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EDITORIAL

Dear Readers,

We are happy to be with you in the last issue of this year. Publication language of our journal is going to be English by 2020, but we will continue to accept articles submitted in Turkish. The year 2019 has been a pretty successful year for us. In addition to national cooperation, we made meetings with international publishing houses and indexes to increase our recognition. The new indexes that we started to be indexed in this year are as follow: **J-Gate, EuroPub, DOAJ, Hinari, GOALI, ARDI, OARE, AGORA, and ProQ.** As is known, **Emerging Sources Citation Index, TUBITAK ULAKBIM TR Index, EBSCO, Cinahl, CiteFactor, TurkMedline, Turk Atıf Dizini, İdealOnline, ROOT INDEXING, J-Gate, EuroPub, DOAJ, Hinari, GOALI, ARDI, OARE, AGORA, ProQuest** were the indexes that we were indexed in. With your contributions, I believe that we will experience better developments in 2020.

In our new issue, we are very happy to be with you once again with beautiful topics. In this issue; the article entitled "The Utility of the Novel Hematologic Parameters for Predicting in Hospital Mortality in Patients with Acute Mesenteric Ischemia" by YILDIZ et al., the article entitled "Adjuvant Chemotherapy in Elderly Patients with Early-Stage Non-Small Cell Lung Cancer" by KEFELI et al., the article entitled "Results of Reverse Obliquity Intertrochanteric Fractures Treated with Third Generation Proximal Femur Nails" by ULKU et al., and the article entitled "Investigation of Foot Pressure Distribution in Asymptomatic Individuals with Mild Hallux Valgus" by TAS et al. are the articles that are in the forefront.

We are also happy that the recent articles that we received were mostly from the faculties of health sciences. It was one of our goals to be a journal where every branch and discipline in the field of health found a place for itself. We are happy to approach our goals step by step in Bezmialem Science with the strength we have received from you.

I would like to thank you again, our esteemed readers, writers and referees for your support on behalf of me and our board of editors.

Kindest regards,

Prof. Dr. Adem AKCAKAYA
Editor-in-Chief

Original Article

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The Utility of the Novel Hematologic Parameters for Predicting in Hospital Mortality in Patients with Acute Mesenteric Ischemia

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ABSTRACT

Objective: Acute mesenteric ischemia (AMI) is recognized as a vascular emergency, having high mortality and requiring rapid and efficient and treatment. In this study, we aimed to assess the levels of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) in patients with AMI and to search for a relationship between the NLR and PLR values and inhospital mortality of patients with AMI.

Methods: In total, one hundred and twenty three patients (67 AMI+ and 56 AMI-) were included in this retrospective study. Sixty seven patients diagnosed as having AMI following computed tomography angiography after presenting to the emergency room were included in AMI (+) group. "Receiver Operating Characteristic" analysis was done to determine the optimal NLR and PLR thresholds in diagnosis and inhospital mortality of AMI. Also, univariate and multivariate analyses were made to evaluate the complete blood count parameters affecting the outcomes of patients with AMI.

Results: It was found that AMI+ patients had higher neutrophil count than AMI- patients, while the lymphocyte count was significantly lower ($p<0.001$ $p<0.001$, respectively). The NLR and PLR values were significantly higher in AMI+patients than in AMI-patients ($p<0.001$, $p<0.001$, respectively). In the multiple logistic regression analysis, only NLR and red cell distribution width were identified as independent predictors of inhospital mortality of patients with AMI [odds ratio (OR)=1.171, 95% confidence interval (CI)=1.020-1.345, p=0.026; OR=1.474, 95% CI=1.028-2.113, p=0.035, respectively]. Although PLR failed to predict inhospital mortality in AMI+ subjects (area under the curve (AUC=0.597, 95% CI=0.459-0.734, $p<0.178$), NLR level greater than 10.1, measured on admission, predicted inhospital mortality in AMI+ subjects (AUC=0.698, 95% CI=0.570-0.827, $p<0.006$).

Conclusion: Neutrophil-to-lymphocyte ratio is a reliable predictive marker in hospital mortality in patients with AMI.

Keywords: Neutrophil to lymphocyte ratio, acute mesenteric ischemia, mortality

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Introduction

Although acute mesenteric ischemia (AMI) accounts only 1% of all hospitalizations with acute abdomen, this rate may increase up to 10% in patients over 70 years of age (1). Primary AMI is a vascular disease and can be classified by etiologies as follows: Mesenteric arterial embolism, mesenteric arterial thrombosis, mesenteric venous thrombosis, and non-occlusive mesenteric arterial ischemia (2). Predisposing risk factors are as follows: Atrial fibrillation, heart failure, coronary heart disease, arterial hypertension, severe valvular cardiac disease and peripheral arterial occlusion (3). In contrast with its low incidence, mesenteric ischemia may be associated with very high mortality rates up to 40-70% (4). Major factors responsible for high mortality include late presentation, absence of simple and reliable biochemical parameters and experienced radiologists, and late diagnosis (5). In contrast with other conditions with abdominal pain of acute onset, AMI is more likely to result in the development of irreversible complications (6). Early diagnosis is of utmost importance for reducing the mortality rate in AMI. Although computed tomography (CT) angiography is considered the gold standard imaging modality in early diagnosis, currently no standard biochemical parameters exist that can be used for routine diagnosis of this condition. Laboratory investigations frequently demonstrate leukocytosis, metabolic acidosis, an elevated D-dimer and elevated serum lactate (7-9). Laboratory studies are non-specific; while abnormal laboratory values may be helpful in bolstering suspicion for AMI, normal laboratory values do not exclude AMI.

Detection of embolic or thrombotic arterial occlusion of the superior mesenteric artery (SMA) in 70 to 80% of patients diagnosed as having AMI after presentation at emergency room has fueled an interest in the use of novel hematological parameters in complete blood count (CBC) including NLR and PLR that indicate the inflammatory and thrombotic status (4,10-13). Although several studies suggested that high NLR and platelet to lymphocyte (PLR) may represent significant markers for early diagnosis of AMI, currently no data exists on the use of NLR and PLR in predicting inhospital mortality in AMI. The aim of this article is to search: (i) whether there exists an association between novel hematologic parameters (NLR, PLR) and AMI; (ii) whether elevated NLR and PLR counts are associated with mortality following AMI.

Methods

Study Design and Setting

In this retrospective study investigating novel CBC parameters that may have a predictive value in AMI mortality, a total of 67 patients diagnosed as having AMI following CT angiography after presenting to the emergency unit of our institute between 2010 and 2014 were included in AMI (+) group . The study was approved by the Local Ethics Committee.

Selection of Participants

A total of 56 age- and gender-matched individuals presenting with abdominal pain but without detection of AMI in CT angiography comprised the AMI (-) group. Patients with active infection, systemic inflammatory conditions, malignancy, advanced hepatic failure, renal failure, thyroid disease, use of corticosteroid or anti-inflammatory medications, and hematological conditions were excluded.

Baseline Measurements

Prior to the initiation of any medical treatments, venous blood samples were drawn from the antecubital vein and were collected in ethylene diamine tetraacetic acid containing tubes. Automatic blood counter (CELL-DYN Ruby, Abbott Diagnostics, Abbott Park, IL, USA) was used for whole blood count. NLR and PLR were calculated as the ratio of the neutrophils and platelets to lymphocytes, respectively, both obtained from the same automated blood samples that were taken at the time of admission to the study. CRP was measured using Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN).

Statistical Analysis

Descriptive statistics included mean, standard deviation, minimum, maximum, and median, while categorical variables were expressed as number and percent. Independent group comparisons were performed using Student t-test for variables with normal distribution and using Mann-Whitney U test in the absence of normal distribution. Percent comparisons between independent groups were performed with chi-square analysis. Risk factors were analyzed using the logistic regression analysis. Receiver operating curve (ROC) curve analysis was used to detect the predictive value of PLR and NLR for AMI mortality. The sensitivity, specificity, and positive and negative predictive value (PPV and NPV) were calculated using different cut-off values. P values less than 0.05 were considered significant, and the confidence interval (CI) was 95%. The statistical analyses were performed using SPSS, version 17 (SPSS, Chicago, Illinois, USA).

Results

Our study included a total of 123 subjects, 67 AMI (+) and 56 AMI (-) patients. All participants presented to the emergency room with abdominal pain. The baseline demographic characteristics and laboratory parameters of patients are shown in Table 1. There were no significant differences between the two groups in terms of age, gender, hemoglobin, platelet, and red cell distribution width (RDW). AMI+ patients had higher neutrophil count than AMI- patients, while the lymphocyte count was significantly lower ($p<0.001$ ve $p<0.001$, respectively). NLR was significantly higher in AMI+ patients than in AMI- patients ($p<0.001$) (Figure 1a). Platelet levels were not found to be significantly different between the groups ($p=0.111$). However, PLR was statistically higher in AMI+ subjects than

in AMI- subjects (p<0.001) (Figure 1b). There was positive correlation between NLR and CRP (r=0.544, p<0.001), and between PLR and CRP (r=0.525, p<0.001).

Of AMI+ patients, 43% had atrial fibrilation (AF) rhythm. A lesion with ≥70% narrowing was present in superior mesenteric artery (SMA) and inferior mesenteric artery (IMA) in 88% and 12% of the patients, respectively. The inhospital mortality rate was 48%. Atrial fibrillation and mortality rates did not differ significantly between patients with SMA or IMA. Univariate logistic regression analysis showed significantly higher mean PLT and PLR values in patients with AF than in patients with sinus rhythm among those with AMI+ (p<0.001, p=0.010, respectively). In the multivariate logistic regression model NLR and RDW emerged as the most significant factors [odds ratio (OR)=1.171, 95% confidence interval (CI)=0.020-1.345, p=0.026, OR=1.474, 95% CI=1.028-2.113, p=0.035, respectively) that predict mortality.

For determination of the best "cut-off" values of NLR and PLR in predicting the inhospital mortality among AMI+ patients, a receiver operating curve (ROC) characteristics analysis was performed. The analysis of PLR failed to determine such cut-off values for predicting inhospital mortality (AUC value of 0.597, 95% CI=0.459-0.734, p<0.178). A NLR level greater than 10.1, measured on admission, yielded an area under the curve (AUC) value of 0.698 (95% CI=0.570-0.827, sensitivity 78%, specificity 61%, p<0.006) (Figure 2a, 2b).

