthetic composite restorations. Yet, the use of mouthwash is also known to help control caries and periodontal illnesses (23,24). Therefore, in our study, we evaluated the staining effect of two different mouthwashes with very high periodontal effectiveness, containing different concentrations of CHX gluconate as well as a mouthwash containing hyaluronic acid.

In the present study, all mouthwashes caused a color alteration on the composite resins. The Chlorhex with a ΔE value of 5.22 was found as the mouthwash that caused the greatest degree of color alteration among all the tested mouthwashes (Table 2). This high value has been characterized as unacceptable for *in vitro* conditions (25). The absorption of the alcohol particles contained in mouthwashes into the resin matrix may result in the softening of the composite resin's surface and so contribute to its discoloration, which may explain the outcomes observed for Chlorhex (26). One of the mouthwashes tested in this study was Oderol. It incorporates zinc, which is an essential element in plaque, saliva, and enamel. Zinc is added to oral healthcare products to control plaque and to prevent calculus formation. In the presence of plaque and saliva, its permanence may persist for hours in the oral environment (27). According to our findings, the specimens immersed in Oderol, which contained a lower amount of CHX gluconate (0.025 mg) than Chlorhex, did not show unacceptable levels of color change. This acceptable color change may be due to the concentration of CHX gluconate mouthwash ranging from 0.2% to 0.12% (28).

The mechanism of staining by CHX gluconate is not known exactly. In the literature, three possible mechanisms were reported (29). 1) CHX may increase the non-enzymatic browning reaction of protein and carbohydrate in the acquired pellicle; 2) CHX denatures components within the dental pellicle, accelerate the formation of pigmented sulfides of iron; 3) CHX precipitates dietary chromagens. According to our results, Chlorhex with a higher concentration of CHX gluconate caused the most severe color alteration of all mouthwashes. The use of CHX gluconate mouthrinse has been reported to cause staining in earlier studies (28,30,31). From this perspective, this study carries confirmatory value.

The use of an SS, which comprises BIS-GMA, UDMA, and TEG-DMA without filler particles, may assist with removing or minimizing the color variations seen in relation to composite resins. Such materials are characterized by low thickness and high usefulness (32). The use of a SS serves to fill any microstructural defects of the surface and so decreases the surface roughness and the presence of bubbles. Different opinions exist as to the SS, which can alter the surface properties, color stability, marginal microleakage, and optical properties of composite resins (33,34).

There are contradictions among the results of previous studies in this area (24,33,35). According to Doray et al. (33), SS can promote the resistance of temporary composite resin immersed in red wine, coffee, and cranberry juice to staining. However, Soares et al. (35) applied a reflectance analysis and showed that the coffee-related surface coloring was higher in the groups that applied SS. Similar to the results of the present study, Lee and Powers (24) stated that the color alterations seen in the sealed group subjected to staining by CHX, tea, and mucin were not notably different when compared to those seen in the unsealed group. Differences in the chemistry, polymerization approaches, and thicknesses of the sealants may impact their sensitivities to coloring. Regarding the current study, an unexpected phenomenon occurred after the polymerization of SSs on the surfaces of the composite samples. Although the initial appearance was a glossy surface, air bubbles formed in the sealant material during the application with a brush, might provide a suitable environment for the discoloration of the material, as they created irregular areas on the restoration surface after polymerization. The variations between the ΔE values of the tested specimens in the present study may also be attributed to the long-term immersion in the mouthwashes, which destroys the integrity of the SS layer. This destroying could lead to microcrack formations and the removal of nonadherent surface particles. The increased thickness of the sealant agent may be responsible for the various degrees of discoloration and deterioration of the surface sealant (24). Based on these, it can be said that the SS application may have no positive effect (33).

The discoloration of composite resins can be considered in relation to their degree of water absorption and the hydrophilicity of the resin matrix (36). If the resin matrix can retain water, it is also able to retain any other liquid, which eventually leads to discoloration. Extraordinary water absorption induces the extension and plasticization of the composite, as well as the hydrolysis of the silane, which in turn forms microcracks and so leads to the decreased durability of the composite restoration (37). Indeed, microcracks or interfacial gaps at the intersection between the filler and the matrix permit stain infiltration and so facilitate discoloration (23,37).

In the formulation of Herculite XRV Ultra, TEGDMA was included to decrease the viscosity of the Bis-GMA resin. While the TEGDMA was included to enhance the handling features, it also resulted in water sorption ability (37). In fact, the hydrophilic characteristics of the TEGDMA lead to extraordinary water sorption, which prompts the extension and plasticizing of the Bis-GMA resin, and causes a reduction in the longevity of the composite resin. It has been reported that hydrophilic materials show a high degree of water absorption and, they become more colored with staining agents than hydrophobic materials (38).

Although Herculite XRV Ultra, a nano-hybrid composite resin, was expected to show less discoloration due to its small particle size, it showed a high degree of discoloration when immersed in mouthwashes. This can be attributed to the structure of the resin matrix (UDMA, TEGDMA, Bis-EMA) and the different sizes of the filler particles.

The results of this study showed that the color changes on composite specimens exposed to different types of mouthwash for 24 hours were not clinically acceptable (ΔE>3.3). Regarding the color parameters (L*a*b*), the results indicated that the ΔL values were negative, which showed that the composite samples became darker in all groups (except for the Chlorhex group). It was reported that color change for dimethacrylate based composites was mostly caused by ΔL* and Δb* (39). In terms of a* axis, the colors of the composite samples immersed in mouthwashes shifted from red towards green after SS application. In relation to the b* axis, the colors of the nanohybrid composite samples shifted from blue towards yellow after SS application (Table 3). Yellowing was most likely due to a reaction involving the many unreacted methacrylate groups (approximately 40%) present within the composite resin. The visual yellowing of BIS-GMA itself during UV illumination in the presence of oxygen implies that some chemical alteration of the resin itself may be one of the principal causes of the yellowing in dental composites (40).

Study Limitations

It is important to recognize the methodological limitations of *in vitro* studies designed to determine color stability. To accelerate the ageing progress, *in vitro* studies aim to simulate the impacts of long-term exposure in an oral environmental. Within the oral environment, the saliva and other liquids may dilute the stains. The first limitation of this study was not keeping the samples in artificial saliva as in the oral environment and not immersing them for a long time. Already, exposure of dental structures and restorative materials to the coloring agents in the oral environment is not continuous and the discoloration can be accelerated by mechanical wear. The second limitation of this study was that mechanical wear was not imitated. Besides, choosing only one composite resin and one surface sealant, and the lack of brushing effect with or without a toothpaste were the other limitations of the study.

Conclusion

Within the limitations of this study, it could be concluded that long-term use of mouthwash causes discoloration on a composite resin restoration. Besides, the application of a low viscosity liquid surface sealant material did not show the excepted effect on the color stability of a nano-hybrid composite resin in terms of three different mouthwashes.

Ethics

Ethics Committee Approval: Ethics committee approval is not required since our study was conducted with composite resin samples and no patient or dental samples were used.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: A.S.G., N.D., Ş.H.S., Design: A.S.G., N.D., Data Collection or Processing: A.S.G., D.S.K., Analysis or Interpretation: A.S.G., N.D., Literature Search: A.S.G., N.D., D.S.K., Ş.H.S., Writing: A.S.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.5363
Bezmialem Science 2022;10(2):226-30

Screening of *mecC* Gene in Methicillin Resistant *Staphylococcus aureus* Isolates

Metisiline Dirençli *Staphylococcus aureus* İzolatlarında *mecC* Geni Taraması

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ABSTRACT

Objective: The diagnosis and treatment of *mecC* positive methicillin resistant *Staphylococcus aureus* (MRSA) isolates pose a significant problem in clinical microbiology and infectious disease practices. The studies on the frequency of *mecC* positive isolates in Turkey is rather scarce. In the present study, we aimed to investigate the presence of *mecA*, *mecC*, *spa* and *pvu* genes in MRSA strains isolated from various clinical specimens submitted to Clinical Microbiology Laboratories of Bezmialem Vakıf Hospital.

Methods: We performed nucleic acid extraction and multiplex polymerase chain reaction (PCR) to 126 MRSA strains to detect *mecC*, *mecA*, *spa* and *pvl* genes.

Results: According to the multiplex PCR results of 126 MRSA strains studied, 126 (100%) had *mecA*, 107 (85%) had *spa*, and 3 (2%) had *pvl* genes. We performed another polymerase chain reaction protocol and *spa* genes were identified in 19 of specimens, which were found negative priorly.

Conclusion: Considering the factors that a university medical center where the study was conducted provided a tertiary healthcare service to a large metropolitan area in Istanbul and none of the isolates carried *mecC* gene might indicate that *mecC* gene carrying MRSA isolates did not pose a significant public health threat in Turkey.

Keywords: *Staphylococcus aureus*, MRSA, *mecA*, *mecC*, *mec*ALGA251, *spa*

ÖZ

Amaç: *mecC* pozitif metisiline dirençli *Staphylococcus aureus* (MRSA) izolatları hem tanı hem de tedavide önemli problemlere yol açan patojenler olarak değerlendirilmektedir. Türkiye'de *mecC* pozitif izolatlar üzerine yapılan çalışmalar nispeten sınırlı kalmıştır. Bu çalışmanın amacı Bezmialem Vakıf Hastanesi'ne teslim edilen klinik örneklerden izole edilip arşivlenen MRSA suşlarında *mecA*, *mecC*, *spa* ve *pvl* genlerinin varlığını araştırarak ülkemizde *mecC* geni taşıyan MRSA izolatları ile ne sıklıkta karşılaşıldığı konusunda bilgi edinmektir.

Yöntemler: Yüz yirmi altı adet MRSA suşuna nükleik asit ekstraksiyonu ve *mecC*, *mecA*, *spa* ve *pvl* genlerinin tespit edilmesine yönelik multipleks polimeraz zincir reaksiyonu (PZR) işlemi uygulanmıştır.

Bulgular: Çalışılan 126 MRSA suşunun multiplex PZR sonuçlarına göre 126'sında (%100) *mecA*, 107'sinde (%85) *spa*, 3'ünde (%2) *pvl* geni tespit edilmiştir. *Spa* geni tespit edilemeyen 19 suşa faklı bir yöntemle tekrar PZR yapılarak bu suşlarda *spa* genleri tespit edilebilmiştir.

Sonuç: Geniş metropolitan bir yerleşim yerine sağlık hizmeti sunan, üçüncü seviye servis ve eğitim hastanesinde izole edilen 126 MRSA suşunun tamamında *mecA* geni tespit edilirken hiçbir suşta *mecC* genine rastlanmamıştır. Bu sonuçlar, ülkemizde *mecC* geni taşıyan MRSA izolatlarının ciddi halk sağlığı problemi oluşturmadığı yönündeki değerlendirmeleri desteklemektedir.

Anahtar Sözcükler: *Staphylococcus aureus*, MRSA, *mecA*, *mecC*, *mec*ALGA251, *spa*

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Received: 11.12.2020
Accepted: 15.07.2021

Cite this article as: Ceylan AN, Sümbül B, Doymaz MZ. Screening of *mecC* Gene in Methicillin Resistant *Staphylococcus aureus* Isolates. Bezmialem Science 2022;10(2):226-30

Introduction

In the 2014 report of the World Health Organization, it was reported that when compared to methicillin susceptible *Staphylococcus aureus* (MSSA), infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) were associated with a significantly higher risk of septic shock, all-cause mortality and bacterial-related mortality, and that they were associated with a longer hospital and intensive care unit stay (1). Considering these data, it is very important to detect the presence of MRSA quickly and accurately in patients.

In clinical practice, MRSA strains are considered resistant to all beta-lactam antibiotics (2). This is because MRSAs produce penicillin-binding proteins (PBP2a') that bind with lower affinity to beta-lactams. PBP2a's are encoded by the *mecA* gene located on the staphylococcal chromosome cassette (*SCCmec*) genetic region (3,4).

The presence of MRSA is detected by phenotypic and genotypic methods in clinical microbiology laboratories. The most common method is to look at the susceptibility of oxacillin/cefoxitin. With this method, the presence of MRSA can be demonstrated the next day. Genotypic methods are used in cases where phenotypic methods are not conclusive (eg, limit values in disc diffusion) or when rapid results are required (5). PCR tests to show the presence of the *mecA* gene in bacteria can give results in hours, and quick card tests can give results in minutes.

In 2011, isolation of *S. aureus* (LGA251) strains isolated from milk and different clinical specimens, which were phenotypically resistant to methicillin but could not be detected by tests for the *mecA* gene, and which were determined to have a different mecA gene, was reported for the first time (5). This different *mecA* gene ($mecA_{LGA251}$) was later named the *mecC* gene (6). These studies have shown that MRSA strains with the *mecC* gene can be overlooked in clinical practice. After this date, similar studies have been carried out from different countries and MRSA strains carrying *mecC* have been reported from many European countries (7).

Studies on the presence of *mecC* in our country have been relatively limited. It is clear that the presence and frequency of MRSA isolates carrying the *mecC* gene is also important in the development of public health policies, as it affects the treatment. Bezmialem Vakıf Hospital as a university hospital providing tertiary healthcare with a central location in Istanbul, accepts patients from all over Turkey. Therefore, it would not be wrong to assume that the data obtained from this hospital will also include some country-wide clues.

In this study, the presence of *mecC* was investigated in MRSA strains isolated and archived in the cultures of various clinical samples in the microbiology laboratory between November 2014 and March 2018 in order to identify MRSA isolates carrying the *mecC* gene in our hospital and to obtain information on the distribution of similar isolates throughout the country.

Methods

Bacterial Isolates and Antibiotic Susceptibility Tests

One hundred and twenty six consecutive MRSA strains isolated and stocked from various clinical samples of patients admitted to the outpatient clinics of Bezmialem Vakıf Hospital and hospitalized between November 2014 and March 2018 were included in the study. These strains were not previously passaged for any reason.

Antibiotic susceptibility of bacteria was determined with the Vitek-2 automated system (bioMerieux, France) in accordance with the manufacturer's recommendations.

DNA Isolation

The MRSA strains in the stocks were passaged on sheep blood agar and incubated overnight. After the incubation, 4-5 colonies were taken from the cultures that grew and placed in 1 mL saline filled Eppendorf tubes and thoroughly mixed with vortex. Bacterial suspensions were incubated at 95 °C for 5 minutes in a heat block. Afterwards, the eppendorfs were centrifuged at 3,000 rpm for 1 minute, and the supernatants were separated to be used in the PCR process and stored at -20 °C.

Detection of *mecC, mecA, spa* and *pvl* Genes

According to the protocol published by the European Union Reference Laboratories National Food Institute in 2012, the primer mix used for the detection of *mecC, mecA, spa* and *pvl* genes was prepared and multiplex PCR was performed according to this protocol (8). PCR conditions were set at 94 °C for 5 minutes, 30 cycles (94 °C 30 seconds, 59 °C 1 minute, 72 °C 1 minute), and 72 °C for 10 minutes. *S. aureus* ATCC 43300 for *mecA* and *S. aureus* NCTC 13552 for *mecC* were used as positive controls.

The PCR for *spa* detection was performed according to the protocol published by Votintseva et al. (9) in 2014 for the strains of which *spa* gene could not be detected.

Results

According to the results of multiplex PCR of 126 MRSA strains studied, *mecA* gene was detected in 126 (100%), *spa* gene in 107 (85%) and *pvl* gene in 3 (2%), and *mecC* gene was not detected in any isolate. An example gel image is shown in Figure 1.

The PCR was applied to 19 strains in which the *spa* gene could not be detected, with a different method for *spa* detection, and *spa* gene could be detected in all 19 isolates (Figure 2) (9).

According to the historical VITEK 2 minimum inhibitory concentration results, all isolates were resistant to cefoxitin and oxacillin, and susceptible to vancomycin and linezolid. Inducible clindamycin resistance was reported in 24 isolates. The susceptibility results of some antibiotics are given in Table 1.

Discussion

The MRSA is one of the most important causes of community and hospital-acquired infections. MRSA outcomes reported from intensive care units are increasing day by day. MRSA is

Table 1.

	The number of susceptible strains	Susceptibility results of MRSA strains for some antibiotics	The number of resistant strains	The percentage of susceptibility
Levofloxacin	32	2	12	70%
Erythromycin	22	53	36	21%
Clindamycin	78		40	66%
Tetracycline	52		66	45%
Trimethoprim/sulphamethoxazole	99		19	84%

MRSA: Methicillin resistant Staphylococcus aureus

an important pathogen due to its multi-antibiotic resistance. In addition to being resistant to all beta lactam antibiotics, it also shows resistance to lincosamide, aminoglycoside and macrolides (10). It is very important to detect MRSA in the laboratory in order to select an appropriate and effective antibiotic.