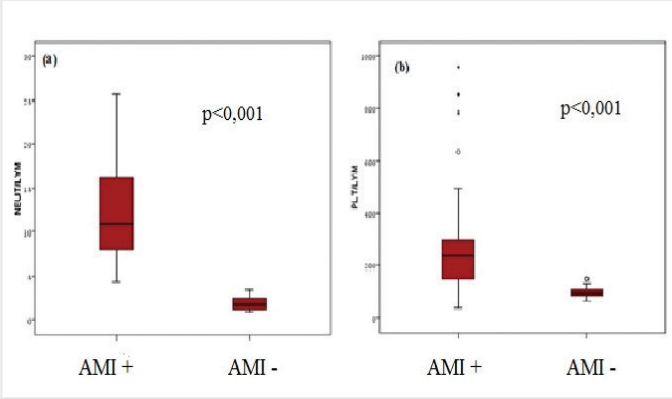

Figure 1. NLR and PLR values in diagnosis of AMI. NLR values in AMI + and AMI - groups (a), PLR values in AMI + and AMI - group (b), AMI; acute mesenteric ischemia, NLR; neutrophil/lymphocyte ratio, PLR; platelet/lymphocyte ratio

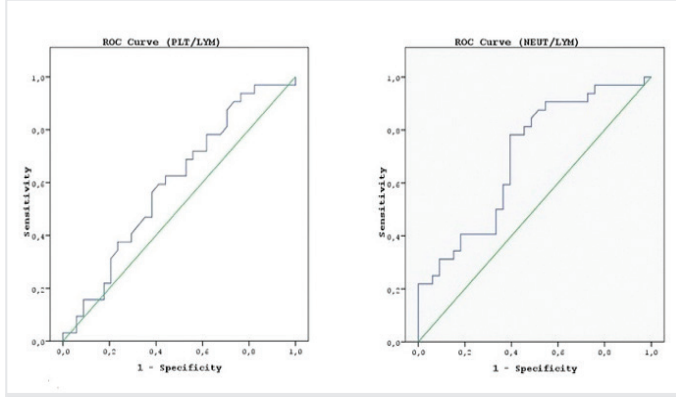

Figure 2. Comparison of receiver-operating characteristic analysis of NLR and PLR in prediction of AMI mortality. AMI; acute mesenteric ischemia, NLR; neutrophil/lymphocyte ratio, PLR; platelet/lymphocyte ratio

Table 1. Demographic and clinical characteristics of study population

Variable	AMI (n=67)	Control (n=56)	p value
Age (mean ± SD)	67±16	62±9	0.61
Men, n (%)	28(42)	24 (43)	0.92
Atrial fibrillation, n (%)	29 (43)	22 (40)	0.86
Diabetes mellitus, n (%)	39 (58)	31 (56)	0.27
Hypertension, n (%)	55 (82)	45 (80)	0.21
Coronary artery disease, n(%)	40 (59)	34 (60)	0.56
Cerebrovascular disease, n (%)	12 (18)	9 (16)	0.8
White blood cell count, ×10⁹/L	16.3±3.5	7.7±1.9	<0.001
Neutrophils, ×10⁹/L	13.8±3.4	4.7±1.5	<0.001
Lymphocytes (ч.L)	2.8±1.2	2.6±0.6	<0.001
Hemoglobin (g/dL)	12.9±2.4	13.2±2.4	0.82
Platelet count, ×10³ µL	300±110	259±61	0.111
Red cell distribution width (%)	17.2±16.5	16±14.3	0.415
Mean platelet volume (fL)	8.9±1.7	7.5±1.9	<0.001
Neutrophil/lymphocyte ratio	12.5±5.8	1.9±0.8	<0.001
Platelet/lymphocyte ratio	269±189	99±20.6	<0.001

AMI: Acute mesenteric ischemia, SD: Standard deviation

Discussion

Our study showed that NLR and PLR levels increased in AMI+ patients. In addition, increased NLR levels, not PLR, predicted inhospital mortality in patients with AMI.

Despite the advances in diagnostic and imaging modalities, mortality rates in AMI remain high, partly due to the failure to consider AMI in differential diagnosis, use of unnecessary diagnostic procedures leading to diagnostic delay, as well as due to the fact that at the time AMI is diagnosed, it is generally too late to administer appropriate treatment (14). Etiology of AMI includes embolic or thrombotic arterial occlusion in 60 to 70% of the cases, non-occlusive ischemia or infarction in 20 to 30%, and mesenteric venous thrombosis in 5 to 10%. SMA is involved in 85% of the cases with embolic or thrombotic arterial occlusion (10). Acute and complete cessation of intestinal blood flow leads to perfusion abnormality in the intestinal wall, resulting in the initiation of a process with high mortality in the absence of urgent treatment due to gangrene, ileus, sepsis, and multi-organ failure following bacterial translocation and infiltration after the collapse of mucosal barrier and bacterial translocation (15). Mortality rate with acute treatment after symptom onset is between 0 and 10%, while this figure rises quickly to 50-60% and 80-100% with delays of 6-12 hours and >24 hours, respectively (16). Despite these high mortality figures, the average delay between symptom onset and establishment of a diagnosis of AMI and initiation of treatment is around 11 hours, showing the need for improved diagnostic performance (10,17). Until now many serum parameters have been tested with regard to their ability to diagnose AMI accurately, and none of these markers have proven sensitive and specific enough to facilitate early diagnosis and to predict early mortality. Similarly, although elevated serum lactate and D-dimer levels are associated with increased mortality in AMI, they are not specific for predicting this increase (9,18).

Ultimately, reduced mortality rate in AMI requires early diagnosis and treatment. Recent studies showed that NLR may represent an important marker for cardiac and non-cardiac conditions, and particularly in detecting the inflammation in malignancies, gastrointestinal conditions, and chronic renal failure (19-22). Toptas et al. (23) reported significantly higher NLR and PLR in patients with AMI as compared to controls where a NLR greater than 4.5 had 77% sensitivity and 72% specificity for a diagnosis of AMI and a PLR greater than 157 had 59% and 65% sensitivity and specificity, respectively. Aktimur et al. (24) found a sensitivity and specificity of 74.3% and 82.9%, respectively for a NLR of ≥9.9 in the diagnosis of AMI and they reported even higher diagnostic yield when NLR and RDW were combined. In another study, a NLR level of greater than 4.68 as a marker of inflammation was associated with acute appendicitis, while higher values (≥5.74) were indicative of complicated appendicitis (25). In the current study comparing NLR and PLR for diagnosis of AMI, it was found that AMI+ patients had significantly higher values than AMI- patients ($p<0.001$, $p<0.001$, respectively). In contrast with the above-mentioned studies, although ROC analysis on the predictive value of NLR and PLR for inhospital mortality in patients with AMI identified a best cut-off value of >10.1 for NLR, no such values could be identified for PLR. In other words, NLR exhibited a significantly higher "area under curve" value. In our participants, the overall inhospital mortality rate was 48% versus 66% in those with a NLR of greater than 10.1, suggesting that NLR, as a strong marker of inflammation, could represent a more valuable indicator of inhospital mortality in patients with AMI as compared to PLR. A significantly increased release of neutrophils into circulation occurs in response to inflammation and neutrophils play a significant role in tissue destruction and injury. In contrast with neutrophils, number of circulatory lymphocytes decreases during inflammation due to increased steroid release due to stress and increased apoptotic cell death (26). In our study, patients who died had statistically insignificant increase in their neutrophil count as compared to patients who survived. (13.9±3.3, 11.6±4.4, $p=0.07$, respectively). However, patients, who died had significantly lower lymphocyte count (1.3±0.3, 3.0±1.3, $p=0.015$, respectively). Also, no significant differences in platelet counts were observed between those patients who did and did not survive (302.2±115.4, 279.5±53.7, $p=0.890$, respectively). Interestingly, despite there was a significant initial difference in neutrophil counts between AMI+ and AMI- patients, no such differences could be identified in platelet counts. Among AMI+ patients who died, there was an insignificant increase in neutrophil counts in conjunction with a significant decrease in lymphocytes. Although the cause of the decrease in lymphocyte counts could not be fully elucidated, it may be associated with the increased release of cortisol secondary to the concurrent occurrence of thrombus and ischemia in the mesenteric artery. Based on our observations, we believe that the increase in NLR in our study is associated with the delay in diagnosis, extent of the intestinal segment with high risk of necrosis, and increased neutrophil count and decreased lymphocyte count secondary to the infection and inflammation. While a lower cut-off value for NLR may represent an important parameter for AMI diagnosis, NLR may predict inhospital mortality at higher cut-off levels. Further prospective studies with larger sample size are warranted for defining the role of these novel hematological parameters in the diagnosis of AMI and in predicting its mortality.

Our study has several limitations. Firstly, it is a single-center and retrospective study. Also, the time from presentation to diagnosis and the type of treatment administered were not assessed. Thirdly, the increase in CRP, a non-specific marker of inflammation, in AMI+ patients led to the consideration of increased NLR and PLR values in AMI patients as a strong marker of inflammation. Although it is interesting to note a significant increase of NLR in both patients with diagnosis of AMI and inhospital mortality of AMI+ patients, its association with clinical course could not be

fully understood. Finally, thrombus formation causing AMI was not confirmed histopathologically.

Conclusion

In this study, high NLR and PLR were found to have significant value in the diagnosis of AMI, while only elevated NLR was significant for predicting inhospital mortality. We believe that as compared with other inflammatory markers, NLR represents a practical, economical, accessible, and useful parameter that can be utilized particularly in the absence of advanced imaging modalities and experienced radiologist assessment. However, further prospective studies are warranted to ascertain the role of NLR as a reliable marker for predicting inhospital mortality in AMI.

Ethics

Ethics Committee Approval: Şişli Hamidiye Etfal Training and Research Hospital 07.08.2018/2066.

Informed Consent: It was not taken due to a retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.S.Y., M.A., G.A., Design: G.E., H.K., S.S., S.S.Y, Data Collection or Processing: G.E., S.S.Y., H.K., M.A., Analysis or Interpretation: H.K., G.A., M.A., S.S., Literature Search: S.S.Y., G.A., H.K., M.A., Writing: G.A., S.S.Y., H.K., S.S., G.E.

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

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Original Article

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Adjuvant Chemotherapy in Elderly Patients with Early-stage Non-small Cell Lung Cancer

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ABSTRACT

Objective: Early-stage non-small cell lung cancer (NSCLC) constitutes approximately 25-30% of newly diagnosed lung cancers. Elderly patients with NSCLC have generallly been underrepresented in clinical studies. We explored adjuvant chemotherapy results in patients ≥65 years with early-stage NSCLC.

Methods: The medical records of 111 elderly patients with early-stage NSCLC were reviewed retrospectively. Collected data included demographic information, clinical assessments and information on treatment. Survival was estimated using the Kaplan-Meier method and prognostic factors were evaluated with log-rank and Cox regression tests.

Results: The median disease-free survival (DFS) was 22.6 months. In univariate analysis, significant association between stage, performance score (PS), adjuvant chemotherapy and DFS was detected ($p<0.05$). Stage, PS and adjuvant chemotherapy were found to have significant effects on overall survival (OS) ($p<0.05$). The median survival for the entire group was 41.6 months. Multivariate analysis showed that stage, PS and adjuvant chemotherapy affected both DFS and OS.

Conclusion: Survival of elderly patients with early-stage NSCLC was significantly influenced by stage, PS and adjuvant chemotherapy. These factors, rather than age, should be considered in the treatment planning for elderly patients with NSCLC.

Keywords: Adjuvant, elderly, non-small cell lung cancer

Introduction

Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related deaths in the US. A total of 224.390 new lung cancer cases and 158.080 deaths from lung cancer were expected to occur in the United States in 2016 (1). In Turkey, lung cancer is the leading cause of cancer in men in all age groups and in male patients older than 50 years. It is the fourth common cause of all cancers in women in all age groups and third common cause in female patients older than 50 years (2). Approximately 85% of all lung cancers are non-small cell, and majority of these cases are metastatic or advanced at diagnosis (3).