The PCR to detect the mecA gene in bacteria and rapid agglutination tests for the presence of PBP2a may miss the presence of mecC (5). Although mecC has not yet been detected in human samples in our country, it has been detected in many European countries. Since the number of studies conducted in our country is very small, it can be said that isolates carrying mecC have not yet been detected in human samples in our country. In this case, it may be recommended to carry out studies looking for MRSA isolates with the mecC gene at regular intervals, to continue the scans, and to carry out studies with a large number of samples and necessary arrangements in order not to miss the possible mecC gene presence in the laboratory diagnosis of MRSA (7). Our study exemplifies such a survey.

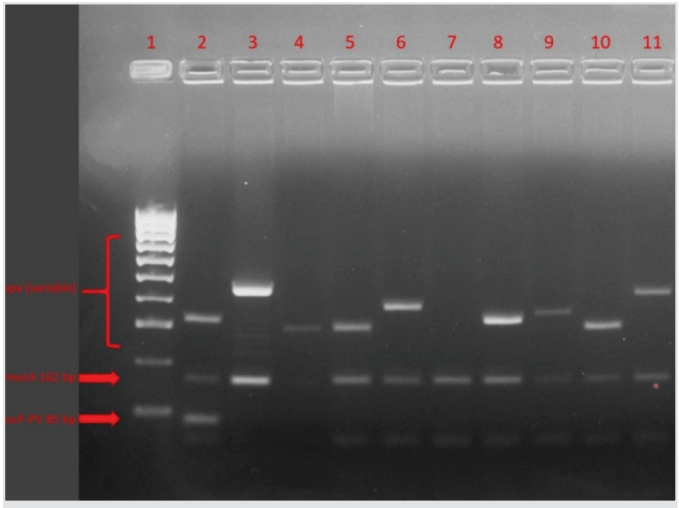

Figure 1. Multiplex PCR gel image
Row1: 100 bp marker.
Row 2: MRSA positive strains for *spa*, *mecA(162bp)* and *pvl(85 bp)* genes from top to bottom.
Row 7: One of the MRSA strains in which the spa gene could not be detected.
Rows 3-6, 8-11: MRSA strains found positive for the spa and mecA genes.
PCR: Polymerase chain reaction, MRSA: Methicillin resistant Staphylococcus aureus

In a study published in 2013, 896 *S. aureus* strains were studied with the VITEK 2 susceptibility system and it was aimed to detect MRSA carrying *mecC*. Of the 455 MRSA *mecA* positive strains, 98% were resistant to oxacillin and cefoxitin, 0.9% were susceptible to cefoxitin, while 1.1% were resistant to oxacillin and susceptible to cefoxitin, and no *mecA* MRSA strains were found to be susceptible to cefoxitin and oxacillin. Of the 62 *mecC* positive MRSA strains, 88.7% were susceptible to oxacillin, resistant to cefoxitin, and 11.3% were found to be resistant to both antibiotics, and similarly, no *mecC* MRSA strains were found to be susceptible to cefoxitin and oxacillin. Of 379 methicillin-susceptible strains (*mecA* and *mecC* negative), 98.8% were found to be sensitive to both antibiotics, while 1.1% were resistant to oxacillin, but sensitive to cefoxitin (11). All 126 MRSA strains in our study were found to have *mecA* gene and according to VITEK 2 results, none of the strains were sensitive to oxacillin or cefoxitin. VITEK 2 antibiotic susceptibility system is a frequently used method in our country. Work needs to be done with other commercial systems as well.

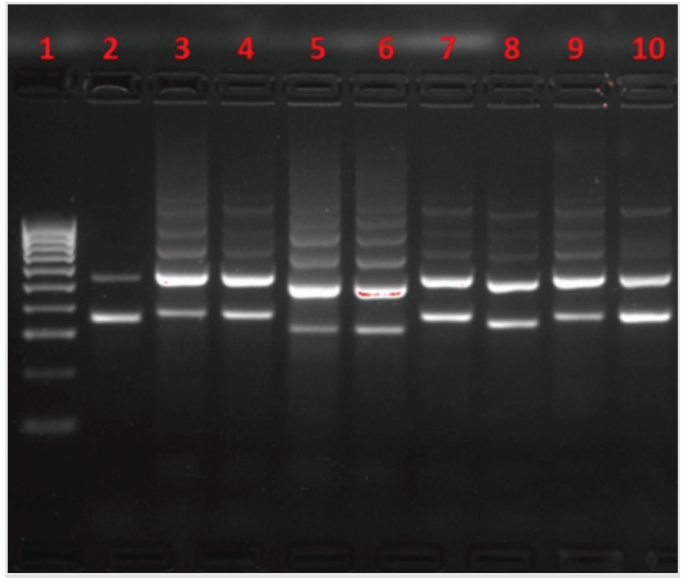

Figure 2. PCR gel image of MRSA strains that were previously found not to have *spa* gene, by using multiplex PCR with new spa primers
Row 1: 100 bp marker
Rows 2-10: *spa* gene regions
PCR: Polymerase chain reaction, MRSA: Methicillin resistant Staphylococcus aureus

Panton-Valentine leukocidin, a cytotoxin known to be secreted from community-acquired *S. aureus*, which causes skin and soft tissue infections and pneumonia, is an important virulence factor (12). Detection of *pvl* gene in a MRSA strain suggests that that strain may be a community-acquired MRSA (13). Data on the origin of the MRSA strains examined in this study were not collected. Community-acquired MRSA strains are often susceptible to clindamycin, tetracycline, trimethoprim-sulfamethoxazole gentamicin, fluoroquinolones, and chloramphenicol, unlike hospital-acquired strains (10). The two MRSA strains we found to have *pvl* gene were also susceptible to these antibiotics. The result of antibiotic susceptibility and *pvl* positivity suggest that these strains may be of community origin.

One of the many virulence factors of *S. aureus* is staphylococcal protein A (*spa*). This protein binds to immunoglobulin (Ig)G and protects the bacteria from phagocytosis. Changes in the IgG binding region of the *spa* gene may cause the *spa* gene not to be detected by classical methods (9). In the multiplex PCR we performed in our study, we could not detect the *spa* gene, although it was repeated twice in 19 of 126 samples. Thereupon, we performed PCR again for the *spa* gene on these 19 isolates according to the protocol published by Votintseva et al. (9) in 2014, and we were able to detect the *spa* gene in all strains (Figure 2).

In many studies conducted in different countries around the world, the presence of *mecC* gene was investigated in *S. aureus* growing from human and animal samples, while *mecC* was not found in some of them (5,7,14). In our country, studies have begun to detect the presence of *mecC* gene in MRSA strains. In the study of Kılıç et al. (15) in 2015, the *mecC* gene was investigated in 1,177 MSSA and 523 MRSA strains isolated from humans, and no *mecC* gene was found in any of the strains. In the study conducted by Cikman et al. (16) in 2018, the presences of *mecA* and *mecC* genes were investigated in 494 MRSA strains isolated from humans, and *mecA* was detected in 315 of them by using PCR, but the presence of *mecC* was not found. In the study conducted by Sayın et al. (17) on cows with mastitis, the presences of *mecA* and *mecC* genes were investigated, and the *mecA* gene was found in 21 of 150 strains and *mecC* gene was found in 7 of 150 strains. In this study, in addition to the previous studies, we discussed MRSA strains from a different region. While we detected *mecA* gene in 126 of 126 MRSA strains isolated from humans in our study, we did not find *mecC* gene.

Studies show that especially molecular methods that look for *mecA* gene in MRSA detection and rapid tests for PBP2a' may miss MRSA due to *mecC* gene, and these methods should be revised to catch *mecC* gene (5). Therefore, laboratories should review the methods by which they detect MRSA to make sure that they do not miss a possible *mecC* positive MRSA with quality control strains. Currently, the number of studies conducted in our country to detect the presence of *mecC* gene in humans and animals is limited. With these data, it would not be correct to say that *mecC* gene was not shown in human samples. In order to show the *mecC* gene status in our country, it is necessary to conduct studies with larger sample numbers from different locations of the country.

Study Limitations

In our study, since the hospital data of the MRSA strains in the stock could not be reached, from which patient samples it was reproduced or outpatient/inpatient interpretation could not be made.

Conclusion

While the *mecA* gene was detected in all of the 126 MRSA strains studied, no *mecC* gene was found in any of the strains. These data support the conclusion that MRSA isolates carrying the *mecC* gene have not yet emerged as an important public health problem in our country.

Ethics

Ethics Committee Approval: Approval was obtained from the Bezmialem Vakıf University Non-Invasive Clinical Research Ethics Committee (02.02.2021/2011-KAEK-42).

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: A.N.C., B.S., M.Z.D., Concept: A.N.C., B.S., M.Z.D., Design: A.N.C., B.S., M.Z.D., Data Collection or Processing: A.N.C., B.S., M.Z.D., Analysis or Interpretation: A.N.C., B.S., M.Z.D., Literature Search: A.N.C., B.S., M.Z.D., Writing: A.N.C., B.S., M.Z.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

DOI: 10.14235/bas.galenos.2021.6570
Bezmialem Science 2022;10(2):231-7

Safety of Cefazolin in Prophylaxis in B-Lactam Allergic Children

Betalaktam Alerjisi Olan Çocuklarda Sefazolin Profilaksisi Güvenliği

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ABSTRACT

Objective: Cefazolin is a first-generation cephalosporin, particularly effective against gram positive agents such as staphylococci and streptococci. Despite this narrow spectrum, it is important in surgical prophylaxis. In this study, we aimed to evaluate the tolerability of cefazolin in patients with confirmed β-lactam allergy.

Methods: Patients who underwent cefazolin allergic work-up due to suspected cefazolin allergy (Group 1) and children with confirmed β-lactam allergy other than cefazolin (Group 2) were included in the study as two groups. β-lactam allergy was confirmed by determining positivity of skin test and/or provocation test with culprit drug and penicillin. Cefazolin skin test was performed in all patients. The provocation test was also applied to all patients with negative skin tests.

Results: A total of 35 patients were evaluated. Eleven of them had a history of suspected allergic reactions after using cefazolin. The remaining 24 had a confirmed β-lactam allergy. Cefazolin allergy was confirmed in 3 patients. We confirmed cefazolin allergy in 1 of 11 children with suspected reactions. Two of the patients with confirmed β-lactam allergy had also cefazolin allergy and both of them had allergy to more than one β-lactam antibiotics.

Conclusion: The majority of patients with a β-lactam allergy can tolerate cefazolin. Our findings imply that children with confirmed allergy to more than β-lactam antibiotics and peniclillin are also sensitive to cefazolin. Children with selective cefazolin allergy are expected to be sensitive to the side chain. A detailed history and comprehensive allergic evaluation are crucial in deciding the use of cefazolin in β-lactam-allergic patients.

Keywords: Drug allergy, beta-lactams, cross reaction, cefazolin

ÖZ

Giriş: Sefazolin, özellikle stafilokoklar ve streptokoklar gibi Gram-pozitif ajanlara karşı etkili olan birinci kuşak bir sefalosporindir. Bu dar spektruma rağmen cerrahi profilakside önemlidir. Bu çalışmada doğrulanmış β-laktam alerjisi olan hastalarda sefazolin tolere edilebilirliğini değerlendirmeyi amaçladık.

Yöntemler: Sefazolin alerjisi şüphesiyle sefazolin alerjisi tetkiki yapılan hastalar (Grup 1) ve sefazolin dışında β-laktam alerjisi olduğu doğrulanmış çocuklar (Grup 2) çalışmaya dahil edildi. Suçlu ilaç ve penisilin ile deri testi ve/veya provokasyon testi pozitifliği belirlenerek β-laktam alerjisi doğrulandı. Tüm hastalara sefazolin deri testi yapıldı. Deri testi negatif olan tüm hastalara provokasyon testi de uygulandı.

Bulgular: Toplam 35 hasta değerlendirildi. Bunlardan 11'inde sefazolin kullandıktan sonra şüpheli alerjik reaksiyon öyküsü vardı. Kalan 24'ünde doğrulanmış bir β-laktam alerjisi vardı. Üç hastada sefazolin alerjisi doğrulandı. Şüpheli reaksiyonları olan 11 çocuktan 1'inde sefazolin alerjisini doğruladık. Doğrulanmış β-laktam alerjisi olan hastalardan 2'si sefazoline de alerjikti ve her ikisinin de birden fazla β-laktam antibiyotiğe alerjisi vardı.

Sonuç: Betalaktam alerjisi olan hastaların çoğu sefazolini tolere edebilir. Bulgularımız, birden fazla β-laktam antibiyotiğe ve penisiline doğrulanmış alerjisi olan çocukların sefazoline de duyarlı olduğunu göstermektedir. Seçici sefazolin alerjisi olan çocukların yan zincire duyarlı olması beklenir. Sefazolin kullanımına karar vermede ayrıntılı bir öykü ve kapsamlı bir alerjik değerlendirme çok önemlidir.

Anahtar Sözcükler: İlaç alerjisi, beta-laktamlar, çapraz reaksiyon, sefazolin

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Received: 08.06.2021
Accepted: 15.06.2021

Cite this article as: Süleyman A, Yücel E, Tamay Z, Güler N. Safety of Cefazolin in Prophylaxis in B-Lactam Allergic Children. Bezmialem Science 2022;10(2):231-7

Introduction

Cefazolin is a first-generation cephalosporin group and a β-lactam antibiotic (1,2). Although cefazolin is not widely used in practice, it is a crucial agent in surgical prophylaxis (3-5). Cefazolin is preferred especially in orthopedic interventions due to its good Gram-positive efficiency and adequate bone penetration (3,6).

Approximately 10% of parents report suspected hypersensitivity to at least one β-lactam antibiotic drug in their children, although the confirmation rate is much lower, most children who are reported to be allergic to β-lactam are actually not allergic to these drug (4). In case of β-lactam allergy, the whole group is avoided due to cross-reaction concerns (5). The natural consequence of this is that surgical prophylaxis in patients with suspected or confirmed β-lactam allergy is performed with alternative agents such as clindamycin or vancomycin instead of cefazolin. This, together with enormous economic and safety issues, leads to the patient being deprived of the first-choice agent (3,7,8).

The first step in the diagnosis of drug allergy is a detailed medical history and physical examination. However, allergy testing is often required for definitive diagnosis. The most commonly used tools for this purpose are drug skin tests and provocation tests. Drug skin tests are generally used in the first stage for allergic evaluation. However, the lack of availability of antigenic determinants of the tested drug and its reactive metabolites causing the reaction, and the lack of validation of the skin test limit the reliability of skin tests. Therefore, drug provocation tests remain the gold standard for the exclusion or confirmation of drug allergy in the absence of contraindications (9,10).

Drug allergies are not permanent and tolerance to drug allergies may develop over time. Especially, cephalosporin allergies can disappear in a shorter time and at a higher rate than penicillin allergies (11,12). In this context, the necessity of avoiding the use of cefazolin in patients with a confirmed β-lactam allergy is controversial. Adult data on this subject indicate that the majority of patients with a confirmed β-lactam allergy can tolerate cefazoline (2,13,14). There is limited data on the use of cefazolin in pediatric patients with confirmed β-lactam allergy. Moreover, it may not always be possible to apply adult data to pediatric patients.

In this study, we evaluated cefazolin allergy in children based on their real-life data. Our aim was to evaluate cefazolin allergy in a group of children with history of suspected reaction to cefazolin and in children with a confirmed β-lactam allergy other than cefazolin.

Method

Study Group and Data Collection

Ethics Committee of İstanbul University approved the study protocol (no: 2020/1325). Informed consents were obtained from the patients and/or their parents. This study was performed according to the regulations of the Declaration of Helsinki.

In the study, children who were admitted to İstanbul University İstanbul Medical Faculty Pediatric Allergy and Immunology Department between May 2017 and May 2021 with suspected cefazolin reaction or confirmed β-lactam allergy other than cefazolin were evaluated.

Patients who underwent cefazolin allergic work-up due to suspected cefazolin allergy (Group 1) and children with confirmed β-lactam allergy other than cefazolin (Group 2) were included in the study as two groups. Patients whose allergic evaluation for cefazolin could not be completed due to uncontrolled asthma, severe skin reaction, and parents' disapproval, and patients with suspected delayed reactions were excluded from the study.