Between 2010 and 2030, a 67% increase in cancer incidence is anticipated for patients aged ≥65 years (4). Specifically, half of the newly diagnosed NSCLC cases occur in patients aged ≥65 years (5). Additionally, elderly patients suffer from approximately twice as many comorbidities compared with the general population which may have a considerable impact on their health and performance status (6).

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In clinical trials, the definition of an elderly patient remains controversial. Epidemiologic literature uses an age of 65 years for the selection of elderly patients (7). It should be noted, however, that there is little knowledge regarding the management of resected elderly patients with NSCLC (8). Therefore, in this study we evaluated the patients ≥65 years of age with surgically resected early-stage NSCLC who had also received adjuvant chemotherapy.

Methods

We retrospectively analyzed the records of the 111 patients who were aged ≥65 years with NSCLC from December 2005 to January 2015 at the Dr. Lütfi Kırdar Kartal Training and Research Hospital and Kocaeli University School of Medicine. Ethics committee approval was obtained from the local committee. Written informed consent was not obtained from patients due to the retrospective nature of the study. All medical records were collected through a detailed review of the patients'charts. The data included demographics, histology, staging, presenting symptoms, treatments, toxicities, and treatment side-effects. TNM classification (7th edition) was used for staging of the patients. Eastern Cooperative Oncology Group (ECOG) performance score (PS) was used for the detection of performance status (9).

These 111 patients that underwent surgery (all of the patients) had no significant comorbidities. Of these, patients who did not receive chemotherapy had stage 1A disease and 1B disease with good prognostic criteria. Eight patients were not given chemotherapy because of having a poor PS after surgery. Nineteen patiens with a good PS had stage 1B disease with poor risk factors and stage 2 disease did not receive chemotherapy. Therefore, out of 111 patients, 84 patients received chemotherapy and these 84 patients were compared with these 19 patients who were offered but did not receive chemotherapy. The response to therapy was determined according to the Response Evaluation Criteria in Solid Tumors criteria (10).

Statistical Analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software was used for all statistical analyses. A p value ≤0.05 was considered to be significant. Toxicity was classified according to the World Health Organization criteria at each cycle of chemotherapy (11). Kaplan-Meier curves were used for the disease-free survival (DFS) and overall survival (OS) analysis and the log-rank test was used for comparisons. A Cox proportional hazard analysis was conducted in order to calculate hazard ratios [95% confidence interval (CI)]. DFS was calculated from the diagnosis of the patient to the date of disease progression, recurrence or death from any cause. OS was calculated from the diagnosis of patient to the date of death from any cause or to the date of the last follow-up.

Results

Data from 111 patients aged ≥65 years old were collected. Ninety-three patients (83.8%) were male and 18 (16.2%) were female. The median age of the patients was 68.0 years (range=65-82 years). Patients had no significant comorbidities. Thirty-eight patients (34.2%) had hypertension, 6 patients had thyroid disorders (5.4%), 5 had renal disease (4.5%) that were easily managable. In the histopathological examinations, 61 (54.9%) of tumors (patients) were detected as squamous cell carcinoma and 33 (29.7%) were adenocarcinoma. Eighty-one percent of the patients were managed with lobectomy, and 19% with segmentectomy or wedge resection. Forty-five percent of the patients had clinical stage 1 and 55% had stage 2 disease. A PS score of 0-1 and 2-4 were recorded in 74.7% and 9.0% of the patients, respectively. Two-thirds of the patients had a smoking history. Approximately, one-third of the patients (32.4%) experienced a weight loss of ≥5% in the last 3 months.

Patients who did not receive chemotherapy, had stage 1A disease and 1B disease with good prognostic criteria and patients with poor PS after surgery did not receive chemotherapy. Therefore, 84 of 111 elderly patients with NSCLC who were eligible for chemotherapy received it. Nineteen patients with poor risk stage 1B disease and stage 2 disease did not receive chemotherapy (Table 1). There were no differences between the characteristics of the patients (p>0.05) (Table 1). Only 9 patients (10.7%) with a PS of 2 received single-agent chemotherapy and 75

Table 1. Characteristics of the patients

Characteristic	Patients (n=84)*	Patients (n=19)±	
Sex			
Male	67 (65.0%)	12 (11.7%)	*p*>0.05
Female	17 (16.5%)	7 (6.8%)	
Age			
Median (range)	67.6 (65-80)	68 (67-82)	*p*>0.05
ECOG PS			
0-1	75 (72.8%)	14 (13.5%)	*p*>0.05
2-4	9 (8.7%)	5 (5.0%)	
Weight loss			
≥5% in previous 3 months	24 (23.3%)	5 (5.0%)	*p*>0.05
≤5% in previous 3 months	60 (58.2%)	14 (13.5%)	
Smoking habitus			
Current or former	63 (61.1%)	12 (12.0%)	*p*>0.05
Never	21 (20.3%)	7 (6.6%)	
Histology			
Squamous cell	52 (50.4%)	12 (11.7%)	*p*>0.05
Adenocarcinoma	20 (19.4%)	6 (5.8%)	
Others	12 (11.7%)	1 (1.0 %)	
Stage			
1	38 (36.8%)	8 (7.8%)	*p*>0.05
2	46 (44.6%)	11(10.8%)	

*Patients that received chemotherapy, ±Patients that did not receive chemotherapy, ECOG PS: Eastern Cooperative Oncology Group Performance Status

patients (89.3%) received combination chemotherapy. Overall, carboplatin-based combinations (51.1%) were most commonly administered. Thirty-six of these 84 patients (42.9%) were treated with carboplatin-paclitaxel, 26 were treated with cisplatin-vinorelbine (31.0%), 4 were treated with carboplatin-vinorelbine (4.7%), 6 were treated with cisplatin-docetaxel (7.1%), 3 were treated with cisplatin-gemcitabine (3.6%), 3 were treated with single-agent docetaxel (3.6%) and 6 were treated with single-agent gemcitabine (7.1%). There were no differences between combination arms regarding to DFS or OS (p>0.05). Seventy-two patients (85.7) that received chemotherapy had ≥3 cycles of chemotherapy. The most frequent toxicities were hematological toxicities (40.4%), nausea-vomiting (22.6%) and neurological toxicities (11.9%). Treatment results are given in Table 2.

Table 2. Chemotherapy modalities and toxicities of the patients

	Patients (n=84)	(%)
Chemotherapy regimen		
Combination	75	(89.3%)
Single agent	9	(10.7%)
Combination therapy		
Carboplatin-paclitaxel	36	(42.9%)
Carboplatin-vinorelbine	4	(4.7%)
Cisplatin-docetaxel	6	(7.1%)
Cisplatin-gemcitabine	3	(3.6%)
Cisplatin-vinorelbine	26	(31.0%)
Docetaxel	3	(3.6%)
Gemcitabine	6	(7.1%)
Chemotherapy cycles		
<3	12	(14.3%)
≥3	72	(85.7%)
Toxicities		
Hematological	34	(40.4%)
Nausea-vomiting	19	(22.6%)
Neurological	10	(11.9%)

The median survival for the overall patient population was 41.6 months (95% CI=33.4-49.8) with a 5-year survival rate of 29.5% (Figure 1). In univariate analysis, stage, PS, adjuvant chemotherapy, and combination chemotherapy significantly affected OS. Patients that received adjuvant chemotherapy showed a significant longer OS (36.1 months vs. 56.4 months, p<0.01) (Figure 1). Patients who received combination therapy showed better survival outcomes than the patients who received single-agent therapy (48.4 months vs. 42.7 months; p<0.05). The median survival of the patients with stage 1 disease was longer than patients who had stage 2 disease (54.4 months vs 34.5 months, p<0.01). The median survival of the patients with a PS of 0-1 was longer than the patients with a PS of 2-4 (46.7 months vs 22.4 months, p<0.01). There were no relationships detected between weight loss, gender, smoking, histopathology, and OS (p>0.05). These data are shown in Table 3.

In multivariate analysis, PS, stage, and adjuvant chemotherapy showed a consistent relationship with OS and DFS (p<0.05) (Table 4). The median DFS was 22.6 months (95% CI=16.7-28.4). Patients that received adjuvant chemotherapy showed a significantly longer DFS (24.1 months vs. 22.5 months, p<0.01). In univariate analysis, significant associations between stage, PS, adjuvant chemotherapy, and DFS were detected (p<0.05) (Table 3). In multivariate analysis, PS, stage, and adjuvant chemotherapy affected DFS (Table 4).

Discussion

Elderly patients represent a complex group based on their comorbidities and reduced functional reserves. Lung cancer is an important health issue in this population (12-15). To date, there has been no standard therapy accepted for NSCLC in the elderly; however JBR 10 trial and the meta-analysis of the Lung Adjuvant Cisplatin Evaluation and JBR 10 trials suggested that elderly patients benefited from treatment with acceptable toxicity (13-15). It is predicted that there will be 67% more patients with lung cancer ≥65 years by 2030. Therefore, in this study we

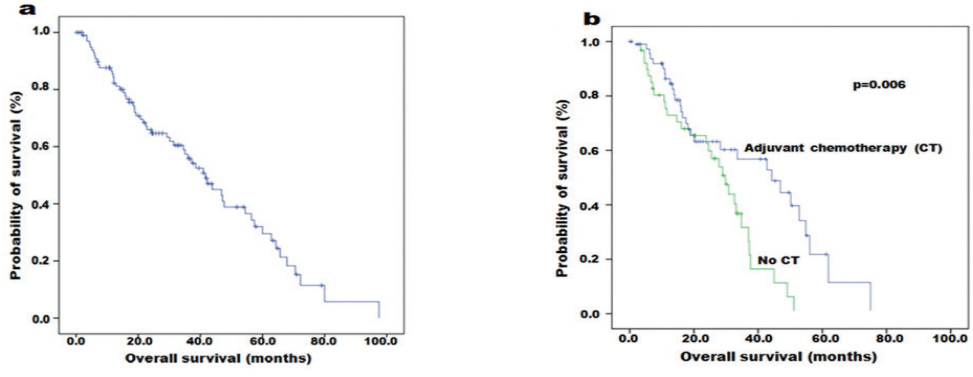

Figure 1. a) Median survival of the patients, b) Survival of the patients who did not receive adjuvant chemotherapy
CT: Chemotherapy

Table 3. Univariate analysis between clinopathological characteristics of the patient group and OS and DFS

Variable	Disease free survival		Overall survival	
	Median (months)	*p* value	Median (months)	*p* value
Stage				
1	24.7	0.01	54.4	0.004
2	19.6		34.5	
Performance status				
0-1	24.9	0.001	46.7	0.008
2-4	16.8		22.4	
Weight loss				
≤5% in previous 3 months	23.7	0.078	48.7	0.092
≥5% in previous 3 months	20.1		39.6	
Adjuvant chemotherapy				
Yes	25.2	0.002	56.4	0.006
No	19.7		36.1	
Chemotherapy regimen				
Combination	24.9	0.06	48.4	0.042
Single agent	20.1		42.7	
Smoking history				
Yes	22.6	0.28	35.7	0.078
No	24.8		47.7	
Tumor histology				
Squamous cell	23.6	0.224	43.0	0.146
Others	21.8		39.7	

OS: Overall survival, DFS: Disease-free survival

Table 4. The multivariate analysis between clinopathological characteristics of the patients and OS and DFS

	Disease-free survival			Overall survival		
Variables	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Performance status	0.079	0.008-0.842	0.033	0.074	0.008-0.830	0.022
Adjuvant chemotherapy	0.072	0.007-0.825	0.024	0.078	0.007-0.826	0.016
Stage	0.080	0.009-0.840	0.044	0.080	0.007-0.854	0.038

OS: Overall survival, DFS: Disease-free survival

adressed our adjuvant treatment results in elderly patients with resected early-stage NSCLC.