Demographic and clinical data of the patients were recorded according to the European Network for Drug Allergy (ENDA) questionnaire (15). Detailed history regarding the culprit drug, spectrum and timing of the symptoms, previous drug reactions and family history of drug allergy, presence of underlying chronic diseases or atopic diseases were obtained from the family and the patients' medical recordings. Informed consent was obtained from the patients and/or their families in the study.

Reactions that were thought to be clinically mediated by immunoglobulin E, such as urticaria, angioedema or anaphylaxis, occurring within six hours were accepted as immediate-type reactions (10).

Diagnostic Evaluation

Tests were performed at the earliest 4 weeks after the suspected reaction. Before the tests were carried out, medications that could affect the results were discontinued as recommended in an appropriate time (9,15,16). The tests with suspected β-lactams were done with one-week intervals.

In patients with confirmed β-lactam allergy, allergic evaluation was performed with the culprit β-lactam and penicillin. Since major determinant of penicillin could not be obtained for all patients, we were only able to perform penicillin skin tests with benzyl penicillin (penicillin G) and aminopenicillin (ampicillin) in patients with suspected β-lactam allergy. Skin tests were performed with recommended concentrations (10,15).

Skin prick test (SPT) with cefazolin was performed to each subject at full-strength concentrations of the drug (20 mg/mL). SPT was considered as positive when the wheal diameter was at least 3 mm or larger than the negative control with surrounding erythema after 20 minutes. In case of a negative SPT, intradermal tests (IDTs) were performed on the volar forearm skin at 2 mg/mL and 20 mg/mL concentrations, as recommended by ENDA, respectively on volar forearm skin. After 20 minutes, IDT was evaluated and considered as positive if the mean diameter of the bleb increased by 3 mm or more with surrounding erythema. Histamine at 10 mg/mL concentrations was used as positive control and 0.9% NaCl was used as negative control as recommended by ENDA (9,10).

If skin tests were negative, the patient was invited subsequently for a drug provocation test (DPT). These tests were performed

only in patients with negative skin tests, and not in patients with positive skin tests due to ethical concerns. Drug provocation tests were carried out in hospital setting in accordance with ENDA recommendations (10). The provocation test was accepted as positive in those who had skin findings, respiratory, cardiovascular or gastrointestinal system findings, or changes in vital signs during or after the test (9,10).

Cefazolin provocation tests were performed in two steps via intravenous route at hospital setting, with a total dose of 50 mg/kg. It was started with 1:10 of the single dose, followed by the remainder after 30 minutes.

Anaphylaxis was diagnosed according to the presence of the clinical criteria (17).

In the presence of compatible history, a positive skin test and/or a provocation test against a β-lactam antibiotic was accepted as β-lactam allergy. If drug skin and provocation tests were negative, drug allergy was excluded.

Statistical Analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) program (Version 23.0. Armonk, NY.). Pearson chi-square test or Fisher's Exact test was used to compare the categorized data. The normality of the distribution of continuous variables was evaluated with the skewness-kurtosis and the Kolmogorov-Smirnov test or Shapiro-Wilks test. Those which did not show normal distribution among continuous variables were given as median and interquartile range (IQR). Nonparametric tests (Mann-Whitney U or Kruskal-Wallis) were used to compare data that did not show normal distribution. A value of $p<0.05$ was accepted to be statistically significant.

Results

Thirty-five children were evaluated for cefazolin allergy. Eleven of the patients (31.4%) in the study group reported suspected allergic reactions after using cefazolin (Group 1). The remaining 24 patients had at least one confirmed β-lactam allergy other than cefazolin and those patients had never used cefazolin (Group 2). Clinical characteristic of the patients in each group are summarized in Table 1.

Cefazolin allergy was detected by using skin tests in 8.5% of the patients (n=3) and it was excluded by using DPT in the remaining of patients (n=31). We found that 9.1% (1/11) of those with suspected cefazolin allergy (Group 1), and 8.3% (2/24) of patients with confirmed β-lactam allergy had allergy to cefazolin (Group 2). The diagnostic approach for cefazolin allergy is demonstrated in Figure 1.

We found that 2 patients were sensitive to both cefazolin and other β-lactams. One of these patients was allergic to ceftriaxone and meropenem in addition to cefazolin, and the other to cefuroxime and amoxicillin-clavulanate (Figure 1). Those two patients were also found to be allergic to penicillin by using skin test and/or drug provocation tests. Cefazolin provocation tests were found negative in all patients with negative skin tests.

Within the group of children with confirmed β-lactam allergy, the presence of cefazolin allergy was found to be significantly higher in patients allergic to more than one β-lactam antibiotics (p=0.015). Comparison of the clinical features of the patients allergic or tolerant to cefazolin is presented in Table 2. We found that confirmed allergy to more than one β-lactam group was a risk factor for cefazolin allergy (p=0.009, 95% confidence interval: 2.7-1402). Characteristics of patients with confirmed cefazolin allergy are illustrated in Table 3.

Discussion

In our study, we found that the vast majority of patients with confirmed β-lactam allergy were tolerant to cefazolin. The only significant risk factor for cefazolin allergy was the presence of confirmed allergy to more than one β-lactam antibiotics.

Table 1. Clinical features and diagnostic results of the patients

	Confirmed β-lactam allergy other than cefazolin n=24	Suspected history of cefazolin allergy n=11	p
Age median (IQR) years	11 (5.2-15)	10 (7.5-11.5)	0.713
Having atopic disease	10 (41.7)	1 (9.1)	0.054
Asthma (± allergic rhinitis)	9	1	
Atopic dermatitis with food allergy	1	0	
Family history of a drug allergy	7 (29.2)	0	**0.045***
Clinical presentations			
Anaphylaxis	9 (37.5)	1 (9.1)	0.084
Urticaria-angioedema	15 (62.5)	8 (72.7)	0.554
Nonspecific finding	0	2 (18.2)	0.092*
Diagnostic results of cefazolin allergy			
Confirmed	2 (8.3)	1 (9.1)	1*
Excluded	22 (91.7)	10 (90.9)	

*Fisher exact test was applied.
IQR: Interquartile range

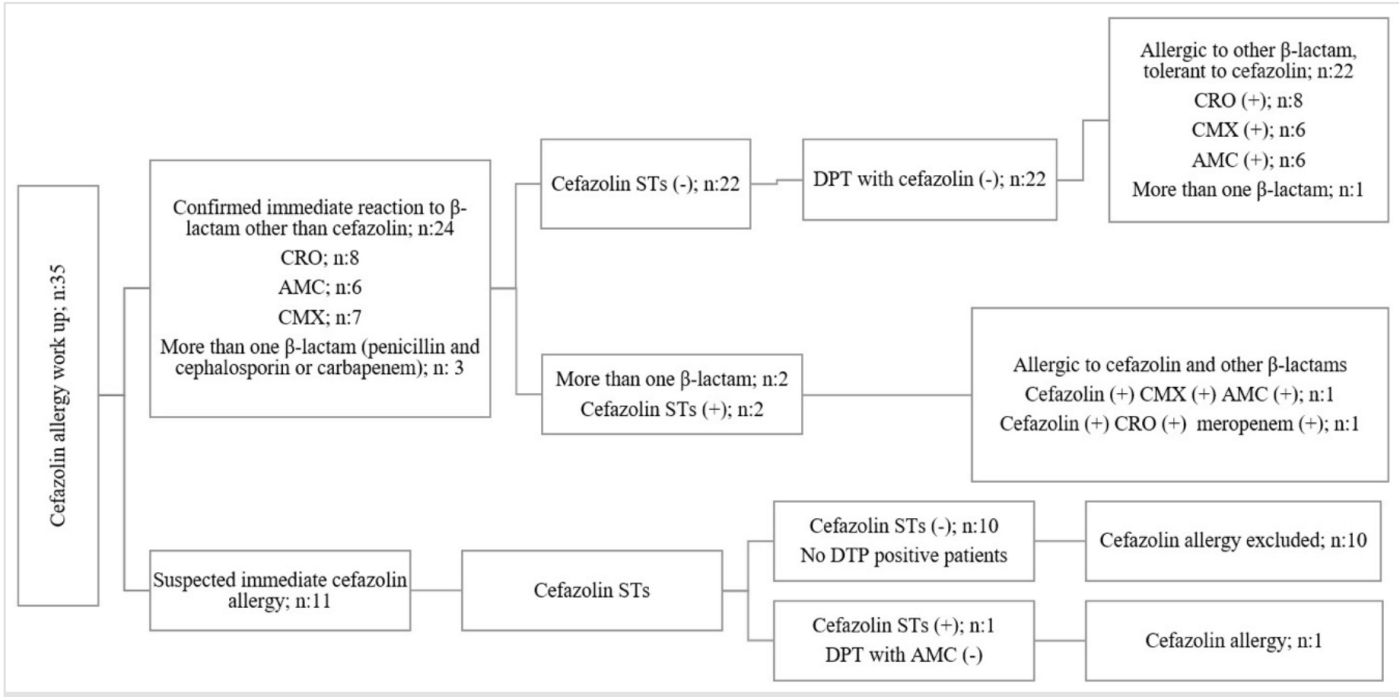

Figure 1. Allergic evaluation results for cefazolin and other β-lactams. Drug allergy diagnosis was confirmed by determining positive response in skin test and / or provocation test. Allergic evaluation for cefazolin was performed in 35 patients. Cefazolin allergy was confirmed in 2 of 24 patients allergic to multiple β-beta lactam antibiotics including penicillin. Cefazolin allergy was confirmed in only 1 of 11 patients who reported suspected allergies to this drug.

AMC: Amoxicillin-clavulanate, CRO:Ceftriaxone, CMX: Cefuroxime, DPT: Drug provocation test, STs: Skin tests

Table 2. Comparison of the clinical features of the patients allergic-and tolerant to cefazolin

	Allergic n=3 (8.5)	Tolerant n=32 (91.5)	p
Age, median (IQR) years	12 (12.5-14)	9 (5-13.5)	0.140
Gender-male	2 (66.7)	17 (53.1)	0.603*
Time between a confirmed β-lactam or suspected cefazolin allergy and diagnostic work-up for cefazolin, median (IQR) months	26 (13.5-34)	12 (9-24)	0.714
Confirmed allergy with more than one β-lactam group, including penicillin	2 (66.7)	1 (3.1)	**0.015***
Cefazolin allergic work-up indication			
Group 1 (suspected with cefazolin allergy)	1(33.3)	10 (31.3)	1*
Group 2 (confirmed with β-lactam allergy except cefazolin)	2 (66.7)	22 (68.8)	
Clinical presentation of β-lactam allergy			
Anaphylaxis	3 (100)	7 (21.9)	**0.018***

*Fisher exact test was applied.
IQR: Interquartile range

Since cefazolin is a β-lactam group antibiotic, its use in patients with confirmed β-lactam allergy may cause the risk of cross-reactions. The safety of cefazolin usage in patients with β-lactam allergy is related to the β-lactam sensitivity pattern. If there is sensitization to common structures, such as β-lactam core, it should be avoided (5,18).

Sensitization patterns of the patients with cefazolin are presented in tree forms; cross-reactivity with penicillin, selective reactivity to cefazolin or cross-reactivity with another cephalosporin (ceftazole) (5,13,18). Cefazolin, which is the most common cephalosporin to cause anaphylaxis in some countries, has unique R_1 and R_2 side-chain groups and does not appear to cross-react with other cephalosporins except ceftazole, a first-generation agent that is only available in some countries. Several studies suggest that skin test positive patients with past immediate reactions to cefazolin often tolerate most of the cephalosporins and other β-lactams (2,13,14,19). In our study, two of the patients with confirmed multiple β-lactam allergy were sensitive to cefazolin. Although the number of our patients with sensitivity

Table 3. Clinical characteristics of patients with confirmed cefazolin allergy

Patient number	Age &gender	Culprit drug	Clinical presentation	Time elapsed, months	Drug skin tests results					DPT results			Confirmed β-lactam*	Possible sensitization pattern
					Penicillins STs		Cephalosporins STs		Carbapenems STs	PV	AM/AMC	Cefazolin		
					PG	AMP/AM	Cefazolin	Other cephalosporins	Meropenem STs					
Patient with confirmed β-lactam allergy, other than cefazolin														
1	12-F	CRO, meropenem	A	26	+	+	+	CRO +	+ [§]	NP	NP	NP	CRO + meropenem, AMP	B-lactam core
2	16-M	AMC-AMP-s, CMX,	A	42	+	+	+	CMX+	NP	NP	+	NP	CXM+AMC	B-lactam core
Patient with suspected cefazolin allergy														
3	13-F	Cefazolin	A	1	-	-	+	NP	NP	-	-	NP	No confirmed beta-lactams	Side chain

A: Anaphylaxis , AM:Amoxicillin, AMC: Amoxicillin-clavulanic, AMP - S: Ampicillin-sulbactam, CRO: Ceftriaxone , CXM: Cefuroxime, DPT: Drug provocation test, F: Female, M: male , NP: Not performed, PG : Penicillin G , PV: Penicillin V, STs:Skin tests

to cefazolin was low, this finding implied that multiple β-lactam allergy might be a risk factor for cefazolin allergy. Based on the fact that β-lactam allergy is the most common drug allergy in children, (4) the risk of cross-reactions with other β-lactams may raise concerns for cefazolin use (5,20-22). Of our two patients with cefazolin allergy; patient no 1 was found to be sensitive to ceftriaxone and meropenem, and patient no 2 to cefuroxime and amoxicillin-clavulanate. This findings together with the presence of penicillin allergy in those children confirmed that the sensitivity of our two patients was to the β-lactam core. On the other hand, all children with confirmed allergy to a β-lactam were tolerant to cefazolin. This could be explained by the sensitivity to the side chains. Similarly, we believed that patient no 3 was sensitive to cefazolin side chains, because he had sensitivity only to cefazolin in the skin test with negative results for penicillin allergy evaluation. The β-lactam sensitivity pattern is directly related to the habit of prescribing a drug in the population (23). In β-lactams frequently used such as amoxicillin-clavulanic acid and cefuroxime or ceftriaxone, sensitivity to the side chains is mostly found (5,23,24). Our results seem to be consistent with the literature for these drugs.

Considering that history of drug reactions are mostly not true and it may disappear over the time even if it is real, (25) it is not appropriate for these patients to avoid cefazolin, which is the first choice especially in surgical prophylaxis. Avoiding this agent and using alternative drugs such as vancomycin or clindamycin is important in terms of increased resistant bacteria in surgical infections (5-8). It is indisputable that this practice mentioned above may cause health and economic problems.

There are some difficulties in confirming the diagnosis of a patient with suspected cefazolin allergy in determining the sensitivity pattern. We could not use benzylpenicilloyl octa-L-lysine and penicillin G at the same time (26). In addition, there are no commercially available reagents for cephalosporin skin tests and therefore tests are usually done with parenteral form of the culprit cephalosporin (12,18,19,24,27). We thought that we overcame the difficulties encountered in diagnosing cefazolin allergy by including only patients with immediate type reaction in our study and by performing a provocation test with cefazolin in all patients with negative skin test results.

Conclusion

In conclusion, our findings showed that patients with confirmed allergy to a β-lactam antibiotic can usually tolerate cefazolin. Children with sensitivity to multiple β-lactam antibiotics and penicillin are expected to be allergic to cefazolin due to sensitivity to β-lactam core. A detailed history and comprehensive allergic assessment are essential to decide on the use of cefazolin.

Acknowledgements: The authors acknowledge Nermin Güler for proofreading and scientific advisor activity.

Ethics

Ethics Committee Approval: Ethics Committee of İstanbul University approved the study protocol (no: 2020/1325).

Informed Consent: Informed consents were obtained from the patients and/or their parents.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: A.S., E.Y., N.G., Design: A.S., E.Y., N.G., Data Collection or Processing: Z.T., N.G., Analysis or Interpretation: Z.T., N.G., Literature Search: A.S., E.Y., N.G., Z.T., N.G., Writing: A.S., E.Y., N.G., Z.T., N.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Review

DOI: 10.14235/bas.galenos.2021.5094
Bezmialem Science 2022;10(2):238-41

Nursing and Care Practice Strategies in the Coronavirus Disease-2019 (COVID-19) Pandemic

Yeni Koronavirüs Hastalığı-2019 (COVID-19) Salgınında Hemşirelik ve Bakım Uygulama Stratejileri

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ABSTRACT

The coronavirus disease-2019 (COVID-19) is a highly contagious disease, which has human-to-human transmission through droplet and contact. It commonly manifests with fever, dry cough, myalgia, and dyspnoea; the severity of this disease may range from mild to severe or critical. Currently, there is no definitive treatment or vaccine for COVID-19. The only evidence-based management of the disease is isolation and supportive care. Therefore, nurses play an important role in the management of the disease. This evidence-based, comprehensive literature review describes the importance of initial assessment, triage, sample collection, patient care with mild to moderate symptoms, critical patient care, and nursing practices in patient management during the COVID-19 pandemic.

Keywords: Coronavirus, nurse, prevention

ÖZ

Yeni koronavirüs hastalığı-2019 (COVID-19), damlacık ve temas yoluyla insandan insana bulaşan oldukça bulaşıcı bir hastalıktır. Genellikle ateş, kuru öksürük, kas ağrısı ve dispne şeklinde kendini gösteren, hafif, şiddetli veya kritik olarak tanımlanan bir hastalıktır. Halen COVID-19 için kesin bir tedavi veya aşı bulunamamıştır. Hastalığın kanıta dayalı tek yönetim şekli izolasyon ve destekleyici bakımdır. Bu nedenle hastalığın yönetiminde hemşirelerin büyük bir rolü vardır. Bu kanıta dayalı, kapsamlı literatür incelemesi; COVID-19 pandemisinde hasta yönetiminde ilk değerlendirme, triyaj, örnek toplama, hafif - orta şiddette semptomları olan hasta bakımı, kritik hasta bakımı ve hemşirelik uygulamalarının önemini anlatmaktadır.

Anahtar Sözcükler: Koronavirüs, hemşire, korunma

Introduction

The coronavirus disease-2019 (COVID-19), which first appeared in Wuhan, China in December 2019, was a virus that spread rapidly all over the world, causing severe acute respiratory syndrome and pneumonia (1).

With the spread of the COVID 19 in the world and the start of the pandemic process, some changes had to be made in health services in many parts of the world. Postponing surgeries, closing outpatient clinics, reducing daily surgeries and emergency services can be counted among the examples to be given. This process was very difficult for health professionals due to high risks arising from the working environment and tragically loses of lives. An issue that changes the perspective in healthcare practices and that we have to pay more attention to is the risks of COVID-19 care and the tragic death of healthcare professionals (2).

The purpose of this review is to provide information about nursing and care practice strategies within the scope of COVID-19 due to the increasing need for the care role, which is the main purpose of nursing, in a short time during the pandemic.

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Received: 09.07.2020
Accepted: 18.03.2021

Cite this article as: Tosun H, Sümre Dalkıran S. Nursing and Care Practice Strategies in the Coronavirus Disease-2019 (COVID-19) Pandemic. Bezmialem Science 2022;10(2):238-41

Nurses During the Pandemic

A multidisciplinary approach is needed in the prevention, control and protection of the pandemic. Nurses working in hospitals designated within the scope of the pandemic play a critical role in the control of COVID-19 (2). The workload of nurses providing care to patients with COVID-19, their families and the community is quite high, and it reaches the upper limit. Nurses serve at the forefront, taking great responsibility in care of patients with COVID-19 that require hospitalization. Nurses who spend a long time with the patients need care practices more than ever in this process (3-5).

The COVID-19 carries a high risk for nurses. Because asymptomatic progression of the disease increases the risk of transmission in some patients. In particular, virulence depends on the severity and degree of infection in the patient. In the literature on the subject, it is emphasized that the daily working hours exceeding 10 hours due to the large number of patients and the low number of health personnel are the reasons for the high incidence of infection in healthcare professionals. In addition, extreme fatigue and stress increase the sensitivity to COVID-19 by causing weakness in immune system (5,6).

As the infection spreads rapidly, there is difficulty in accessing personal protective equipment, so the rate of infection spread among healthcare professionals is increasing. Thus, the rate of transmission of infection between visitors, staff and patients increases. All personnel in the department will have to stay in quarantine for 14 days in case of contamination in any healthcare professional (5,6).

In order to prevent transmission from the patient to the healthcare professionals, necessary precautions should be taken, covering the entire process from the patient's admission to the hospital until discharge. The team involved in the care of patients with COVID-19 should consist of nurses experienced in patient care. Inexperienced nurses must work under the guidance of this group and there must be a leader coordinating the team. When the COVID-19 pandemic first appeared, it was important to share the duties among nurses, to ensure employee safety, and therefore to ensure the sustainability of health services (5).

Nurses, one of the most reliable health professional groups, play a key role in providing the necessary education on the prevention of diseases and reducing the spread of false information. It is among the duties of nurses to be aware of the symptoms of the disease, to provide and maintain infection control measures, and to provide the best care to the patient and their relatives. For this reason, the COVID-19 pandemic is a period in which nurses use their consultancy and educator roles at the highest level (3,7).

Points that the healthcare professional should pay attention to during the COVID-19 pandemic (8):

- Triage protocols should be established for acute respiratory tract infection symptoms at first admission.
- Hospital entry points should be restricted.
- In hospital applications, individuals should be given a face mask and should be told to wear it constantly.
- A quick anamnesis should be taken from the patients.
- Patients with suspected COVID-19 should be isolated quickly and safely.
- The healthcare team must use protective equipment.
- They should wash their hands with soap and water or alcohol-based hand washing products.
- While coughing and sneezing, the mouth must be covered with a handkerchief.
- Touching the face with unclean hands should be avoided.
- Frequently touched objects and surfaces should be cleaned and disinfected.
- Close contact with individuals should be avoided, a distance of 1.5 m should be left in between
- Closed and crowded areas should be avoided.
- Protective equipment should be used to minimize the risk of exposure of healthcare professionals to COVID-19.
- Two separate areas should be created for healthcare professionals to put on and take off their protective equipment.
- Protective equipment can only be tolerated for a few hours as it raises body temperature.
- Care providers should notify the competent units when they have complaints of dry cough, fever, sore throat or come into contact with individuals diagnosed as having COVID-19. They should be tested immediately and quarantined for 14 days according to the test result.
- Shifts should be arranged for nurses to rest. This arrangement should be every 2-3 hours for the health personnel who care for isolated patients.
- Hospitals should be designed as intensive care units, including operating rooms.
- All nurses should be trained to work in the intensive care unit.
- Nurses should be organized according to their knowledge, skills and experience, in such a way that they can care from complex patients to stable patients.
- Patient records are very important for both the hospital and the patient and their relatives. All daily notes should be kept electronically by physicians and nurses.
- Due to the increased stress and panic environment during the pandemic, the mental and psychosocial health of caregivers is as important as their physical health.
- Avoid contact with patients who are coughing, have high body temperature and have difficulty in breathing.

- Avoid sharing objects that come into contact with the mouth.
- Shaking hands should be avoided.
- Standard rules such as hand and respiratory hygiene, safe waste management and sterilization of equipment should be followed.
- In order to reduce the workload of nurses, nurse planning should be done in such a way that one nurse per patient.

Things to do in care and treatment for individuals diagnosed as having COVID-19 (8-12);

- If the patient's hemodynamics is good, prone position should be given. In the literature, it is stated that applying the prone position for a minimum of 12 hours a day increases oxygenation and reduces mortality.
- In individuals with severe acute respiratory distress, it is recommended to keep the individual in the prone position for 12-16 hours in order to maintain mechanical airway patency.
- Vital signs should be followed up at frequent intervals.
- Bilateral lung sounds should be listened to.
- The patient's pain and consciousness should be evaluated.
- It is recommended that patients in the intensive care unit stay in negative pressure rooms.
- It is recommended that at least 3 specialists (physician and nurse) be assigned to ensure a safe prone position for each patient.
- Visitor entry should be prohibited in intensive care units. However, video calls can be made to ensure communication between the patient and their relatives. Negative pressure system must be applied to reduce droplet and aerosol exposure in patients hospitalized with the diagnosis of COVID-19 in intensive care units. In cases where there is no negative pressure system, the room doors should be kept closed and the number of rooms should be increased.
- Patient rooms should be single occupancy, if there is no single room, a distance of at least 1-1.5 meters should be left between patient beds.
- Every material used for patients should be patient-specific.
- Oxygenation of patients should be monitored frequently and it should be ensured to maintain it within normal limits.
- It is very important to intubate patients at the right time. For this reason, the respiratory functions of the patients should be monitored frequently.
- Non-rebreathing reservoir masks should be used in patients without dyspnea, and non-invasive respiratory support systems should be used in patients with dyspnea.
- Heart rhythm and rate, blood pressure and other organ functions should be monitored continuously by monitoring sick individuals.
- Due to the long-term prone positioning, sick individuals should be followed up frequently against the risk of pressure injury.
- Fluid therapy should be carefully managed and balanced solutions should be selected in terms of the risk of dehydration. Edema must be checked.
- Considering comorbidities, especially in elderly patients, is very important in terms of care planning.
- It is very important to follow the patient's hourly intake and output. If there is a urinary catather, it should be taken care of.
- Evaluation of capillary refill rate, body temperature and perfusion of sick individuals is very important.
- If the patient is not intubated, oral feeding should be supported, enteral nutrition should be preferred in patients whose oral intake cannot be maintained.
- Gastrointestinal system functions and aspiration risks of individuals must be evaluated. Individuals over 65 years of age who are at risk of aspiration and those with advanced distension should be supported with parenteral nutrition.
- Patients should be followed up for the risk of developing coagulopathy. Low molecular weight heparin prophylaxis is recommended for the prevention of thromboembolism in individuals in line with the physician's request.
- The patient must be followed up for the development of acute renal failure.
- The patient should be followed up for early signs of sepsis.
- Regular blood gas analysis is very important.
- The entrances to the patient's room should be reduced as much as possible.
- Oral care must be applied once every 4 hours.
- Aspiration of patient secretions should be ensured.
- All contaminated materials used after care should be disposed of in the medical waste box and removed from the area.

It is very important to apply individualized care when planning nursing care for patients with COVID-19.

Conclusion and Recommendations

COVID-19 is a highly contagious viral disease with a high mortality rate. It is only possible with a qualified individualized nursing care that individuals hospitalized due to the diagnosis of COVID-19 are affected by adverse situations as little as possible. The responsibilities of nurses in this regard are quite high and of critical importance. For this reason, if the nurses know both the interventions to be applied for the sick individual and the necessary measures to protect themselves during the period from the patient's admission to the discharge from the hospital, negative experiences, recovery time and contagiousness can be reduced.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: S.S.D, H.T., Design: S.S.D, H.T., Data Collection or Processing: S.S.D, H.T., Analysis or Interpretation: S.S.D, H.T., Literature Search: S.S.D, H.T., Writing: H.T., S.S.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Review

DOI: 10.14235/bas.galenos.2021.6186
Bezmialem Science 2022;10(2):242-6

Pharmaceutical Care Services During the COVID-19 Pandemic in Turkey: Proposal of a Holistic Approach

COVID-19 Pandemisinde Türkiye'deki Farmasötik Bakım Hizmetleri: Bütüncül Bir Yaklaşım Önerisi

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ABSTRACT

Community pharmacists, who are among the primary healthcare service providers, are regarded as the closest and most accessible healthcare professionals. Unfortunately, patient counseling, one of the most important pharmacy profession tasks, cannot be performed in exceptional cases, which can endanger patient safety. The coronavirus disease-19 (COVID-19) pandemic process we are facing today is an example of these particular situations in Turkey. Partial curfews imposed within the scope of COVID-19 pandemic measures have also increased problems. Therefore, there is a need for a pharmaceutical care service network that will enable individuals who do not have access to pharmacies, who are in risk groups, or whose mobility is restricted due to pandemic or disaster, to reach their prescribed medicines safely under pharmacist consultancy. In this regard, establishing a pharmaceutical care service network on a scientific and systematic basis may solve the current problems, mostly encountered during the COVID-19 pandemic in Turkey. Designing a network within the framework of vehicle routing and assignment problems is important in ensuring patients' rapid access to prescribed medications and preventing unfair competition among pharmacies.

Keywords: COVID-19, community pharmacists, telehealth

ÖZ

Birinci basamak sağlık hizmeti sunucuları arasında yer alan eczane eczacıları, en yakın ve en erişilebilir sağlık çalışanları olarak kabul edilmektedir. Eczacılık mesleğinin en önemli görevlerinden biri olan hasta danışmanlığı maalesef hasta güvenliğini tehlikeye atabilecek istisnai durumlarda yapılamamaktadır. Bugün karşı karşıya olduğumuz koronavirüs hastalığı-19 (COVID-19) salgın süreci, Türkiye'deki bu özel durumların bir örneğidir. Koronavirüs pandemi önlemleri kapsamında uygulanan kısmi sokağa çıkma yasakları da karşılaşılan bu sorunları artırmıştır. Bu nedenle eczanelere erişimi olmayan, risk grubunda bulunan, salgın veya afet nedeniyle hareket kabiliyeti kısıtlanan bireylerin reçeteli ilaçlarına eczacı danışmanlığında güvenle ulaşmasını sağlayacak bir farmasötik bakım hizmet ağına ihtiyaç duyulmaktadır. Bu bakımdan bilimsel ve sistematik bir temelde farmasötik bakım hizmet ağı kurmak, Türkiye'de özellikle COVID-19 salgını sırasında karşılaşılan güncel sorunları çözebilir. Araç rotalama ve atama problemleri çerçevesinde bir ağ tasarlamak, hastaların reçeteli ilaçlara hızlı erişimini sağlamak ve eczaneler arasında haksız rekabeti önlemek açısından önem arz etmektedir.

Anahtar Sözcükler: COVID-19, eczane eczacıları, telesağlık

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Received: 09.03.2021
Accepted: 17.06.2021

Cite this article as: Arslan M, Akbal Dağıstan Ö, Şar S. Pharmaceutical Care Services During the COVID-19 Pandemic in Turkey: Proposal of a Holistic Approach. Bezmialem Science 2022;10(2):242-6

Introduction

Pharmacists, who are primary health care providers, are regarded as the closest and most accessible healthcare professionals all around the world. In terms of public health, especially community pharmacists, hospital pharmacists, and clinical pharmacists come to the fore. There are different pharmacy specialties in many different countries, such as clinical pharmacy, family pharmacy, intensive care pharmacy, home care pharmacy, and disaster pharmacy. Among these specialties, family pharmacy and home care pharmacy gain more importance in terms of continuous and controlled follow-up of patients' drug treatment processes.

In the literature, many studies are revealing the pharmacist's necessity to provide drug consultancy service. Triller et al. (1) found that the home care pharmacy program positively affected patient outcomes. Walus and Woloschuk (2) conducted a pilot application in an ongoing home care practice that included home visits, training, information transmits via telephone, and recording patient data in a database. As a result of a survey conducted with pharmacists participating in this practice, several non-negligible pharmacists need to be observed. Pharmacists are included in the ongoing practice through a private insurance company. It was stated that it would be beneficial for pharmacists to expand and maintain this practice. Dilks et al. (3) revealed that the provision of pharmacy services at home increased patient safety, especially for the elderly, improves patients' quality of life, and reduced health expenditures. In addition to home health care services, telepharmacy applications, which are legally carried out in many countries as a product of constantly developing technologies, also come to the fore in pharmacist-patient counseling. Poudel and Nissen (4) stated that telepharmacy practices carried out within the legal rules framework would contribute to the management of drug treatment processes and access to drug and drug information for patients who could not access pharmacist counseling due to geological or demographic constraints. The fact that the United States e-commerce company Amazon, which has attracted considerable attention and discussed recently, has started to offer online pharmacy services in the USA, is an important example that should be addressed in terms of telepharmacy services. Especially during the COVID-19 pandemic, it is seen that telepharmacy services have increased in many countries. Margusino-Framiñán et al. (5) stated in the study they conducted in Spain that the telepharmacy services offered during the COVID-19 pandemic would optimize clinical outcomes and reduce transmission risk. Tortajada-Goitia et al. (6) applied a questionnaire to pharmacists to evaluate hospital pharmacies' telepharmacy services during the COVID-19 pandemic in Spain. They found that the telepharmacy services provided the continuity of many patients' health care in this process (6). Ibrahim et al. (7) put forth that telepharmacy increased the rate of patients' access to pharmaceutical care services and reduced the likelihood of drug-related side effects during the COVID-19 pandemic in the United Arab Emirates.