The results of previous studies have demonstrated the benefit of chemotherapy in elderly patients with resected NSCLC (16). Früh et al. (15) showed that adjuvant cisplatin-based chemotherapy should not be withheld from elderly patients with NSCLC purely on the basis of age. Although our study included only small number of patients, our results were commensurate with the results of the aforementioned studies, all of which indicated that adjuvant chemotherapy and combination chemotherapy were well-tolerated and provided clinical benefits in elderly patients with early-stage NSCLC.

Patients treated with adjuvant chemotherapy had higher DFS and OS. Cisplatin- and carboplatin-based combination chemotherapy appeared to be tolerated well. In the database analysis conducted by Cuffe et al. (13), 3759 patients ≥65 years receiving adjuvant chemotherapy showed better OS. Although JBR 10 confirmed a survival benefit for cisplatin-vinorelbine in patients ≥65 years, we found a trend towards using carboplatin-based combinations (14). These findings are consistent with those of a new study conducted in 2789 patients with resected NSCLC (16). When platinum chemotherapies were compared, superiority with respect to toxicities and efficacy were not detected. Similar findings were detected in two recent population-based analyses (13,17). Collectively, these results indicate that patients ≥65 years with resected early-stage NSCLC benefit from adjuvant chemotherapy.

Approximately 25-30% of all NSCLC cases are diagnosed at an early stage (18). With the increasing usage of computed

tomography, the incidence of early cancers is expected to increase (19). Surgical removal at this early stage represents the maximal opportunity for long term survival in lung cancer (20). Five-year relative survival rates for localized lung cancer were 54% and 26.5% for regional lung cancer (21). Five-year survival after lobectomy for stage 1 NSCLC was found to range from 45% to 65%, depending on the stage and the location of the cancer (22). Demirci et al. (23) evaluated 26 patients with NSCLC who were older than 70 years and underwent surgery and received adjuvant treatment. The median OS was 21.8 months for stage 1B, 35.4 months for stage 2A, 27.6 months for stage 2B and 21.8 months for stage 3A disease (23). In our study, the median survival for the total study population was 41.6 months with a 5-year survival rate of 29.5%. The median survival of the patients with stage 1 and 2 disease were 54.4 and 34.5 months with 5-year survival rates of 45.9% and 27.1%, respectively. Consistent with the aforementioned studies, our results confirmed the importance of stage.

Performance status is a predictor of OS in cancer patients, and is generally used to inform cancer treatment decisions (24,25). Inal et al. (24) evaluated prognostic factors for OS in elderly (≥65 years) patients with advanced NSCLC who received first-line cisplatin-based chemotherapy. They found PS as an important prognostic factor in elderly patients with advanced NSCLC (24). Also, the PS has already been considered as an important prognostic factor in elderly patients with advanced NSCLC in other studies (25,26). Unal et al. (25) investigated the effect of various the prognostic factors on survival in NSCLC patients ≥65 years. They found that PS in addition to stage and white blood cell and platelet count significantly influenced survival (25). In our study, we determined the importance of PS on survival in early-stage NSCLC for both DFS and OS.

Patients with comorbidity do not receive standard cancer treatments such as surgery, chemotherapy, and radiation therapy as often as patients without comorbidity, and their chance of completing a course of cancer treatment is lower (27). In our study, we observed that patients that were chosen for operation were carefully selected patients that had minimal comorbidities. This may cause bias for our results. Another limitation of our study was having limited number of patients especially compared with recently published studies (16). This may explain the relatively lower survival times compared with these studies.

Based on our study results, we recommend the usage of combination chemotherapy regimens in elderly patients with a good PS. It is important to note that the type of adjuvant chemotherapy used did not have an impact on survival. In conclusion, adjuvant chemotherapy should not be withheld based on age alone in patients with early-stage NSCLC.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the local committee.

Informed Consent: Written informed consent was not obtained from patients due to the retrospective nature of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: U.K., S.K., D.I., U.I., Design: U.K., S.K., K.U., Data Collection or Processing: S.K., D.I., A.S., Ö.A., Analysis or Interpretation: U.K., U.I., D.A., A.S., Ö.A., Ö.O., Literature Search: S.K., D.I., A.S., U.A., U.I., D.A., E.Ö., K.U. Writing: U.K.

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Original Article

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Comparison of Third Generation Proximal Femoral Nails in Treatment of Reverse Oblique Intertrochanteric Fractures

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ABSTRACT

Objective: The purpose of the study is to evaluate the treatment results of reverse oblique AO/OTA 31A1 fractures that have highly mechanical instability risk with two third generation intramedullary nails.

Methods: Twenty-eight patients (8 men, 20 women) treated by third generation proximal femoral nails [proximal femoral anti-rotation (PFNA)® or Intertan®] followed minimum one year were included. Average age was 65.0 (31-93) years. Clinical and radiological results, screw migration at one year and complications were recorded.

Results: Mean operation time was 72.2 and 72.5 minutes, flouroscopy time was 64.4 and 64.7 seconds, mobilisation time was 2.1 and 2.2 days, full weight bearing time was 8.6 and 8.5 weeks, tip-apex distance was 20.1 and 20.2 mm, fracture healing time was 10.5 and 10.2 weeks, Harris hip score at one year was 80.5 and 83.5, neck-shaft angle difference at one year was 1.6 and 1.1 mm, screw migration at one year was found in 10 and 3 patients and mean migration distance was 3.1 and 0.4 mm for PFNA and Intertan nails respectively. No complications recorded that needs secondary intervention. Fracture healing obtained in all patients.

Conclusion: Reverse oblique intertrochanteric fractures can be effectively treated with third generation intramedullary nails. More screw migration was seen in PFNA than Intertan nails after the operation in this study.

Keywords: Reverse oblique fracture, instability, 3. generation, proximal femoral nail

Introduction

In older patients with intertrochanteric fractures, the main target is immediate surgical intervention and faster rehabilitation (1,2). Dynamic nails, proximal femur nails and fixed-angle proximal femur locking plates are the most common treatment options. Osteosynthesis material must be strong enough to carry loads because in the older population, restriction of load-carrying can be difficult.

Since reverse obliquity fractures are often accompanied by lateral femoral cortex fracture, they are more likely to have instability and are classified as 31 A3 according to the AO/OTA classification (3,4). Dynamic hip nails, which are the gold standard for stable fractures, are generally not considered suitable for such fractures (5-8). Whereas intramedullary hip nails are biomechanically stronger and more reliable (5-8).

Since problems such as Z effect and implant failure were observed in the second generation proximal femur nails, third generation hip nails with superior implant design and stability were introduced. There are studies that support the reliability of third generation nails especially in unstable trochanteric fractures (9-12). In this study, the clinical and radiological results of patients treated

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with two different third generation proximal hip nails in AO/OTA 31 A3 class reverse obliquity fractures were retrospectively compared.

Methods

Between January 2006 and January 2012, 33 patients with AO/OTA 31 A3 reverse obliquity fractures were treated by surgical method. Since 1 patient was a patient with multiple traumas, 1 patient had a pathological fracture, 1 patient was not followed up and 2 patients died, they were not included in the study. Twenty-eight patients were followed for a period of at least 1 year. Of 28 patients, 20 (71.4%) were female and 8 (28.6%) were male. The median age was 65 (31-93) years. The average follow-up duration was 19.4 months (12-60). All patients underwent pelvic anteroposterior (AP) X-ray and hip AP-lateral X-ray of the operated side on the first postoperative day. Patients were followed up with the same radiological imaging methods and physical examination on the 1st, 2nd, 3rd, 6th, 12th months after surgery and then annually (Tables 1 and 2).

Table 1. Demographics of patients

	PFNA®	Intertan®	Total
	16	12	28
Fall	13 (81.3%)	9 (75.0%)	22 (78.6%)
Sports injury	2 (12.5%)	0 (0.0%)	2 (7.1%)
Falling from high	1 (6.3%)	2 (16.7%)	3 (10.7%)
Traffic accident	0 (0.0%)	1 (8.3%)	1(3.6%)

Table 2. AO/OTA subgroup analysis of patients

	PFNA®	Intertan®	Total
Fracture type	16	12	28
A3-1	2 (12.5%)	1 (8.3%)	3 (10.8%)
A3-2	5 (31.3%)	4 (33.3%)	9 (32.1%)
A3-3	9 (56.3%)	7 (58.3%)	16 (57.1%)

All operations were performed by the same surgeon with the same closed technique on the traction table. Sixteen patients received proximal femoral nail anti-rotation (PFNA®-Synthes, Oberdorf, Switzerland) (Figure 1) and 12 patients received intertrochanteric antegrade nail (Intertan®-Smith-Nephew, Memphis, TN) (Figure 2). In PFNA® cases, nails were 24 cm long and 130° angled. In Intertan® cases, nails were 20 cm long and 130° angled. In 2 cases where the neck-shaft angle was low on the intact side, 125° Intertan® nails were preferred.

Total operation and fluoroscopy times, mobilization and total load delivery times, tip-apex distances and fracture recovery times were recorded during operations. Also, Harris hip score (1st year) (13), neck-shaft angle change in the first year, nail migration in the first year and complications were noted. Complications requiring revision surgery, such as deep infection, inability to heal and shortening by more than 15 mm, were considered major complications.

Calculations were performed on AP-Lateral X-rays of the pelvis and hip AP-lateral X-rays in the postoperative 1st day and 1st year. Neck-shaft angle was calculated on pelvis AP X-rays and tip-apex distance was calculated on hip AP-lateral X-rays. Lateral protrusion difference between the postoperative first day and the postoperative first year of dynamic screw was considered as nail migration in the first year. The functional evaluation of the patients in the first year was performed using Harris hip score. Patients' complaints, such as feeling the presence of the nail and restlessness, were considered as implant discomfort.

All patients underwent proper mobilization, exercise program, standard antibiotics and thromboembolism prophylaxis after surgery. All patients were allowed to give as much burden as could be tolerated. Walkers and crutches were not used when the patient did not need them. For our study, Acıbadem University ATADEK ethics committee approved the meeting dated 22.12.2016 with the decision number 2016-/20/15.