The World Health Organization also sees digital health solutions as one of the most promising approaches to the COVID-19 pandemic; such as mobile applications used for contact tracing to monitor the outbreak and used to deliver and gather instant information or online tools to provide medical consultancy (8). Besides, the International Pharmaceutical Federation (FIP) has published a "Call to Action" on 16.04.2020 to support pharmacists and pharmacy workers who are at the frontline of the fight against coronavirus/COVID-19. In this call, the points where pharmacists can play an active role in the management of the pandemic process have been put forward such as: (i) *Providing logistical and financial support for home delivery of medicines and medical devices by pharmacists, especially to high-risk groups, such as older adults, patients with non-communicable diseases and patients with immunocompromising conditions (congenital or acquired), who have been advised to stay at home. This can be done in partnership with postal services or with other logistical partners*. (ii) *Allowing pharmacists and pharmacy staff to conduct routine pharmacy tasks remotely as necessary, including through telepharmacy consultations* (9).

Turkey at a Glance

When home care pharmacy services or telepharmacy services are evaluated in Turkey, it can be seen that there is a gap. In Turkey, homecare health services are provided by the Turkish Ministry of Health since 2005. The medicines required for the patient during the delivery of home healthcare services are covered by the service unit; however, pharmacists have not been included in these units (10). During the regular home visits, medications were prescribed to the patients benefiting from this service between 2012 and 2017 by 213,336 specialist physicians. However, the prescriptions were either delivered to the patients via their relatives or their neighbors who collected them through local pharmacies or home care service workers. Since these prescribed medicines could not be delivered directly from the pharmacist, patients used their medicines without a primary pharmacist consultation.

Many studies conducting in Turkey on home care services have discussed the drug-related problems that arise during the provision of these services. Aslan et al. (11) stated that problems such as complications due to misuse of medicines might occur during home healthcare services. Yeniçeri conducted a study with 255 volunteers over 65 years old who registered for home care services. It was found that the rate of polypharmacy in patients was 48.6%, and 53.4% had at least one inappropriate drug use. It was also revealed that the frequency of drug side effects and inappropriate drug use increased due to polypharmacy (12). Similarly, Sargın (13) conducted a study with 317 patients over 65 years old who registered for home care services. As a result, polypharmacy was detected in 54.9% of the participants, and 49.5% had at least one inappropriate drug use. Besides, the study showed that the prevalence of inappropriate drug use increased with polypharmacy (13).

In the light of this information, it is seen that drug-related problems are experienced in-home healthcare services in our country, and the role of pharmacists in this regard has started to attract attention. In extraordinary situations such as pandemic, natural disasters whereby patients have difficulty for accessing to

their medicines, these problems draw even more attention and the pharmaceutical care need arises (14).

As it is known, pharmacists play an active role in both drug and vaccine development studies and the protection of public health. Due to the legislation on pharmaceutical in Turkey, pharmacists should also take an active role in drug procurement processes in extraordinary situations and crisis periods (15). COVID-19 cases are gradually increasing in Turkey. In line with the Turkish Republic, Ministry of Health's data, the number of patients diagnosed with the COVID-19 in the country as of February 13, 2021, was 2,579,896 (16). This frame has made community pharmacies one of the health institutions with the highest patient traffic during the pandemic period. Not only meeting the prescriptions of patients diagnosed with COVID-19, but also the increase in demand for immune-boosting products, vitamins, personal protective equipment, and hygiene products significantly increased the demand for pharmacist consultancy (17).

Partial curfews imposed within the scope of COVID-19 pandemic measures have also caused problems in individuals' access to drugs and pharmacist counseling. Also, due to the quarantine process that COVID-19-positive patients must comply with, drug use cannot be provided under pharmacist consultation during their home treatment process. Along with lack of proper consultancy, high treatment doses of drugs used in COVID-19-positive patients according to diagnostic treatment guidelines, reduce patient compliance. For example, Favipiravir (only 200 mg tablet form markedly available in Turkey) should be used as 2x1,600 mg: 8 tablets in the morning and 8 tablets in the evening for the loading dose for the first 2 days, followed by 2x600 mg as a maintenance dose (3 tablets in the morning and 3 tablets in the evening) (18). Unfortunately, most of the patients who receive treatment at home do not want to use this drug, which requires high doses, due to incorrect/inadequate information. This situation negatively affects the patient's compliance with the treatment, aggravates the clinical picture of the disease, and increases hospital re-/admissions and hospitalization rates. In this context, it is predicted that a system where direct pharmacist-patient communication is provided will be beneficial.

When the subject is handled from a different perspective, pharmacy visits of COVID-19 positive patients make community pharmacies areas with a high risk of contamination. It should also be noted that the first healthcare provider who died in our country due to COVID-19 was also a community pharmacist (Pharm. İhsan Giray). It was also known that there were 51 pharmacists and 19 pharmacy employees who died as of May 10, 2021, due to COVID-19 (16). Unfortunately, many pharmacists and pharmacy workers continue to get infected by COVID-19.

The filiation technique is used in Turkey to screen the chain of contacts in COVID-19. Patients who are tested positive for COVID-19 and their close contacts are visited by filiation teams consisting of three members (physician, health professional, and assistant personnel) appointed by the Turkish Ministry of Health (16). These teams give the first prescriptions of these patients created by the patient's family physician to them at their home. However, since all of these groups do not include pharmacists, these quarantined patients have problems in the follow-up of their medication therapy and access to medicines.

According to the pharmaceutical legislation in Turkey, FIP's aforementioned advice, related to COVID-19 practice implementation focused on the delivery of medicines to the patient residences, is not legal. However, it is a known fact that home delivery of medicine and over the counter products, is an uncontrolled issue during this pandemic period (9,19). Illegal pharmaceutical couriers who are not even a healthcare professional or who do not work in the health sector draw attention at this point. Inevitably, the delivery of drugs to patients by such couriers who have no expertise in drugs, will negatively affect patients' treatment process. Additionally, with the circular issued during the COVID-19 pandemic period, the Vefa Coordination Group was established under government control to meet citizens' basic needs who were not able to go out due to various reasons such as an ongoing lockdown or heath related issues and did not have anyone to meet their needs (16). Obviously, this practice allows that specified group of patients to have access to their medical needs in terms of medications, over-the-counter products etc. Still, the fact that pharmacist consultancy cannot be obtained in this practice also paves the way for the negativities due to incorrect/non-rational use of drugs. Another shortcoming in the current operation is that there is unfair competition among pharmacies, as there is no systematic appointment and distribution strategy for pharmacies where needs are met.

Proposed Approach; Establishment of a Pharmaceutical Care Service Network

During the COVID-19 pandemic process, the Turkish Ministry of Health has put into a telehealth service project that includes medical consultations, clinical diagnosis, and health service delivery. Within the scope of the project, which has started to be piloted in five cities, people who should be in quarantine can make an appointment through the Central Physician Appointment System, talk to physicians by online communication tools such as video conference, and receive their prescriptions via SMS. This system emphasized the importance of digital applications during extraordinary situations such as the pandemic period (16). Therefore, in parallel with this running project, it is thought that with an integrated system approach comprising the teleconsultation services by pharmacists, patient safety can be ensured by both the safe access of the patients to the medicines and the continuous patient counseling. Also, Aysu and Rabuş (20) emphasized the importance of such systems in the COVID-19 pandemic for the sustainability of pharmaceutical care.

In this regard, establishing a pharmaceutical care service network on a scientific and systematic basis may be a solution for the current problems, mostly encountered during the COVID-19 pandemic in Turkey. Designing a network within the framework of vehicle routing and assignment problems is important in

ensuring rapid access of patients to prescribed medications and preventing unfair competition among pharmacies. Providing such a holistic system that facilitates the access of individuals to their prescription medicines under pharmacist consultancy offers solutions from various perspectives including minimizing the drug-related problems that can be encountered at home health care services, increasing success of drug treatment and patient compliance, and reducing the health expenses of patients caused by non-rational use of drugs.

By integrating such a holistic approach to the healthcare system, pharmaceutical care services can reach to patients in extraordinary situations such as pandemics or natural disasters and in particular rural areas that cannot make any personal contact with a pharmacist directly. Thus, pharmaceutical care services' sustainability will be ensured under all conditions. From a different perspective, implementing such a system, especially in delivering pharmaceutical services in developing countries will also help achieve some of the FIP Development Goals to transform global pharmacy such as Goal 18: Access to medicines, devices & services, Goal 19: Patient safety, and Goal 20: Digital health (21).

The study's main purpose is to evaluate the problems experienced in accessing drugs in Turkey, especially during the COVID-19 pandemic process, and offering a solution. With this study, the need for online, legal, and reliable pharmacy systems to provide solutions to the problems experienced by individuals in accessing their prescribed medicines in such extraordinary conditions is put forth.

The authors also want to emphasize that they have started a project on this subject with a specialized working group within the scope of digital health applications. Therefore, the information presented in this study is one of the milestones of the relevant project.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.A., Ö.A.D., S.Ş., Design: M.A., Ö.A.D., S.Ş., Literature Search: M.A., Ö.A.D., Writing: M.A., Ö.A.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Review

DOI: 10.14235/bas.galenos.2021.6398
Bezmialem Science 2022;10(2):247-60

Radiological Appearences of Benign Soft-tissue Tumors of the Hand and Wrist with Special Emphasis on MRI

El-El Bileği İyi Huylu Yumuşak Doku Lezyonlarının MR Görüntüleme Bulguları

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ABSTRACT

Tumoral and pseudotumoral lesions of the hand and wrist are commonly encountered in routine clinical practice. Although most of them are benign, radiological differential diagnosis of these lesions is difficult, because of their nonspecific imaging findings, except ganglia, localized type of tenosynovial giant cell tumors and lipomas. Digital radiography, computed tomography, and ultrasound may be useful in identification of the lesions in the wrist and hand, but magnetic resonance imaging with superior contrast and spatial resolution is the most important imaging modality.

Keywords: MRI, hand, tumor, wrist, benign, lipoma, fibroma

ÖZ

El ve el bileğinin tümoral ve psödotümöral lezyonlarına rutin klinik uygulamada sıklıkla rastlanır. Çoğu iyi huylu olmasına rağmen, gangliyonlar, lokalize tenosinoviyal dev hücreli tümörler ve lipomlar dışında özgün olmayan görüntüleme bulguları nedeniyle bu lezyonların radyolojik ayırıcı tanısı zordur. El bileği ve eldeki lezyonların tanımlanmasında dijital radyografi, bilgisayarlı tomografi ve ultrason yararlı olabilir, ancak üstün kontrast ve uzaysal çözünürlüğe sahip manyetik rezonans görüntüleme en önemli görüntüleme yöntemidir.

Anahtar Sözcükler: MRG, el, tümör, el bileği, benign, lipom, fibrom

Introduction

Tumoral and pseudotumoral lesions of the hand and wrist originating from various kinds of tissues such as bone, cartilage, tendon-tendon sheath, synovium, muscle, nerve and connective tissue are commonly seen in routine clinical practice. The World Health Organization classification for soft-tissue tumors is a commonly used classification system and provides uniformity for the reporting and treatment of different tumors and tumor-like lesions (1) (Table 1). Soft-tissue lesions of the hand and wrist range from non-neoplastic-inflammatory conditions to benign and malignant tumors. Most of them are benign and usually present with painless lumps and nodules (2). Except lesions with specific imaging findings (e.g. ganglia, localized type of tenosynovial giant cell tumors and lipomas), radiological features of these tumoral lesions frequently do not allow clinicians for a definitive diagnosis (3-5). Therefore, hand surgeons often prefer excision of the lesion before reaching an exact radiological and pathological diagnosis, because of patient's cosmetic worries and functional loss. To reduce the list of the differential diagnoses, thorough clinical examination,

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Cite this article as: Yılmaz TF, Toprak H, Atasoy B, Sönmez FC, Aralaşmak A, Gürkan V. Radiological Appearences of Benign Soft-tissue Tumors of the Hand and Wrist with Special Emphasis on MRI. Bezmialem Science 2022;10(2):247-60

Received: 07.05.2021
Accepted: 02.07.2021

taking a good anamnesis, location of the lesion, and associated systemic diseases such as rheumatologic disorders are important (6,7). X-ray and computed tomography (CT) are helpful in demonstrating calcifications within the lesion and evaluating the bony structures. Cystic nature of the lesion can be determined with a high-frequency transducer ultrasound (US). Internal vascularization of the lesion may be demonstrated with color Doppler imaging (8). Magnetic resonance imaging (MRI) with its superior contrast and spatial resolution should be the preferred imaging method in diagnosis and characterization of hand and wrist mass lesions. MRI can accurately determine the signal characteristics of the lesions that reflect the underlying pathological structure of the mass. MRI also determines the enhancement patterns of the lesion and accurate localization related to surrounding anatomical structures (2-6,9-14). In this pictorial review, the radiologic, especially MRI, findings of common and unusual hand and wrist soft-tissue lesions will be illustrated and a systematic MR-based approach for the work-up of these lesions and distinctive MRI features that help clinicians in differential diagnosis will be discussed.

Benign Soft-tissue Mass Lesions of the Hand and Wrist

Ganglion Cyst

Ganglion cysts are non-malignant cystic lesions that arise in relation with joints or ligaments. The most common palpable painless lumps located in the hand and wrist area are ganglia (15). Histopathologically, ganglion cysts consist of a thin fibrous tissue capsule with no real synovial overlaying, and include mucinous substance filled with gelatinous fluid. Previous trauma, mucosal degeneration and synovial herniation are possible reasons which result in ganglion cyst formation, although exact etiology of ganglion cyst is unclear. Characteristically, they are adjacent to the underlying joint capsule, tendon sheath or bursae. They tend to arise in young females. They mostly occur in dorsal aspect of the wrist where they generally originate from the scapholunate joint or ligament. Lesser specific regions comprise volar aspect of the wrist where they emerge from the radio-scaphoid or scaphotrapezial joint. In the fingers, the most frequent site for ganglia is third and fourth fingers where they arise from metacarpophalangeal joints and distal interphalangeal joints (2). On US and color Doppler US, ganglion cysts typically present as anechoic and avascular noncompressible cystic lesions

Table 1. WHO classification of soft-tissue tumors

Tumor type	Tumor	Patient age	Anatomic location
Adipocytic	Lipoma	Adulthood (5^{th}-7^{th} decades)	Subcutaenous; intramuscular; intermuscular
Fibroblastic/myofibroblastic	Nodular fasciitis, myositis ossificans, fibroma of tendon sheath	Young adulthood (3^{th}-5^{th} decades)	Nodular fasciitis: deep subcutaneous region or in the fascia Fibroma of tendon sheath: Tendon sheath
Fibrohistiocytic	Giant cell tumor of the tendon sheath	Adulthood (4^{th}-6^{th} decades)	Tendon sheaths
Smooth muscle	Angioleiomyoma (vascular leiomyoma)	Adulthood (4^{th}-7^{th} decades)	Subcutanous; tunica media of the veins
Skeletal muscle	Rhabdomyoma	Young adulthood (3^{th}-5^{th} decades)	Head and neck region
Pericytic (perivascular)	Glomus tumor	Adulthood (4^{th}-5^{th} decades	Tip of fingers; neuromyoarterial apparatus
Vascular	Hemangioma, epitheloid hemangioma, angiomatosis, lymphangioma	Childhood	Subcutanous; striated muscle; multicompartmental
Chondro-osseous	Juxtacortical chondromas, soft-tissue chondromas	Adolescent, young adulthood (2^{th}-4^{th} decades	Juxtacortical chondromas: Periosteum of tubular bones Soft-tissue chondromas: Origin unknown
Lesions not included in the WHO classification of soft-tissue tumors			
Lesion type	**Lesion**	**Patient age**	**Anatomic location**
Tumorlike	Ganglion cyst, hematoma, seroma, abscess, epidermal inclusion cyst, foreign body granuloma, anomalous muscle	Ganglion cyst: Young women Epidermal inclusion cyst: 4^{th}-6^{th} decades	Ganglion cyst: Adjacent to joint capsule, tendon sheath, bursae Epidermal inclusion cyst: Subcutaneous
Benign	Peripheric nerve sheath tumor (PNST): schwannoma (neurilemoma), neurofibroma, perineurioma	Adulthood (3^{th}-5^{th} decades)	Extending along nerves (superficial or deep)

(7). Floating internal echoes and internal septations may be present within the lesions. MRI displays a well-circumscribed unilocular or multilocular masses of fluid signal adjacent to a joint or tendon sheath. Although the signal of the lesions may be variable depending on the quantity of proteinaceous substance and presence of hemorrhage. On contrast-enhanced T1-weighted sequences, slight enhancement of the thin wall and septa may be observed. But there is commonly no enhancement of the interior contents of the cyst unless there is rupture (7,16) (Figure 1). The absence of pericapsular soft tissue contrast enhancement is a clue for differential diagnosis of a ruptured ganglion cyst from other solid soft-tissue lesions (7). A thin pedicle from ganglion cyst to adjacent joint or tendon sheath is often present.