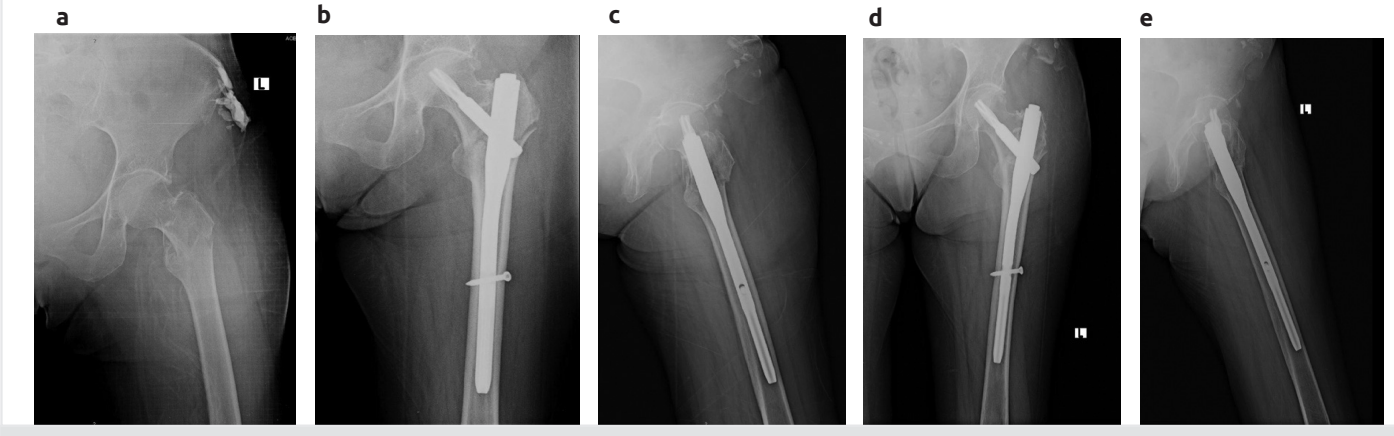

Figure 1. A 71-year-old female patient had a history of fall. (a) Third generation PFNA® (Synthes) was applied to the patient who was diagnosed as having a type AO 31 A3 fracture with impaired lateral cortex integrity. Early post-operative x-rays (b,c) and x-rays in the first year (d,e) are shown

Statistical Analysis

In our study, SPSS software was used for all statistical analyses. The normal distribution of the data was analyzed using the Kolmogorov-Simirnov test. During the analysis of data other than descriptive statistical methods, quantitative data showing normal distribution were compared using student tests. Data that did not show normal distribution were also compared using the Mann-Whitney U test. Qualitative data were analyzed using chi-square and Fisher exact chi-square tests. Significance level was considered as $p<0.05$.

Written and oral informed consents of all patients included in the study were obtained.

Results

There was no statistically significant difference between nails in terms of operation times ($p>0.05$). In addition, there were no differences between nails in terms of fluoroscopy times, mobilization and total load delivery times, tip-apex distances, fracture recovery times and Harris hip scores in the first year. There was also no difference between nails in terms of the neck-shaft angle change in the first year. Mean nail migration in the first year was statistically significantly different in PFNA® compared with Intertan®.

In four cases (2 with Intertan®, 2 with PFNA®), hematoma formation occurred laterally in the thigh. In 3 of these cases, the hematoma was spontaneously resorbed while in 1 patient who underwent Intertan®, drainage was required with local anesthesia. Four patients (2 with Intertan®, 2 with PFNA®) felt discomfort due to the implant, yet in none of these patients implant was needed to be removed. Two patients (1 with Intertan®, 1 with PFNA®) suffered from long-term groin pain. Healing of fracture was achieved in all patients. None of the patients had major complications, such as infection and dislocation. In our study,

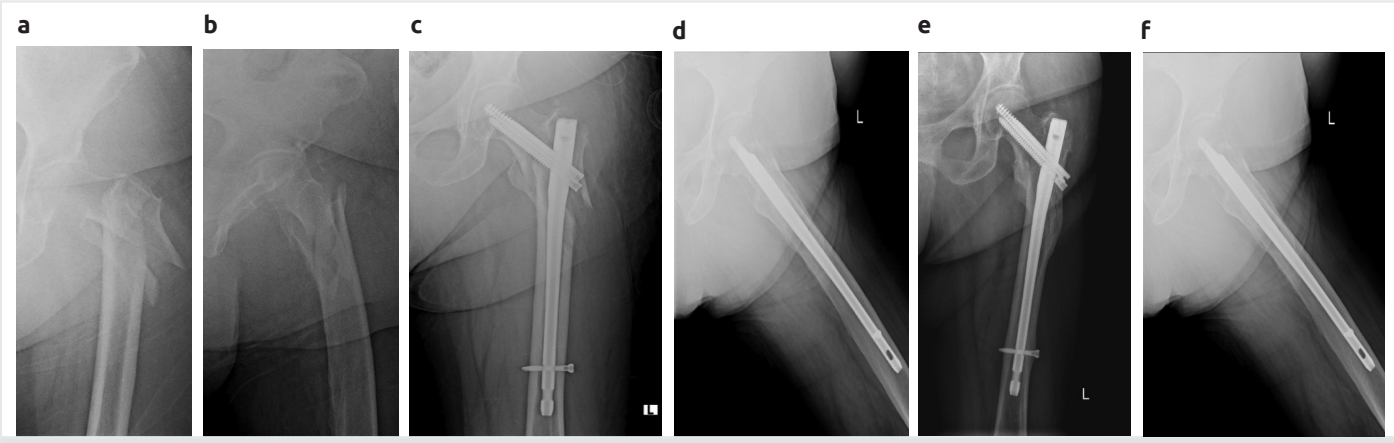

Figure 2. Third generation Intertan® (Smith and Nephew) was applied to a 58-year-old female patient who was diagnosed with type AO 31 A3 fracture with impaired lateral cortex and trochanter minor integrity (a,b). Early post-operative x-rays (c,d) and x-rays in the first year (e,f) are shown

Table 3. Results and complications

	PFNA® (n=16) Average ± SD (Median)	Intertan® (n=12) Average ± SD (Median)	+p
Operation time (dk)	72.18±12.10	72.50±11.18	0.945
Fluoroscopy time (sn)	64.37±15.72	64.75±13.15	0.947
Mobilization time (day)	2.06±0.85	2.16±0.93	0.762
Full load time (week)	8.56±1.89	8.50±1.62	0.928
Type-apex distance (mm)	20.12±2.09	20.16±1.64	0.955
Boiling time (week)	10.50±2.47	10.16±2.16	0.713
Harris hip score (1. year)	80.50±4.22	83.50±4.52	0.083
Neck-shaft angle difference (1. year) (degree)	1.62±2.50 (0)	1.08±1.72 (0)	0.684
Migration (1. year)	10 (62.5%)	3 (25.0%)	0.049*
Migration (1. year) (mm)	3.12±2.55 (4.5)	0.41±0.79 (0)	0.011*
Complications	2 implant disorder 2 hematoma 1 groin pain	2 implant disorder 2 hematoma 1 groin pain	

+: Students t-test, *p<0.05. SD: Standard deviation

first year control X-rays showed nail migration in 3 (25%) of 12 patients with Intertan® and 10 of 16 patients with PFNA®. Mean nail migration was measured as 3.1 mm for PFNA® and 0.4 mm for Intertan® (Table 3).

When mean nail migration was statistically compared, there was significant difference in favor of Intertan® in terms of both patient number and migration distance. However, nail migration in the PFNA® group did not result in re-operation. No patients from either group felt clinically leg shortening.

Discussion

Early mobilization is very important in trochanteric femur fractures in elderly patients (1-3). Since mobilization without giving load is difficult in older patients, osteosynthesis material must be strong enough to share the load.

Reverse obliquity trochanteric fractures have potential to lead to mechanical instability. Lateral cortex fractures of the proximal femur are a major cause of instability (4,14). Implants used to treat these fractures should support the lateral cortex, preventing instability. Therefore, dynamic hip nails are not recommended in reverse obliquity trochanteric fractures, and intramedullary hip nails are preferred in treatment (15-17).

In reverse obliquity intertrochanteric fractures, older generation intramedullary hip nails have been applied relatively more successfully than alternatives. However, screw slip has become a common problem in the second generation hip nails, where 2 screws are placed in the femoral head (18). Although the cause is not known for certain, one of the 2 lag screws sent to the femoral head shows backward migration. The other screw also shows migration to proximal side (Z effect). This has become a significant disadvantage in the second generation proximal femur nails. Park et al. (19) reported that in 4 of 21 cases with proximal femoral nails, the femoral nail showed migration, and 3 of them required revision surgery. The PFNA® and Intertan® used in our study are also third generation proximal femur nails.

Implant selection has been highlighted as critically important in the successful treatment of reverse obliquity hip fractures, with proper placement of the chosen implant and good reduction as a whole (20,21).

In stable trochanteric fractures, usually longitudinal traction and internal rotation are sufficient for adequate reduction. However, in reverse obliquity fractures, these maneuvers may not be sufficient for reduction because the proximal components, including the trochanter major, remain in the lateral. Sometimes, reduction loss can occur when placing the nail. When we encountered this problem in our study, reduction was made using Steinmann pin and spike pusher and closed technique was applied in all cases. Reduction with Steinmann pin was applied percutaneously.

Another cause of reduction loss is the false trochanteric entry site (16). This crucial step should be applied without error, because it is very important to maintain reduction and to place the implant correctly. Therefore, the ideal entry location should be determined even if it causes more radiation exposure with repeated procedures. Along with the placement of nails, determining the bone entry location was the most time-consuming stage in our surgeries, resulting in radiation exposure.

One of the most useful methods for determining the accuracy of the implant position is the measurement of the tip-apex distance defined by Baumgaertner et al. (20). The tip-apex distance is suggested to be below 25 mm. Many studies confirm that this is important for successful surgery and reduces the risk of implant failure (19,20). For this reason, the position of the hip nail is just as important as the ideal bone entry location. This step should not be skipped and should be applied until the optimal position is achieved.

In all our cases, the tip-apex distance was below 25 mm. None of the patients had problems with implant failure. Our search for the ideal bone entry location and hip nail position naturally increased our fluoroscopy time. Our average fluoroscopy time was 64.5 seconds (64.4 s for PFNA®, 64.7 s for Intertan®).

If the gap between the proximal and distal main parts does not close and adequate compression is not achieved, the hip nail tends to slip backwards and impaction occurs when the patient gives load. This occurs more frequently with PFNA®. Due to its strong compression capacity, the Intertan® nail does not leave large enough space for impaction to occur. In our study, 3 (25%) of the 12 patients with Intertan® had nail migration, while 10 of the 16 patients with PFNA® had this migration. After 1 year, mean nail migration difference was found statistically significant for PFNA ($p<0.05$). Mean nail migration was 3.1 mm for PFNA® and 0.4 mm for Intertan®. On the other hand, none of the PFNA® migrations required revision surgery.

Although the complication rate was 35.7% in our study (31.2% in PFNA® and 41.7% in Intertan®), all of these complications were minor complications. Three were reversibl hematomas. Four of them were implant discomfort that did not require extraction, and 2 were spontaneous groin pain. None of the other complications required revision, except for one case that required hematoma drainage under local anesthesia. In all cases, fracture healing was achieved.

Study Limitations

The minimum follow-up period of our study was 1 year and the number of patients was low.

Conclusion

According to our assessment, reverse obliquity trochanteric fractures can be successfully treated using third generation intramedullary hip nails such as PFNA® and Intertan®.

Intertan® nail reduces the risk of nail migration because it provides strong compression. As a result, implant selection, ideal entry location determination, ideal nail positioning and surgical technique are important to achieve successful results.

Ethics

Ethics Committee Approval: For our study, Acıbadem University ATADEK ethics committee approved the meeting dated 22.12.2016 with the decision number 2016-/20/15.