Epidermal Inclusion Cyst

Epidermal inclusion cysts (EICs) are the most encountered type of cutaneous lesions representing with well-encapsulated mobile painless nodules. Congenital misplacement of residual ectodermal tissues, obstruction of the pilosebaceous unit by adjacent inflammation or tumor, human papilloma virus infection, and traumatic or surgical implantation of epithelial components into the dermis are proposed mechanisms for epidermal inclusion cyst formation (17). There is a mild male predominance, and middle -aged adult population is affected mostly. Because hands are particular body regions with increased risk of penetrating injuries, EICs commonly occur in the hands (11). There are imaging features of EICs related to the cyst maturation and cyst content composed of keratin and cholesterol. US and color Doppler US show well-circumscribed heterogenous mildly echogenic avascular mass lesions. On MRI, EICs appear hyperintense on both T1-weighted and T2-weighted images secondary to high lipid concentration (Figure 2) (12). Small low-signal foci on T2-weighted sequences may be present secondary to existence of internal keratin debris and microcalcifications. Peripheral thin rim enhancement can be observed on post-contrast T1-weighted images. Absence of central enhancement on postcontrast T1-weighted images is the characteristic MRI finding of non-complicated EICs (12). Ruptured EICs may simulate inflammatory or neoplastic lesions with thick and irregular peripheral rim enhancement and surrounding soft-tissue edema (18).

Giant Cell Tumor of the Tendon Sheath

Giant cell tumors of tendon sheath (GCTTS) or localized type tenosynovial giant cell tumors are benign and slow-growing masses of unknown etiology arising from tendon sheaths. They are the most common solid lesion arising from soft-tissues in the hand (10). They frequently present between 4th and 6th decades with a slight female dominance. The volar aspects of first three fingers are frequently affected and much less commonly the wrist is affected. Flexor tendons are affected twice as often as extensors (13). On plain radiography, osseous abnormalities accompany the lesion approximately in 20% of patients. The most common osseous abnormality is extrinsic cortical erosion with scalloping. Calcifications and periosteal reaction are rarely seen (19). GCTTSs present as multilobulated hypoechoic solid masses on US examination. During dynamic US examination, they do not move with the adjacent tendon when the affected digit is flexed or extended (20). On color Doppler US, increased blood flow is commonly seen (21). MRI is more reliable and GCTTS characteristically present as well-defined lesions adjacent to or surrounding

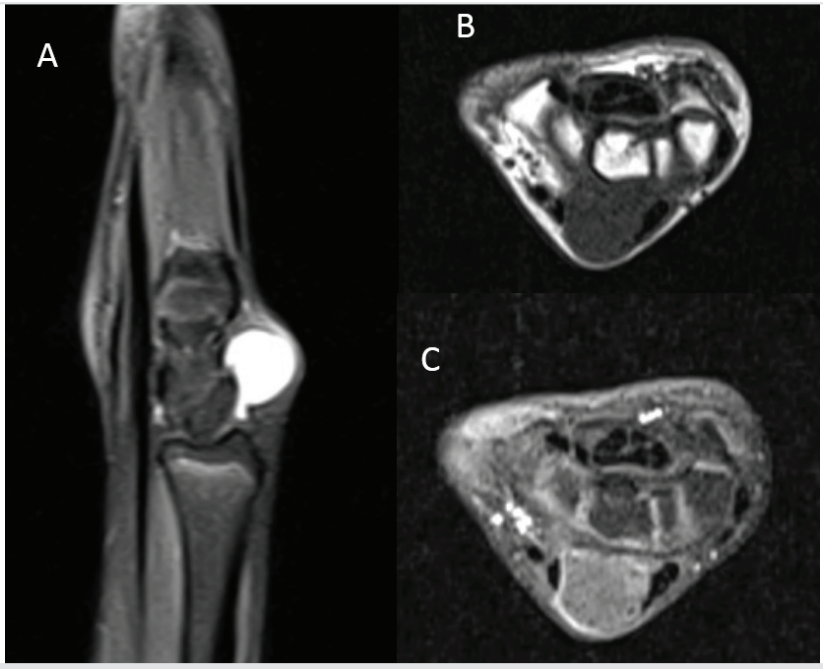

Figure 1. Ganglion cyst in a 12-year-old female presenting with slowly growing painless hand lump. (a) Sagittal fat saturated PDW sequence demonstrates a smooth, well circumscribed lesion with homogeneous low signal adjacent to lunate bone. (b) The lesion is hypointense on axial T1w sequence. (c) After contrast administration, faint rim enhancement is seen with no internal enhancement

a tendon. On T1-weighted sequences, the mass is generally hypointense, and nearly isointense compared to skeletal muscle. Hemosiderin deposition caused by chronic hemorrhage results in prominent hypointensity on T2-weighted sequences (2). On gradient echo sequences blooming artifacts may be present. The pathognomonic finding of GCTTSs on MRI is marked low signal intensity on T2-weighted images and blooming artifact caused by hemosiderin deposition. GCTTSs commonly show hyperintensity on STIR images (10). Existence of abundant proliferative capillaries within the collagenous stroma is the cause of vivid enhancement in GCTTSs (Figure 3) (11).

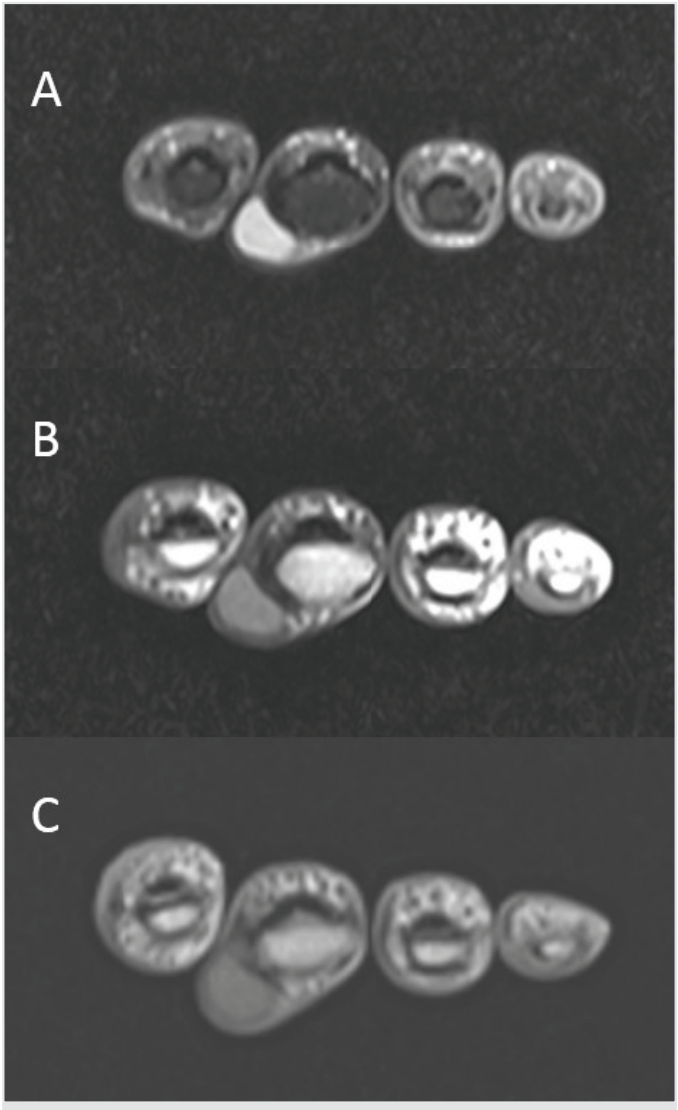

Figure 2. a-c. Epidermal inclusion cyst in a 45-year-old male presenting with a lump over the third proximal interphalangeal joint. Axial PD-weighted fat-saturated (a) MR image demonstrates a smooth, well-demarcated subcutaneous hyperintense lesion. Axial precontrast (b) and postcontrast (c) T1-weighted MR images demonstrate a mildly hyperintense mass lesion due to high lipid content with no contrast enhancement

MR: Magnetic resonance, PD: Proton-density

Fibroma of the Tendon Sheath

Fibromas of the tendon sheaths (FTS) are rare lesions of the tendon sheath and consist of fibroblasts within a dense fibrous stroma. They generally manifest as firm, painless, slow- growing masses, and preferentially located in the hand and wrist area (22). They frequently present in 3rd-5th decades and are seen three times more common in males (23). FTS are usually well-defined lesions and have low signal on all MRI sequences secondary to presence of abundant fibrous tissue (2). Due to fibrous component, they may demonstrate no contrast enhancement or demonstrate minimal contrast enhancement (Figure 4). However, signal intensity on T2w sequence and contrast enhancement may be variable, most probably due to different ratio of fibrous and cellular tissue. If a fibroma of the tendon sheath presents as hypointense signal on all MR sequences, it can be confused with GCTTS. On GRE sequence, GCTTSs usually demonstrate "blooming artefact" of accentuated low signal intensity, which is not an expected finding in FTS (2,3).

Leiomyoma

Leiomyomas are solitary, benign, slowly growing tumors that originate from nonstriated muscle. Leiomyomas are commonly found in genitourinary system, and gastrointestinal tract. Locations other than these regions including hand and wrist region are rare. Soft-tissue leiomyomas can be classified into three main groups. The most encountered subtype, the cutaneous leiomyoma, is quite small; therefore it is seldomly interpreted radiologically. The second form, deep soft tissue leiomyoma, is located in deep soft tissues of extremities or the retroperitoneum. The third form, angioleiomyoma, is a rare benign tumor arising from smooth muscle of the veins (3). They present as painful subcutaneous nodules in fingers and they are histopathologically characterized by multiple thick-walled blood vessels with thickened non-striated muscle coating (24). On T1- weighted sequences, angioleiomyomas present as well-demarcated subcutaneous mass lesions hypointense compared to fat and isointense compared to muscle with homogenous strong enhancement after intravenous application of contrast medium. On postcontrast T1-weighted images, a vessel leading up to the lesion can be observed. On T2-weighted sequences, they present as heterogenous high signal masses (3) (Figure 5).

Peripheral Nerve Sheath Tumors

Peripheral nerve sheath tumors (PNSTs) are tumors that originate from nerve sheaths. They can be benign or malignant. The benign forms are classically classified as schwannomas and neurofibromas. Schwannomas are fusiform shaped masses originate from the schwann cells which envelope the peripheral nerves and contain cellular and myxoid components. Schwannomas are encapsulated and localized eccentrically to the parent nerve, and surgical resection can mostly be performed. In contrast, neurofibromas are non-encapsulated tumors consisted of schwann cells and fibroblasts (25). Neurofibromas are fusiform shaped masses located centrally within the involved nerve and tend to separate nerve fibers; therefore surgical excision of the parent nerve with the tumor is required because neurofibromas

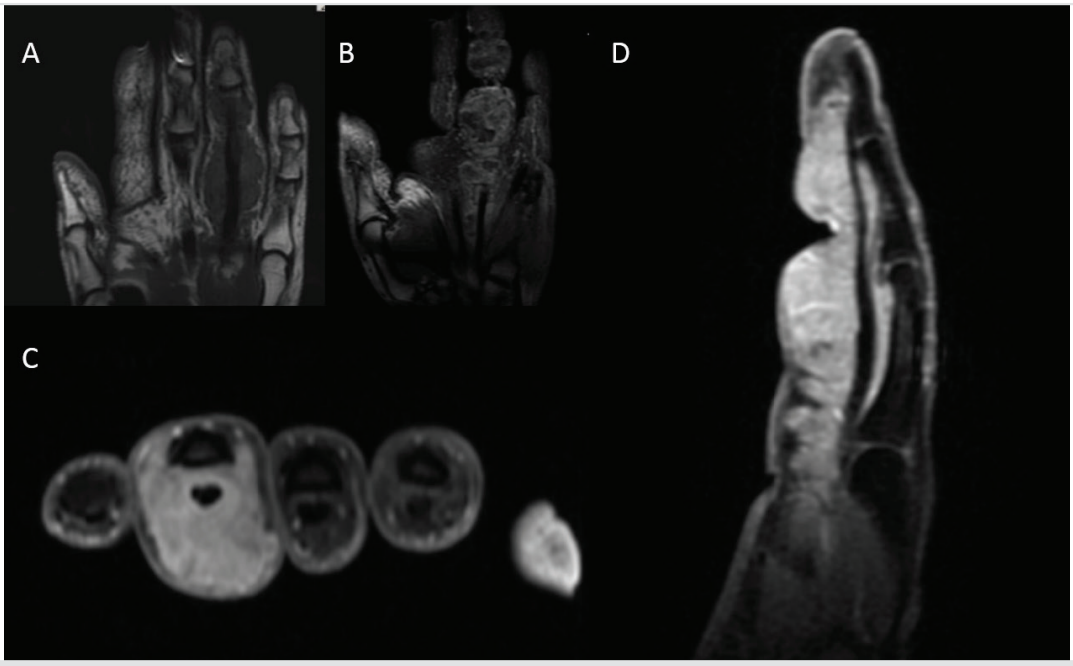

Figure 3. GCCTS of flexor tendon sheath in a 59-year-old-male presenting with a soft-tissue mass in the fourth finger. A lobulated hypointense mass is seen enveloping the flexor tendons of the fourth finger on coronal T1-weighted MR image (a). The mass has heterogenous signal intensity with low signal areas on coronal PD-weighted fat-saturated MR image (b). A significant homogenous contrast enhancement of the mass is observed on the axial and sagittal fat-saturated T1-weighted MR image (c,d)

MR: Magnetic resonance, GCCTS: Giant cell tumors of tendon sheath

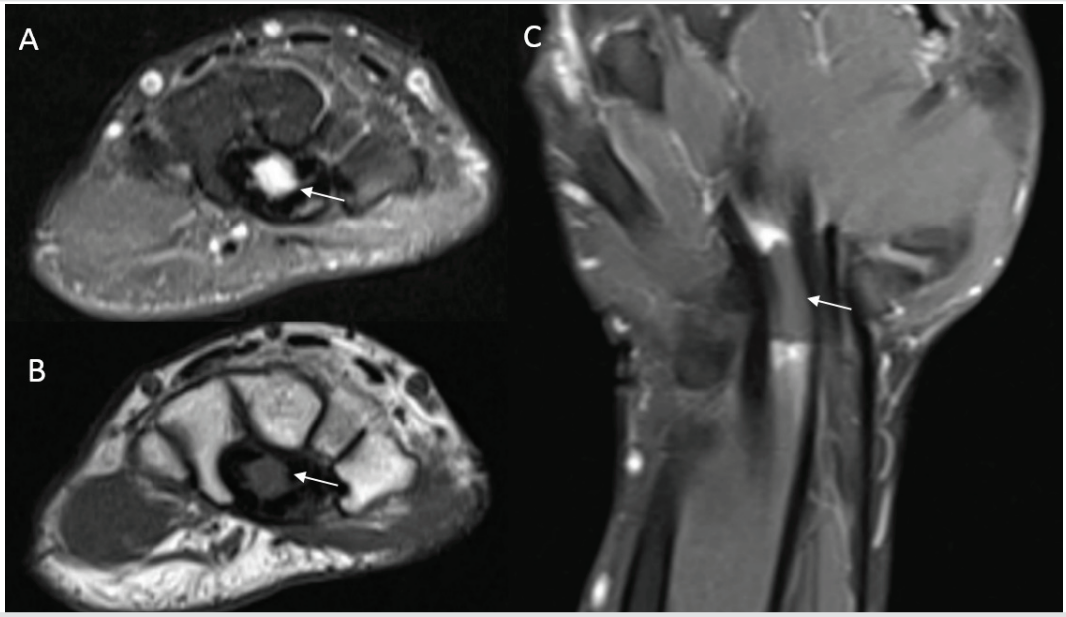

Figure 4. Fibroma of the tendon sheath in a 49-year-old male presenting with a 8-month history of a lump in the left hand on the radial part of the first carpal bone. Axial PD fat-saturated MR image (a) shows a hyperintense mass between the superficial and deep flexor digital tendons. Axial (b) T1-weighted sequence shows a hypointense mass, which is slightly hyperintense compared to muscle. Fat-saturated, contrast-enhanced, coronal T1-weighted sequence (c) reveals foci of contrast enhancement at the anterior and posterior part of the lesion