Informed Consent: Since our study was retrospective, no consent was obtained from the patients.

Peer-review:: Externally peer-reviewed.

Authorship Contributions

Concept: T.K.Ü., O.T., M.S., Design: T.K.Ü., A.G., Data Collection or Processing: T.K.Ü., M.S., Analysis or Interpretation: A.K., A.G., O.T., Literature Search: O.T., T.K.Ü., Writing: T.K.Ü., O.T., M.S.

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

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Original Article

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Investigation of Foot Pressure Distribution in Asymptomatic Individuals with Mild Hallux Valgus

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ABSTRACT

Objective: The purpose of the present study was to investigate plantar pressure distribution during static standing in asymptomatic individuals with mild hallux valgus and to compare the results with healthy controls group.

Methods: This study included a total of 25 asymptomatic individuals with hallux valgus (22 females, 3 males) between the ages of 19 and 51 years. The controls group comprised of 28 health individuals without hallux valgus (25 females, 3 males) between the ages of 20-51 years. Static plantar pressure distribution and force measurement during static standing in individuals were assessed using Tecscan MatScan System (Tekscan, Inc., South Boston, Massachusetts, USA).

Results: It was found that both groups have similar maximum pressure (p=0.669), mean pressure (p=0.950), heel maximum force (p=0.660), midfoot maximum force (p=0.894), metatarsal maximum force (p=0.824), contact area (p=0.695), force-time integral (0.498), pressure-time integral (p=0.769) and center of force (p=0.178).

Conclusion: The results of the present study show that asymptomatic individuals with or without mild hallux valgus have similar plantar pressure distribution. These results suggest that plantar pressure distribution do not affect the development of hallux valgus or changes of hallux position do not change plantar pressure distribution in asymptomatic individuals with mild hallux valgus.

Keywords: Hallux valgus, plantar pressures, orthopedics, foot

Introduction

Hallux valgus is a foot deformity characterized by the lateral deviation of the big toe that accompanies the medial deviation of the first metatarsal bone (1). Hallux valgus, one of the most common foot deformities in the adult population, is associated with many demographics and biomechanical abnormalities such as age, female sex, use of narrow-nosed shoes, short achilles tendon, ligament laxity, and increased pronation in the back foot (2-5). Hallux valgus deformity is often accompanied by callus formation in the big toe, subluxation of the first metatarsophalangeal joint and pain (6). These symptoms that accompany hallux valgus can cause a significant reduction in the person's quality of life (7,8).

Plantar foot pressure distribution measurement provides important information about foot and ankle function during walking or other functional activities (9). Plantar pressure distribution is frequently used in the evaluation and treatment plan of individuals with hallux valgus as well as neurological and musculoskeletal diseases associated with foot disorders (9-13). There is a correlation between the presence of hallux valgus and the reduction in the medial longitudinal arch (14). It is thought that changes in the position of the big toe as well as a decrease in the medial longitudinal arch in individuals with hallux valgus

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may cause changes in plantar pressure distribution. When the literature is examined, it is observed that changes in plantar pressure distribution in individuals with hallux valgus are the subject of many studies (13,15-20). However, when these studies are examined, it is observed that the plantar pressure distribution of elderly individuals with severe hallux valgus was investigated during walking (13,16-18,20) and/or the presence of pain was not questioned in the evaluated individuals (13,15-18). Changes in plantar pressure distribution in asymptomatic individuals with young and mild hallux valgus were not the subject of any studies. Therefore, the aim of this study was to investigate plantar pressure distribution in static standing posture in asymptomatic individuals with mild hallux valgus and compare it with healthy individuals without hallux valgus. The hypothesis of this study is that the plantar pressure distribution of individuals with mild hallux valgus will differ from the control group.

Methods

Power Analysis

Power analysis was conducted to determine the number of cases in this study. In order to perform this study with 80% power and 5% error margin, it was found that in case the peak pressure value of the control group is 140 kPa and the standard deviation is 40 kPa, a minimum of 15 individuals in each group are needed to detect a deviation of 40 kPa at the peak pressure (15,17).

Subjects

This study was conducted with the participation of 53 individuals, including 6 males and 47 females in the age range of 19-51 years. The hallux valgus group consisted of a total of 25 individuals, including 3 males and 22 females with asymptomatic hallux valgus who did not suffer any pain associated with hallux valgus in the age range of 19-51 years. The control group was made up of 28 individuals, including 3 males and 25 females without hallux valgus in the age range of 20-51 years. Assessments within the scope of this study were performed in the Toros University. Individuals with asymptomatic hallux valgus and the control group were formed from the relatives of the researcher. In the study, patients with plantar fasciitis, foot injuries such as achilles tendinopathy, rheumatic diseases such as rheumatoid arthritis or osteoarthritis, history of major trauma or surgery involving the foot and/or lower extremities and significant postural disorder involving the foot and/or lower extremities were not included. In order to carry out this study, the necessary approval was obtained from the Toros University Non-interventional Ethics Committee (decision No: 2018-03/05). Individuals who read and approved the informed consent form were included in this study.

Hallux Valgus Assessment

The individuals 'big toe position was evaluated using the Manchester Scale, which was defined as valid and reliable in clinical evaluation (21,22). The scale consists of 4 phases. Stage 0 shows that there is no deformity and it is the normal big toe position. Stage 3 indicates the presence of severe hallux valgus. Only asymptomatic individuals with Stage 1 hallux valgus were included in the study. The assessment of the position of the big toe according to the Manchester scale of individuals was done by a physiotherapist with 27 years of experience in foot health and diseases. Also the hallux angle of the toe of individuals was measured using goniometric measurement. Goniometric measurements were made while individuals were in a standing upright position. While the first metatarsophalangeal joint was designated as the pivot point of the goniometer, a lever of the goniometer was placed on the metatarsal bone and its other arm was placed as parellel to the proximal phalanx. The hallux angle was recorded in degrees.

Measurements of Foot Pressure Distribution

Measurements of static foot plantar pressure distribution of the subjects were evaluated using the Tecscan MatScan systems, which were reported to be reliable and valid (Texcan, Inc., South Boston, Massachusetts, USA) (23,24). Measurements were made with bare feet, standing in a comfortable upright position for 30 seconds. The peak pressure (kPa) average pressure (kPa), peak force of the heel (Ibs), mid-foot peak force (Ibs), forefoot peak force (Ibs), contact area (cm^2), the force-time integral (Ibs*s), pressure-time integral (kPa*s) and the change in the center of force (cm) were recorded (Figure 1).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows-version 22 software. The compatibility of variables with normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirrov/Shapiro-Wilk tests). Demographic data and the parameters of the pedographic analysis results were evaluated using descriptive analyses and were presented using the median and interquartile range. Since it was determined that the parameters evaluated did not show normal distribution, these parameters were compared between the groups using the Mann-Whitney U test. Cases where p value is less than 0.05 were evaluated as statistically significant results.

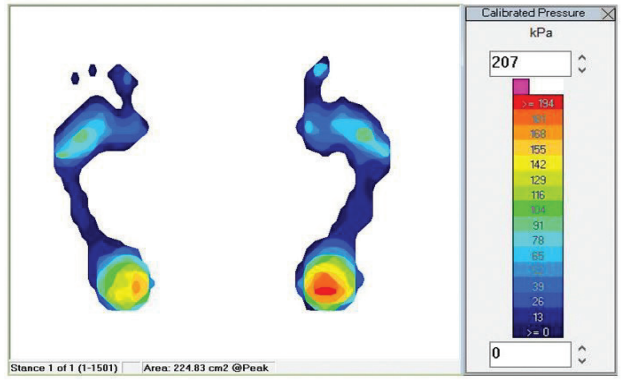

Figure 1. Plantar pressure distribution map obtained from pedographic analysis

Results

Age (p=0.858), height (p=0.412), weight (p=0.762) and body mass index (p=0.581) of both groups included in the study were found to be similar (Table 1).

It was found that the hallux angle of individuals with hallux valgus increased compared with the control group (p<0.001). Plantar pressure distribution analysis results showed that the peak pressure (p=0.669), average pressure (p=0.950), heel peak force (p=0.660), mid-foot peak force (p=0.894), forefoot peak force (p=0.824), contact area (p=0.695), the force-time integral (p=0.498), pressure-time integral (p=0.769), and changes in centre of force (p=0.178) parameters were similar in both groups (Table 2).

Discussion

This study was planned to investigate plantar pressure distribution in asymptomatic individuals with mild halllux valgus. The results showed that, unlike the hypothesis of the study, the results of plantar pressure analysis of individuals with and without hallux valgus were similar. Similar to the results obtained, Iliou et al. (15) showed that average of the pressures in the metatarsal region was similar in individuals with mild hallux valgus and without hallux valgus, and that the average pressure significantly increased at first and second metatarsal heads in individuals with moderate or severe hallux valgus. Unlike the results obtained, Hida et al. (13) reported decreased peak force, contact area, contact time and force-time integral compared and increased forefoot peak pressure and force values in individuals with moderate and severe hallux valgus compared with the control group during walking. Mickle et al. (16) reported increased peak pressure, pressure-time integral and peak pressure in the first metatarsal region in the aged population with hallux valgus. Galica et al. (17) reported increased peak pressure in the front foot and abnormal pressure distribution in the rear foot in the geriatric population with hallux valgus. Wen et al. (20) reported an overload in the first and second metatarsal bones, a decrease in the amount of pressure around the hallux and a collapse in the medial longitudinal arch. As seen here, the studies in the literature differ markedly from the results we obtained. There may be several major reasons for this. First, a significant portion of these studies were conducted on individuals of advanced age with significant hallux valgus. In addition, individuals with pain were included in these studies or the pain of individuals was not questioned. In addition, in all studies, foot pressure distribution of individuals with hallux valgus was evaluated during walking and foot pressure distribution in static condition was not evaluated.

In this study, the foot pressure distribution of individuals was evaluated in the static state and it was found that the change in the foot pressure center of individuals with hallux valgus was similar to that of the control group. These results indicate that there is no loss of postural stability in asymptomatic individuals

Table 1. Median values of demographics of groups (interquartile range/ %25-%75 percentile)

Parameters	Control Group	HV Group	*p*
Age (year)	35.5 (25.5-42.5)	31.0 (25.0-46.0)	0.448
Gender (F/M)	25/3	22/3	-
Height (m)	1.62 (1.58-1.65)	1.62 (1.57-1.68)	0.140
Weight (kg)	63.5 (55.8-70.1)	62.2 (55.4-67.2)	0.670
BMI (kg/m^2)	23.8 (21.9-26.1)	23.4 (21.4-25.6)	0.408
Hallux angle (°)	12 (10-13)	21 (19-22)	<0.001

BMI: Body mass index, HV: Hallux Valgus, F: Female, M: Male

Table 2. Median values of plantar pressure analysis results of groups (interquartile range/ %25-%75 percentile)

Parameters	Control Group	HV Group	*p*
Peak pressure (kPa)	132 (102-164)	124 (103-162)	0.238
Average pressure (kPa)	108 (80-139)	108 (81-128)	0.568
Heel peak force (lbs)	30.9 (27.7-38.9)	31.0 (25.0-42.6)	0.566
Mid-foot peak force (lbs)	20.4 (16.2-28.6)	21.4 (15.6-28.8)	0.254
Front foot peak force (lbs)	32.7 (27.1-37.4)	30.2 (26.4-39.0)	0.910
Contact area (cm^2)	91.8 (77.5-99.2)	91.5 (84.0-98.0)	0.827
Force-time integral (lbs*s)	359.1 (325.7-407.4)	385.1 (324.1-445.9)	0.930
Pressure-time integral (kPa*s)	187.0 (165.5-198.0)	181.0 (170.0-206.0)	0.665
Change in centre of force (cm)	6.10 (5.2-8.2)	7.0 (6.1-8.4)	0.121

kPa: Kilopascal; lbs: Pound

with mild hallux valgus. Similar to the results we obtained, Kavlak (25) found that hallux valgus did not affect static and dynamic balance in the geriatric population. Hurn et al. (26) examined the relationship between the balance performance on one leg and the severity of hallux valgus in a study and found that there was no loss of balance in individuals with mild and moderate hallux valgus and that the amount of mediolateral oscillation increased in individuals with severe hallux valgus.

This study had some limitations. Firstly, this study was conducted only in young and asymptomatic individuals with mild hallux valgus. If geriatric individuals and individuals with different severity of hallux valgus were included in the study, differences in plantar pressure distribution could be revealed in line with the progression of hallux valgus. Second, the pedographic analyses performed in this study were performed by a non-blind evaluator, but we believe that the measurement using standard methods minimalized possible biases. Finally, the majority of the individuals evaluated in this study consisted of women. However, similar gender distribution of individuals with hallux valgus in our study and in studies in the literature suggests that this would not result in a significant change in results.

Conclusion

As a result, it was found that pressure and force distributions of asymptomatic individuals with mild hallux valgus and the control group without hallux valgus were similar. The results suggest that in individuals with asymptomatic mild hallux valgus, hallux valgus development does not affect plantar pressure distribution, or that plantar pressure distribution is not associated with hallux valgus development. Furthermore, similar changes in the center of force in asymptomatic individuals with mild hallux valgus and in the control group indicate that postural control is not affected in asymptomatic individuals with mild hallux valgus.

Ethics

Ethics Committee Approval: Toros University Non-Interventional Ethics Committee (decision No: 2018-03/05).

Informed Consent: Individuals who read and approved the informed consent form were included in this study.

Peer-review: Internally peer-reviewed.

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Original Article

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Is it Possible to Diagnose Endometrium Cancer with the Levels of Prolactin, Eotaxin, E-selectin and Ca 125?

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ABSTRACT

Objective: The aim of this study to evaluate possible serum markers for distinction of endometrial carcinoma (EC) from benign uterine gynecologic diseases.

Methods: The study group consisted of 65 patients who were diagnosed with histologically confirmed EC were compared with 65 women who had operation indication for benign uterine diseases with fasting blood serum levels which were taken prior to surgery in terms of prolactin, eotaxin, e-selectin and Ca 125 concentrations.

Results: Serum prolactin (20.7±18 and 16.2±13), eotaxin (219.4±95 and 205.15±80) and e-selectin (67.2±29 and 61.5±29) levels were not significantly different between EC and the other group ($p>0.05$). Ca 125 levels (36.6±40 and 18.8±9) were significantly higher in EC group when compared with the benign uterine patology group ($p<0.05$).

Conclusion: Although serum levels of prolactin, eotaxin, e-selectin were higher in EC group, those were not statistically significant. Although, serum Ca 125 levels were siginificantly higher in EC group, diagnostic role of Ca 125 is limited. There is currently no marker to distinguish EC from benign uterine pathologies.

Keywords: Prolactin, eotaxin, E-selectine, Ca 125, endometrial cancer marker

Introduction

Endometrium cancer (EC) is the most common gynecologic cancer in developed countries (1). The survival rates of EC are much higher as in many other cancers in case of early diagnosis and given appropriate surgical and medical treatment (2). The diagnosis of EC is most commonly made as a result of endometrial sampling in the patient suffering from bleeding in menopause or irregular bleeding. Although endometrial sampling is a procedure made from natural holes in the body, it is an invasive procedure and its cost is significantly higher when compared with a simple blood test. It also has a complication risk.

Mortality risk of EC increases due to histological grade of tumor, lympho-vascular area invasion, tumor size, cervical involvement and metastasis to lymph nodes (3). The importance of tumor markers and some cytokines, chemokines, adhesion molecules and growth factors have been studied many times in cancer diseases. The development of tumor cells has been shown to increase with the effect of cytokines [interleukin (IL)-4, 6, 10), chemokines (IL)-8, eotaxin], growth factors, adhesion molecules (selectins, integrins) and enzymes such as nitric oxide synthetase and cyclooxygenase-2 (4). Although immune cells have tumor suppressor effects in early stage cancers, they are able to increase tumor spread and metastasis due to some phenotypic modifications

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in advanced stages (5). Chemokines are proteins also involved in the cancer process that regulate the distribution and traffic of immune cells. There are studies showing the stimulating effects of chemokines in some human cancers (6). Furthermore, increased chemokine receptor expression has been reported to be associated with poor prognosis and metastasis (7). Eotaxin-1 is a chemokine that has been discovered as an eosinophile selective chemoattractant. Eotaxin mRNA expression has been shown in many tissues, including the endometrium (8). The direct angiogenic effect of eotaxin has been shown to explain its role in metastasis and tumor invasion (9).

L-selectin (CD62L), E-selectin (CD62E), P-selectin, and integrins are molecules likely to be effective in the progression of tumor cells. Endothelial E-selectin is involved in wound healing and infections by providing leukocyte participation along with P-selectin. However, this role of selectins can have unintended consequences in pathological situations such as chronic inflammation and cancer (10). Many studies have shown increased carcinogenetic potency in prolonged inflammatory diseases such as chronic pancreatitis, inflammatory bowel disease and prostatitis (11). Increased selectin and selectin receptors have been identified in many tumor cells. This causes selectin to interact with tumor cells, attracting these cells to systemic circulation and metastasis (12).

Tumor markers are substances in the form of hormone, enzyme, metabolite, immunoglobulin, or protein which are produced by tumor or tissue in supraphysiological levels and their quantitative measurements can be done in patient's tissue, blood or other body fluids with biochemical or immunochemical methods. They may also include tumor-related antigens, oncogene, and oncogene products. An ideal tumor marker should have high sensitivity and specificity, allowing recognition and curative treatment of the tumor while the tumor is still small or the patient is asymptomatic.

Tumor markers are used to recognize tumors as early as possible, improving clinical outcomes and survival. Ca 125 is a tumor marker used in diagnosis and follow-up of ovarian cancer, and it is known that levels of Ca 125 are elevated in advanced stage EC (13). Ca 125 is a marker belonging to the mucin family of glycoproteins and located on the surface of mesothelial cells, which can be elevated in many benign conditions (endometriosis, tuboovarian abscess) other than ovarian and EC (14). Therefore, it has limited sensitivity and specificity in the diagnosis of ovarian and EC (15).

Prolactin is a protein molecule that acts as a hormone and cytokine that enables the production of milk secreted from the pituitary gland. The anti-apoptotic or mitogenic efficacy of prolactin in breast and glial cells is known (16). There are studies showing that prolactin may be effective in prostate cancer, lymphoma and leukemia, besides breast cancer (17-19). Prolactin is also synthesized in the endometrium, myometrium and cervix. There is only one study in the literature showing that prolactin increases in EC (20). There are also a limited number of studies in which prolactin levels are evaluated in cervical and other gynecologic cancers, but no clear association between prolactin levels and cancer has been shown in these studies (21).

Although EC is the most common gynecological malignancy, it is not ideal to screen the entire population due to the lack of an ideal method to sample the endometrium under examination conditions and the lack of a specific blood test of EC. Especially, postmenopausal women using oral contraceptives containing estrogen only, women with Lynch syndrome [hereditary non-polyposis colorectal cancer (HNPCC)] and with chronic anovulatory cycles due to polycystic ovary syndrome can be taken in screening programme.

In this study, we compared serum prolactin, e-selectin, eotaxin and Ca 125 levels between patients with benign gynecologic pathologies and patients with EC diagnosis and evaluated the possibility of using these cytokines in diagnosis of EC without intervention.

Methods

This study was conducted in the XX institution with the approval of the Ethics Committee of the institution (protocol number 2009/1920) as a prospective single-center study. Informed consent forms were obtained from all patients included in the study. All procedures were carried out in accordance with the Helsinki Declaration.

The study group was composed of 65 patients who were histologically diagnosed as having EC, while the control group was composed of 65 patients who did not have gynecological and non-gynecological malignancies and who were operated for benign reasons such as myoma and uterine prolapse. The additional diagnosis was made by the evaluation of tissue samples taken by the endometrial curettage method, by gynecopathologists and this diagnosis was confirmed by hysterectomy materials examined after the operation. It was confirmed after the operation that the pathology results of the patients operated due to benign pathologies were also benign.

Five millilitres of fasting blood samples were taken from both groups on the morning of the surgery. On the same day, prolactin, Ca 125, Ca 19-9 and Ca 15-3 concentrations were measured in the E170 modular system (Roche Diagnostics, Mannheim, Germany) by electrocemoiliminusance method.

For measurement of serum eotaxin and E-selectin levels, blood samples taken in gel tube were centrifuged at 4000 rpm and the upper phases were separated and samples were stored at -80 ºC until the study day and E-selectin concentrations (eBioscience, Vienna, Austria) and eotaxin levels (Invitrogen, California, USA) were measured using commercial kits using the sandwich enzyme immunoassay method.

Statistical Analysis

For statistical analysis, SPSS 15.0 (SPSS Inc. Chicago, IL., USA) package program was used. The distribution of variables between groups was presented with average and standard deviation values. The compatibility of variables with normal distribution was

evaluated by Kolmogorov-Smirnov test. The Mann-Whitney U test was used to evaluate the difference between the two group averages. Statistical significance level was selected as p<0.05.

Results

The mean age of the EC group was 56.8±12 and the mean age of the benign uterine pathology group was 54.6±9 years. There were no statistically significant differences between the groups in terms of age (p>0.05).

There were no statistically significant differences between groups in terms of prolactin level (20.7±18 ng/mL vs 16.2±13 ng/mL), eotaxin level (219.4±95 pg/mL vs 205.1±80 pg/mL) and E-selectin level (67.2±29 ng/mL vs 61.5±29 ng/mL) (p>0.05).

However, there was significant difference between the EC group and the benign uterine pathology group in terms of Ca 125 levels (36.6±40 u/mL vs 18.8±9 u/mL) (p<0.05) (Table 1).

Discussion

Preoperative tumor marker analysis is important not only for diagnosing the tumor but also for therapeutic follow-up. With effective new biomarkers, the clinician will be able to diagnose early and evaluate treatment effectiveness. In our study, we evaluated markers that could distinguish EC from benign uterine pathologies. In our study, we found that prolactin, eotaxine, E-selectin and Ca 125 levels were higher in the serum of patients with EC than in the serum of patients with benign uterine pathologies, but only increase in Ca 125 was statistically significant. The elevations in prolactin, eotaxine and E-selectin levels were not statistically significant.