MR: Magnetic resonance, PD: Proton-density

can not be separated from the nerve fibers. PNSTs are among the common lesions of the wrist and hand. In the hand and wrist region, schwannomas originate from deeper and thicker nerves (especially the ulnar nerve), so they generally arise along the flexor aspect, while neurofibromas tend to involve smaller cutaneous nerves (11). PNSTs usually present as slow growing, painless, solitary small mass lesions (<5 cm). Neurofibromas usually occur in 3rd and 4th decades and schwannomas generally occur in 4th and 6th decades. PSNTs are rarely associated with neurofibromatosis. On MRI, signal pattern of PNSTs is nonspecific. They are isointense or mildly hyperintense compared to muscle on T1-weighted sequences and prominently hyperintense on T2-weighted sequences. PNSTs usually demonstrate significant enhancement following intravenous gadolinium (Figure 6). Sometimes, especially neurofibromas may show no enhancement (26). Heterogenous enhancement may be seen in malignant PNSTs and in the presence of cystic degeneration. "Target sign" with peripheral hyperintense rim surrounding a hypointense center on T2-weighted sequences secondary to central fibromatosis surrounded by myxomatous tissue is more usually observed in neurofibromas, however can also be occasionally observed in schwannomas (25). Other MRI findings especially present in larger tumors include; a fusiform lesion in the characteristic location of the nerve, "string sign" representing the parent nerve entering and exiting the lesion, "plit fat sign" due to the rim of fat that encircles the tumor, and "fascicular sign" secondary to small ring-like components on T2-weighted sequences corresponding to fascicular fibers inside the

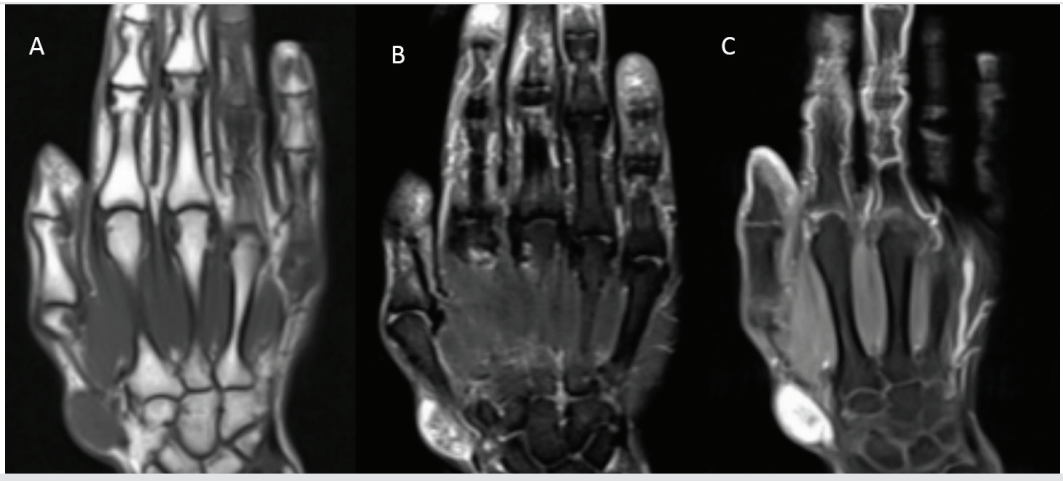

Figure 5. a-c. Angioleiomyoma in a 64-year-old man presenting with a painful mass in the radial aspect of the first carpometacarpal joint. Coronal T1-weighted image (a) shows a well demarcated hypointense mass which is isointense compared to muscle. The mass is heterogenous hyperintense on coronal PD fat-saturated image (b). Coronal, contrast-enhanced, fat-saturated T1-weighted MR image (c) demonstrates strong contrast enhancement

MR: Magnetic resonance

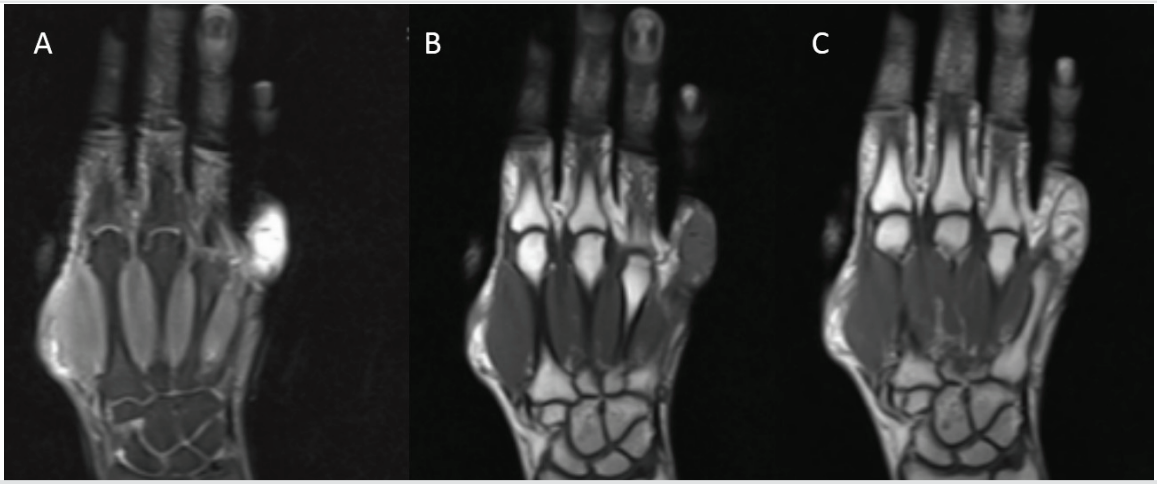

Figure 6. a-c. Schwannoma in a 55-year-old man with a painless mass in the dorsal part of the proximal phalanx of the 5th finger. Coronal fat-saturated PD-weighted MR image (a) shows a subcutaneous, well demarcated fusiform hyperintense lesion. The lesion is hypointense on T1-weighted MR image (b). Coronal contrast-enhanced T1-weighted MR image (c) shows strong contrast enhancement

MR: Magnetic resonance

tumor (27). Malignant change is very rare in PNSTs. Features such as large tumor size (>5 cm), the presence of peritumoral edema, infiltrative margins, marked heterogeneity and fast growth should raise the suspicion of malignancy (26,28).

Hemangiomas and Vascular Malformations

Hemangiomas and vascular malformations are among the commonest soft-tissue lesions throughout the body. Vascular malformations are common in the hand. They are located deeply in the hand, but they are usually located subcutaneously in the fingers (11). Most lesions represent as isolated lesions, while some lesions represent as ill-defined infiltrating lesions involving several anatomic structures. International Society for the Study Vascular Anomalies (ISSVA) classifies vascular anomalies into two major groups according to cellular turnover and histopathological characteristics: benign vascular tumors (hemangiomas) and vascular malformations. Vascular malformations are not true neoplastic lesions; in fact, they are errors of vascular morphogenesis with a normal rate of endothelial turnover. Vascular malformations are subdivided into low-flow (venous, lymphatic, capillary, and mixed) and high-flow types (fistula and arteriovenous malformations) dependent on predominant histologic component and hemodynamic features (29-31).

On USG, hemangiomas appear as iso/hyperechoic well-defined solid mass lesions. However, they can show hyperechoic areas consistent with fatty component. Anechoic vascular channels can also be present on USG. Phleboliths may be seen as bright echogenic foci with posterior acoustic shadowing inside the lesion. On color Doppler US, hypervascularity may be detected in hemangiomas (8,29). On MRI, hemangiomas are typically iso to hypointense compared to muscle on T1-weighted sequences and have high signal intensity on T2-weighted sequences due to increased fluid content secondary to slow blood flow in vessels (Figure 7) (32). Larger lesions (>2 cm) appear more heterogenous on all sequences, because they contain nonvascular components such as fat, smooth muscle, fibrous tissue, myxoid stroma, hemosiderin, and thrombus. Fluid-fluid levels may also be observed in larger masses. Serpentine vascular flow void areas are sometimes present. A feeding vessel or early draining vessel may also accompany the lesion. Enhancement on post-contrast series is variable from mild to heterogeneous, but usually there is strong enhancement following intravenous gadolinium (33).

Capillary malformation, also called port wine stain, is the most common form of vascular malformation. A clinical diagnosis is sufficient in most of the patients; so imaging is rarely needed. Imaging reveals asymmetric skin thickening with no associated mass lesion and apparent vascular channels. Slow flow vascular malformations (lymphatic and venous) are usually multiseptated and display iso to low signal intensity on T1-weighted sequences and high signal intensity on T2-weighted sequences. Venous malformations are composed of enlarged slow-flowing vascular channels with no solid tissue content. Venous malformations show delayed enhancement. They are characterized by phleboliths which results in signal void foci on all pulse sequences. In lymphatic malformations, multiple lymphatic fluid included areas with intervening septa are seen histopathologically. They tend to be more infiltrative than the venous form. On MRI, lesions with micro or macrocsyts are present. Microcystic form of lymphatic malformations show no enhancement but macrocystic form show septated enhancement after intravenous contrast material is injected (29). Arteriovenous malformations (AVMs) are vascular lesions with abnormal linkage between arteries and veins. Central nidus is present in AVMs whereas there is no central nidus in arteriovenous fistulae. On MRI, they present as dilated vascular channels without a soft tissue component. High flow vascular malformations are characterized by arterial feeders, draining veins, and arterial or early venous enhancement (29). Phleboliths may also be present in high flow vascular malformations (34). Doppler US or MR angiography may be applied to demonstrate the high flow and evaluate arterial supply and venous drainage pattern in high flow vascular malformations.

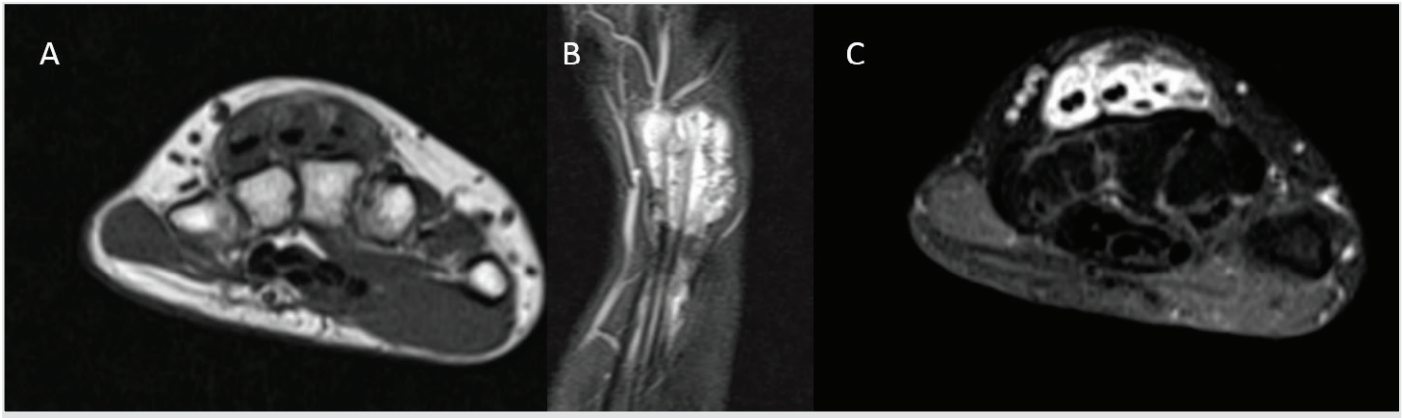

Figure 7. a-c. Hemangioma in a 35-year-old female with a swelling history on the volar part of the hand over the carpal bones and the carpometacarpal joints. Axial T1-weighted MR image (a) shows a hypointense lesion surrounding the extensor tendons of the hand. This lesion is hyperintense on coronal fat-saturated T2-weighted MR image (b). Axial, contrast-enhanced, fat-saturated T1-weighted MR image (c) shows avid and near homogenous contrast enhancement

MR: Magnetic resonance

Glomus Tumor

Glomus tumors are small hamartomas originating from the neuromyoarterial apparatus inside the glomus bodies which are responsible for thermoregulation. Thus, glomus tumors are mostly present at the finger tip, either in the pulp or under the fingernail. They account up to 5% of soft tissue tumors of the hand and characteristically occur in the fourth or fifth decade of life, equally in both sexes (35). Typical clinical presentation is finger pain and tenderness exacerbated by temperature changes and mild trauma. Large lesions located in the nail beds may be seen as red-blue spots. X-rays demonstrate smooth extrinsic erosions of dorsal aspect of the fingers if the lesions are large enough. On MRI, they present as small, smooth-contoured masses with low or intermediate signal on T1-weighted images and with homogenously high signal intensity on T2-weighted images, and show uniform strong enhancement following intravenous contrast material injection (Figure 8) (10). The imaging properties of glomus tumors resemble to vascular tumors, but typical distal location and relatively small size may help clinicians in differential diagnosis.

Nodular Fasciitis

Nodular fasciitis is a rapidly growing benign reactive fibroblastic mass thought to be related with trauma. It is commonly located on volar aspect of hand (36). It usually occurs in young adults. It is composed of various amount of myxoid, cellular, or fibrous tissues which result in variable MRI appearance of the lesion (22). Lesions with high cellularity or myxoid component appear hypointense or nearly isointense compared to skeletal muscle on T1-weighted sequences and high signal on T2-weighted sequences. On the contrary, those with high fibrous component are hypointense on all MRI sequences. Contrast enhancement is frequently uniform, but it may also be peripheral in lesions with an extensive extracellular myxoid matrix and central cystic degeneration (22). Fascial tail sign is an important diagnostic feature of nodular fasciitis which is characterized by linear extension of the lesion along the adjacent fascia on MRI (13). Due to rapid growth pattern and nonspecific MRI findings, nodular fasciitis may mimic a malignant soft tissue lesion. Differential diagnoses should include soft tissue sarcoma, fibrous histiocytoma, peripheral nerve sheath tumor and early stage of myositis ossificans.

Extraosseous Chondroma

Extraosseous chondromas consist of articular chondromas, juxtacortical chondromas (also known as periosteal or parosteal chondroma) and soft tissue chondromas. Nearly all juxtacortical and soft tissue chondromas occur in the hands and feet. They are usually located in fingers. Soft tissue chondromas and juxtacortical chondromas only differ in their anatomical location (3). Young and middle-aged adults are most frequently affected. X-rays demonstrate an ovoid or lobulated soft-tissue mass with sharply demarcated borders. Calcifications occur up to in 50% of chondromas which may be central or peripherally located. A surrounding shell of continuous or discontinuous bone may also be present on plain films or CT (37). MRI appearance of chondromas depends on tumor contents and the grade of matrix calcification. On T1-weighted images these tumors are isointense to hypointense compared to skeletal muscle. On T2-weighted sequences they appear typically hyperintense due to high fluid content of chondroid matrix. If calcification is present, hypointense foci can be seen on both sequences. After intravenous gadolinium administration, septal or peripheral enhancement is seen similar to enchondromas (Figure 9) (38).

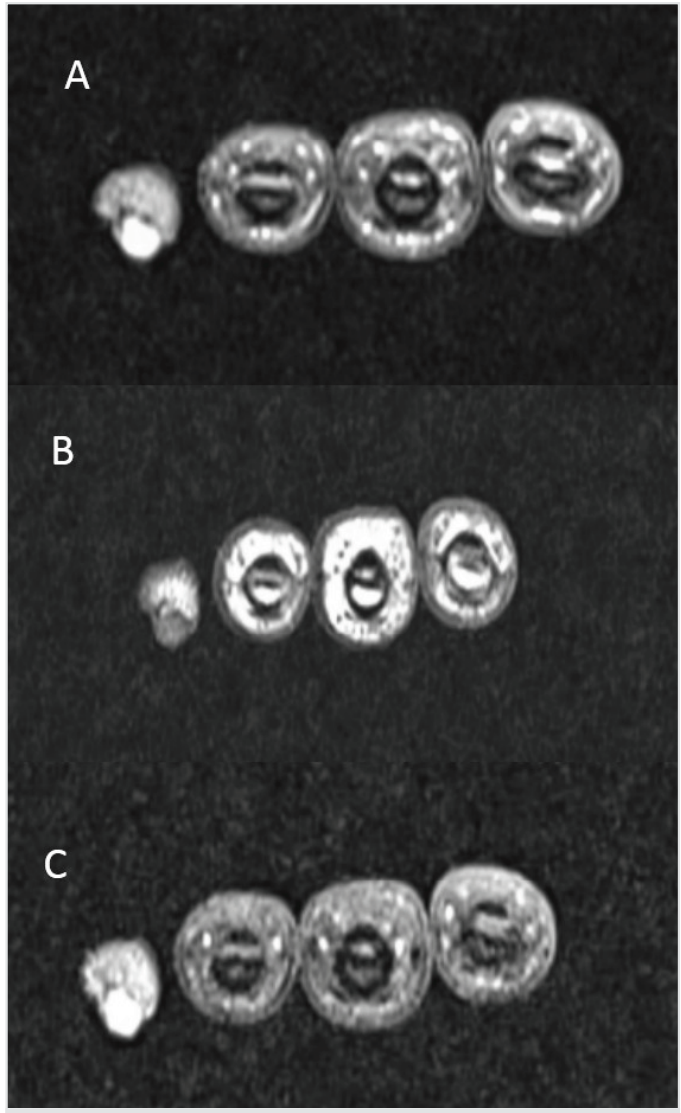

Figure 8. Glomus tumor in a 35-year- old female with a painful lump on the tip of the right 5th finger. Axial, PD-weighted fat-saturated sequence (a) shows a smooth-well circumscribed subcutaneous lesion of extremely high signal intensity. Axial T1-weighted sequence (b) shows intermediate signal intensity lesion. After contrast administration, intense and homogenous enhancement is present on axial contrast-enhanced fat-saturated T1-weighted MR image (c)

MR: Magnetic resonance, PD: Proton-density

Lipoma

Lipomas are consisted of benign mature adipocytes. In spite of the fact that lipomas are the most common soft tissue tumors in the body, they are relatively uncommon in the hand and wrist region (39,40). When they are located in upper extremity, they are frequently located in subcutaneous region, however, may also be located in muscles, bones, and tendons. In hand and wrist, they typically present as slow-growing painless masses at the thenar or hypothenar eminence. On US, they appear usually as homogeneous solid mass lesions with or without a capsule and are predominantly hyperechoic or isoechoic compared to surrounding fat tissue. On Doppler US, they show no vascularity or show minimal vascularity (8). On MRI, lipomas have the similar MRI signal pattern as the subcutaneous fat tissue. So, they are seen as encapsulated masses that are hyperintense on both T1-weighted and T2-weighted sequences (3). They show homogenous signal loss on the STIR or fat-saturation sequences. Lipomas usually show no contrast enhancement after intravenous contrast material administration. Thin (less than 2 mm) septae and nonfatty components with low signal intensity on both T1- and T2-weighted sequences can be seen inside the lesions. Minimal contrast enhancement of septa may be present. It can be hard to differentiate benign lipomas from atypical lipomatous tumor (synonymous with well-differentiated liposarcoma in the extremities) or liposarcomas. Thick enhancing septations (>2 mm) or nodules and persistent areas of high signal on STIR or fat-saturated sequences should raise the suspicion of liposarcoma (41).

Lesions Associated with Rheumatic Diseases and Metabolic Disorders

Rheumatoid nodules (RN) are typically encountered in subjects with severe long-standing rheumatoid arthritis (RA), although, infrequently, they may be also observed in other autoimmune disorders including systemic lupus erythematosus, ankylosing spondylitis, and rheumatic fever. Infrequently, RNs precede the articular findings of rheumatic disorders. Histopathologically, RNs are composed of chronic inflammatory cells and palisading fibroblasts with or without central necrosis. They frequently arise on the dorsal aspect of the hand and fingers inside the subcutaneous tissue (particularly at sites of compression, traction, and repetitive microtrauma), however the bursae, joints, tendons, and ligaments can also be included (12). RNs are observed approximately in 20% of subjects with RA. MRI features of RNs are non-specific. On MRI, RNs present as ill-defined masses inside the subcutaneous tissue demonstratingalow to intermediate signal intensity on T1-weighted sequences. The signal intensity of the nodules on T2-weighted images is variable. Solid nodules appear hypointense, while cystic lesions appear hyperintense on T2-weighted sequences (7). Contrast enhancement is also variable. Solid nodules show intense and homogenous contrast enhancement. Nodules with cystic components show weak or peripheral contrast enhancement (Figure 10) (12).

Crystal deposit diseases including gout and pseudogout (hydroxyapatite and calcium pyrophosphate dehydrate deposition disease) may rarely present as focal periarticular soft-tissue mass at the hand and wrist region (7). Clinical and imaging findings of gout and pseudogout are usually specific. Soft-tissue masses with or without calcifications causing juxtacortical erosions with overhanging edges may be seen on hand X-rays. On T1-weighted sequences, gouty tophi and pseudogout tend to be hypo to isointense. On T2-weighted MR sequences, related to degree of inflammation and presence of calcification, they demonstrate heterogenous hypo or hyperintense signal intensity (3,7). Gouty tophi can show diffuse or peripheral contrast enhancement.

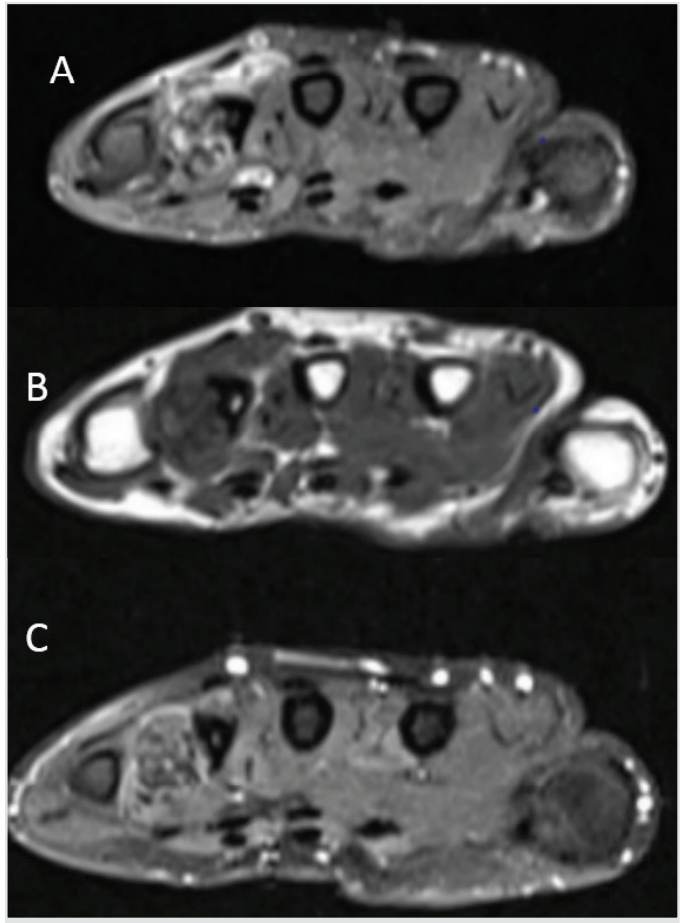

Figure 9. Juxtacortical chondroma in a 37-year-old female presenting with a painless mass lesion on the distal part of the 4th metacarpal bone, who has described slow growth over the previous 1 year. Lesion appears hyperintense due to high fluid content of chondroid matrix and includes hypointense foci consistent with calcifications on axial PD-weighted fat-saturated sequence (a). Axial T1-weighted image (b) shows a hypointense lobulated heterogenous mass. After contrast administration, heterogenous enhancement is observed on axial contrast-enhanced fat-saturated T1-weighted MR image (c)

MR: Magnetic resonance, PD: Proton-density

Table 1. WHO classification of soft-tissue tumors

Tumor type	Tumor	Patient age	Anatomic location
Adipocytic	Lipoma	Adulthood (5^{th}-7^{th} decades)	Subcutaenous; intramuscular; intermuscular
Fibroblastic/myofibroblastic	Nodular fasciitis, myositis ossificans, fibroma of tendon sheath	Young adulthood (3^{th}-5^{th} decades)	Nodular fasciitis: deep subcutaneous region or in the fascia Fibroma of tendon sheath: Tendon sheath
Fibrohistiocytic	Giant cell tumor of the tendon sheath	Adulthood (4^{th}-6^{th} decades)	Tendon sheaths
Smooth muscle	Angioleiomyoma (Vascular leiomyoma)	Adulthood (4^{th}-7^{th} decades)	Subcutanous; tunica media of the veins
Skeletal muscle	Rhabdomyoma	Young adulthood (3^{th}-5^{th} decades)	Head and neck region
Pericytic (perivascular)	Glomus tumor	Adulthood (4^{th}-5^{th} decades	Tip of fingers; neuromyoarterial apparatus
Vascular	Hemangioma, epitheloid hemangioma, angiomatosis, lymphangioma	Childhood	Subcutanous; striated muscle; multicompartmental
Chondro-osseous	Juxtacortical chondromas, Soft-tissue chondromas	Adolescent, young adulthood (2^{th}-4^{th} decades	Juxtacortical chondromas: Periosteum of tubular bones Soft-tissue chondromas: Origin unknown
Lesions not included in the WHO classification of soft-tissue tumors			
Lesion type	**Lesion**	**Patient age**	**Anatomic location**
Tumorlike	Ganglion cyst, hematoma, seroma, abscess, epidermal inclusion cyst, foreign body granuloma, anomalous muscle	Ganglion cyst: Young women Epidermal inclusion cyst: 4^{th}-6^{th} decades	Ganglion cyst: Adjacent to joint capsule, tendon sheath, bursae Epidermal inclusion cyst: Subcutaneous
Benign	Peripheric nerve sheath tumor (PNST): schwannoma (neurilemoma), neurofibroma, perineurioma	Adulthood (3^{th}-5^{th} decades)	Extending along nerves (superficial or deep)

Figure 10. a-c. Rheumatoid nodule in a 40-year-old male, with a 5-year history of known rheumatoid arthritis. Sagittal PD-weighted fat-saturated sequence (a) reveals a well-demarcated subcutaneous lesion composed of solid and cystic components. The solid component is mildly hyperintense and the cystic component is hypointense on coronal T1-weighted sequence (b). After contrast administration, solid component shows mild to moderate contrast enhancement on coronal contrast-enhanced fat-saturated T1-weighted MR image (c)

MR: Magnetic resonance

Table 2. Characteristic MRI findings that may help identify benign soft-tissue tumors and tumor-like lesions of hand and wrist region

Lesion type	Speficic finding (If present)	T1 signal intensity	T2 signal intensity	Contrast enhancement pattern
Ganglion cyst	A thin pedicle extending to adjacent joint space	A well-circumscribed unilocular or multilocular mass with similar to fluid (low signal)	A well-circumscribed unilocular or multilocular mass with similar to fluid (very high signal)	Slight enhancement of the thin wall
Epidermal inclusion cyst	Volar subcutanous location	Moderate high signal	High signal	No central enhancement Thin peripheral enhancement or Absent peripheral enhancement
Giant cell tumor of the tendon sheath	Well-defined lesions surrounding a tendon	Hypointense	Marked low signal intensity "Blooming artefact" on gradient-echo sequences	Moderate to significant enhancement
Fibroma of the tendon sheath	Well-defined lesions adjacent to a tendon	Hypointense	Hypointense "Blooming artefact" absent	No or mininimal contrast enhancement Moderate to marked diffuse enhancement
Angioleiomyoma	Subcutanous nodules A vessel leading up to the lesion on postcontrast T1-weighted images	Hypointense to fat and isointense to muscle	Heterogenous high signal	Strong enhancement
Peripheral nerve sheath tumor	"String sign" "Split fat sign" "Fascicular sign" "Target sign"	Isointense or slightly hyperintense to muscle	Hyperintense; hyperintense rim and central area of a low signal in target sign lesions	Variable (generally intense; central in target sign lesions
Hemangioma	Lobulated, septated, multicompartmental; Well-circumscribed or poorly defined margins	Iso to hypointense to muscle Hyperintense regions in fat containing lesions or lesions with hemorrhage Fat within and at the periphery of the lesion is diagnostic Phleboliths seen as low-signal intensity foci	High signal intensity Typically high T2 signal intensity in areas of slow flow Serpentine vascular flow void areas in high flow lesions Fluid-fluid levels in larger lesions Phleboliths seen as low-signal intensity foci	Variable from mild to heterogeneous; usually strong enhancement
Venous malformation	Slow-flowing vascular channels with no solid tissue component Multiseptated lesion	Iso to hypointense to muscle In the presence of phlebolits; signal void foci	High signal intensity No evidence of high flow velocity signal voids In the presence of phlebolits; signal void foci	Delayed enhancement.
Lymphatic malformation	Multiseptated lesion Infiltrative Lesions with multiple micro or macrocsyts	Iso to hypointense to muscle	High signal intensity Fluid-fluid levels	Microcystic form of lymphatic malformations show no enhancement. Macrocystic form show septal enhancement

Table 2. Characteristic MRI findings that may help identify benign soft-tissue tumors and tumor-like lesions of hand and wrist region

Lesion type	Speficic finding (If present)	T1 signal intensity	T2 signal intensity	Contrast enhancement pattern
Arteriovenous malformation	Dilated vascular channels without a soft tissue component and with an intervening central nidus	Dilated hypointense vascular channels	Dilated hypointense vascular channels	Intense enhancement of arterial feeders and draining veins. Early venous enhancement
Glomus tumor	Small, smooth-contoured mass at the finger tips	Hypo or intermediate signal	Homogenously high signal	Uniform strong enhancement
Nodular fasciitis	Fascial tail sign	Variable (Nearly isointense to muscle, or hypointense)	Variable (hyper or hypointense)	Uniform; rarely peripheral
Extraosseous chondroma	Solitary ovoid or lobulated soft-tissue mass with sharply demarcated borders adjacent to phalanx	Isointense to hypointense to skeletal muscle Hypointense foci in the presence of calcifications	Typically hyperintense due to high fluid content of chondroid matrix Hypointense foci in the presence of calcifications	Septal or peripheral enhancement
Lipoma	Subcutaneous mass lesion located at the thenar or hypothenar eminence Homogenous signal loss on the STIR or fat-saturation sequences Infiltrating or insinuating margins tend to suggest benign lipoma rather than liposarcoma	Similar signal to subcutaneous fat tissue; hyperintense	Similar signal to subcutaneous fat tissue; hyperintense	No contrast enhancement
Rheumatoid nodule	Ill-defined masses in the subcutaneous tissue	Iso to hypointense to muscle	Variable. Solid nodules are hypointense, cystic lesions are hyperintense	Variable. Solid nodules demonstrate intense and homogenous contrast enhancement. Cystic nodules demonstrate weak or peripheral contrast enhancement
Gout-pseudogout	Nodular masses in the subcutaneous tissue	Hypo to isointense to muscle	Heterogenous hypo or hyperintense signal intensity	Diffuse or peripheral contrast

Table 3. MRI features which indicate malignancy

Diameter >5 cm
Lobulation
Peritumoral edema
Bone and neurovascular bundle involvement
Fascial edema; extension through the fascia
Skin thickening
Skin contact
Hemorrhage
Necrosis
Contrast enhancement: Type 2 curve pattern
Thick rim enhancement
Solid internal enhcencement
Presence of thickened septae (>2 mm)
MRI: Magnetic resonance imaging

Lesion Characterization Based on MRI and Specific Features that are Useful in Differential Diagnosis of Benign Lesions from Malignant Counterparts

The MRI with its high contrast and spatial resolution is the most important imaging modality in radiological examination of hand and wrist mass lesions. Characteristic MRI findings that may help identify benign soft-tissue tumors and tumor-like lesions of hand and wrist region are demonstrated in Table 2. By noting the signal characteristics and determining the lesion location, a confident diagnosis can often be made in certain lesions such as lipomas, ganglion cysts, GCSTs, and PNSTs. Beyond mentioned certain lesions, the exclusion of malignancy should be the first and most important step for the treatment algorithm. In literature, various MRI features of hand and wrist soft-tissue lesions that may allow differentiation between benign and malignant lesions were reported (Table 3) (4-6,13-14,42). Knowing these various MRI features, rendering biopsy of these lesions that may lead to unnecessary mortality and morbidity redundant. Histologic examination should be the definite and

final method to establish the diagnosis especially in lesions which demonstrate indeterminate MRI characteristics.

Conclusion

Wrist and hand may be involved by different types of soft-tissue lesions. Most of these lesions are benign. Patients' history, clinical evaluation and diagnostic imaging, especially the MRI with its high soft-tissue contrast would play an important role in appropriate management of these lesions. By noting the signal features and defining the lesion localization, an exact diagnosis can often be made in particular lesions such as lipomas, ganglion cyts, GCSTs, enchondromas, and PNSTs.

Peer-review: Externally peer reviewed.

Authorship Contributions

Data Collection or Processing: H.T., B.A., F.C.S., A.A., V.G., Analysis or Interpretation: V.G., Writing: T.F.Y., H.T., A.A.,

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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