It is known that prolactin is not just a hormone secreted from the pituitary gland, it has paracrine and autocrine activity in many tissues and prolactin is produced in the secretory phase in the endometrium (16). Although the role of prolactin in carcinogenesis is not known, studies have shown that it increases in breast and prostate cancer (19,22). There are a limited number of studies showing increased levels of prolactin in EC in the literature. Yurkovetsky et al. (20) evaluated patients with EC with a comprehensive serum marker panel in their study. As a result of that study, prolactin was found to be 98% specific and 98% sensitive in the diagnosis of EC (20). Kanat-Pekkaş et al. (21) also showed that prolactin levels were significantly higher in patients with EC, but it was indicated that the use of prolactin alone was a remote possibility for screening or as a marker. In our study, prolactin was found to be high in patients with EC compared to patients with benign uterine pathologies, but this difference was not statistically significant.

Blockade of eotaxine signaling pathway increases chemotherapeutic efficacy which supports the effectiveness of this chemokine in cancer growth and metastasis (23). Nolen et al. (24) showed that eotaxin decreased in ovarian cancer and it was also reported that eotaxin decreased in patients with EC in the panel which was evaluated in the study by Yurkovetsky et al. (20). In our study, eotaxin levels were higher in patients with EC than in patients with benign uterine pathologies. However, this difference did not reach a statistically significant level.

In our study, we found no significant difference in E-selectin level. There are publications in the literature showing the relationship between increase in E-selectin level in breast and colon cancer and metastatic disease (25). The only study in which E-selectin was evaluated in EC was the study of Yurkovetsky et al. (20), in which E-selectin was found to decrease significantly in EC. Furthermore, even if significant changes in the level of E-selectin are detected, this cytokine may increase in many chronic diseases, which reduces the likelihood of being used as a cancer marker (26).

In our study, Ca 125 was the only marker we found significantly higher in the serum of patients with EC. This marker produced by coelomic epithelium may increase in other gynecologic malignancies, especially ovarian tumors, and in some benign conditions (endometriosis, pelvic infection, pregnancy, myoma) (27,28). It was found to increase in 11-33% of patients with EC (29). Although increase in Ca 125 in EC is generally associated with extra-uterine propagation, many studies have shown that it correlates with stage, deep myometrial invasion, positive peritoneal cytology, and lymph node metastasis (30). It is also known to be a marker of disease activation in the follow-up process after treatment. Serum Ca 125 levels above 70 U/mL was claimed to be associated with prognosis in the multicenter study in 413 patients with EC by Kim et al. (30). In our study, the mean Ca 125 level was 36.6 in the EC group. Given the finding in our study and literature, it should be underlined that Ca 125 may be important for follow-up in EC, but its use area is limited due to its low specificity as a screening parameter.

The most important limitation of our study was that our patient numbers were partially low. Evaluations in a larger study group will contribute greatly to the use of these parameters in EC.

Table 1. Comparison of serum levels of biomarkers between groups

	EC (n=65) Median** (min-max)	BP (n=65) Mean (min-max)	p*
Prolactin (ng/mL)	20.7 (3-40)	16.2 (3-40)	>0.05
Eotaxin (pg/mL)	219.4 (130-320)	205.1 (120-292)	>0.05
E-selectin (ng/mL)	67.2 (37-102)	61.5 (29-95)	>0.05
Ca 125 (u/mL)	36.6 (4-76)	18. (7-28)	<0.05

*Mann-Whitney U test, **Median (min-max), min: Minimum, max: Maximum, EC: Endometrium cancer

Conclusion

Prolactin, eotaxine and E-selectin levels are not different between patients with EC and patients with benign uterine disease. It is not possible to use these hormones and cytokines as serum markers and to use them for screening and diagnosis in EC. Although Ca 125 has been found high in patients with EC, it is still not qualified for screening or diagnosis due to low specificity. This issue with contradictory results in the literature seems likely to be clarified when re-evaluated with studies with higher number of cases.

Ethics

Ethics Committee Approval: This study was conducted prospectively in the Department of Obstetrics and Gynecology of Istanbul Medical School with the approval of the ethics committee (protocol number 2009/1920).

Informed Consent: Informed consent forms were obtained from all patients included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.B., Design: Ö.T., Data Collection or Processing: S.B., Analysis or Interpretation: S.B., Literature Search: Ö.T., Writing: Ö.T.

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

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Original Article

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The Relationship Between Risky Health Behaviors and Satisfaction with Life in University Students

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ABSTRACT

Objective: This study was conducted for the purpose of determining relationship between risky health behaviors and satisfaction with life in university students.

Methods: The study was designed as a cross-sectional type. The sampling of the study was createdconsisted of 856 students accepting an education in the different departments of a university in the Mersin in the 2017-2018 spring semester. Three types of data collection tools (Student Introduction Form, Risky Health Behaviors scale (RHBS), and Life Satisfaction scale (LSS) were used in the collection of data in the study. Frequency, mean, standard deviation, t-test, ANOVA and the Pearson correlation analysis were used in the analyses.

Results: The mean risky health behaviors scores (58.68±7.53) of students in our study were found to be high, and their mean satisfaction with life scores (16.75±4.57) were found to be moderate. The mean risky health behavior scores are high and mean life satisfaction scores are low for male students, those whose grade point averages are 2.51 and below, those whose parents education level is secondary education and below, those who live away from their family, those whose incomes are less than their expenses, and those whose general health status and interpersonal relationship level is "poor".

Conclusion: Negative, good and moderate correlations were found between life satisfaction and the RHBS psychosocial and nutritional subdimensions, respectively., for the students. It was determined that as the mean total RHBS, psychosocial and nutrition sub-dimension scores of the students increased, their satisfaction with life decreased.

Keywords: University student, risky health behavior, life satisfaction

Introduction

Risky health behaviors are defined as behaviors that are life-threatening; that result in disease, disability, or death; that prevent one from being a physically, psychosocially, economically, and sexually healthy adult. It is frequently unclear what consequences they will create. In some situations, they include involuntarily making choices (1-4).

Adolescence is a risky process in the emergence of risky health behaviors. The emergence of the needs of students receiving their university education this term, such as the acceleration of physical and psychosocial developments, the development of their autonomy, the increase of their personal responsibilities and their communication within the peer group, and gaining and confirming a place in the peer group, and coming face to face with pressures and different options within the group can trigger the emergence of risky health behaviors (3,5). In these types of situations, the triggering of risky health behaviors frequently originates from being unable to handle the pressure originating from their peer group and difficulties in adapting to a new lifestyle period. Social setting support is very important in adolescents to handle risky health behaviors effectively. It will be easier to

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deal with risky health behaviors in a social environment (e.g. family/friends) that supports the cognitive and psychological development of adolescents, that is a positive role model in behaviors aimed at the development of health, and in which health education is valued (5,6).

Some risky health behaviors gained in adolescence can result in disease, disability, labor loss, economic loss, and death (3,4). Previous studies have determined that the risky health behaviors gained in the adolescence lead to cancer, cardiovascular diseases, cirrhosis, and substance abuse in adulthood (6,7).

Risky health behaviors in university-aged adolescents show variation. These types of behaviors can be sorted as aggressiveness, tendency towards fighting, substance use, burglary, school absence, unprotected sexual relations, unbalanced nutritional habits, and inactivity (2,8,9). Viener et al. (10) reported in their study that 51.2% of college students smoke cigarettes, drink alcohol, and engage in risky sexual behaviors. Mahallik et al. (11) determined in the study they conducted that the rate of risky sexual behaviors in males in the 20-year-old age group increased significantly (11). It is reported in the literature that the average risky health behavior scores of adolescents are high (2,9). It was determined that the highest average risk scores for the risky social behaviors of adolescents were in the physical activity, nutrition, and hygiene subdimensions and that the lowest average risk score was in the substance use subdimension (9,12,13). The tendency of university-aged adolescents towards risky health behaviors is growing every day (2). It is emphasized that variables such as change in family structure, advancement of technology, societal educational inadequacy, protection-prevention programs not being solution-focused (9), age, gender, substance use, academic success, income level, and dormitory living are influential in the increase of risky behaviors in this group (2,5,10,12,14).

Outlook on life and satisfaction obtained from life are important in the emergence of risky health behaviors in university-aged adolescents. Because, satisfaction with life is a subjective datum that contains an individual's cognitive, psychological, and social evaluations with regard to his or her own living space (15). Therefore, a university-aged adolescent individual positively assessing his/her life expresses his/her satisfaction with his/her life that he/she experiences greater positive affection (16-18). This situation reveals the general idea regarding the satisfaction with life regarding the criteria that individuals specify for a quality, happy, and satisfying life and is a significant finding (16). Satisfaction with life can be affected by some individual traits such as age, gender, income level, education level, religious-cultural beliefs, marital status, and familial and social support (19). Goals and desires regarding the future of students, especially of the college-aged ones, affect their satisfaction with life. However, the decisions for goals and desires, worldviews, passions, and desires to work of the students in their university years which are not fully matured can create stress by negatively affecting their satisfaction with life. Previous studies reported that the academic success, communication with intrafamilial and social environments, and self-respect of university students affected satisfaction with life (16-18). Previous studies have reported that parental support and closeness increase the quality of life in university students and inhibit tendencies towards risky health behaviors (15,19). It was reported that the tendency towards risky health behaviors was high in college students who experienced stress, anxiety, hopelessness, and emotional loneliness and that their satisfaction with life was negatively affected based on this (20). In light of the literature, it is thought that there could be a relationship between satisfaction with life and risky health behaviors that may develop in students in their university years who are in adolescence. Accordingly, this study was conducted for the purpose of determining relationship between risky health behaviors and satisfaction with life in university students.

Methods

Design

This study was a cross-sectional type study. This study was conducted with volunteer students who were receiving an education in 15 departments (five departments in the field of health, 10 departments outside of the field of health) that provide undergraduate education in different areas at a university found in the Mersin province in the spring semester of the 2017-2018 academic year.

Sample and Setting

The population of the study comprises 1.103 students currently receiving an education at a university. The sample size of the study was decided using the "known-population sample size" formula. Disciplines that provide education in the field of health and in a field other than health were divided into categories. The number of students to be taken into each category of sampling was determined in accordance with the weight of the category over the number of students found in the categories. With the simple random sampling method, 509 students receiving an education in the field of health and 347 students receiving an education in a field other than health were included in the study. The study was completed with a total of 856 students.

Students who were 18 years of age and over and who volunteered to participate were included in the study. Students who did not volunteer to participate were not included in the scope of the study.

Data Collection Tools

Three types of data collection tools were used in the collection of data in the study. Data-collection tools: "Student Introduction Form", "Risky Health Behaviors scale (RHBS)", and "Life Satisfaction scale (LSS)". The researchers gathered the data by distributing the surveys to the students. The researchers prepared the "Student Introduction Form", the first data collection tool, by conducting a literature review, in order to identify the data relating to the socio-demographic characteristics of the students (1,4,7,10,16). This form contained questions that covered the characteristics of the students such as age, gender, class, income level, tendency to get in fights, expectations for the future, and

loneliness. The second form was the "RHBS". Çimen (21) developed the scale in 2003, and it is used in the determination of risky health behaviors in adolescents. The five-point likert-type scale is composed of 35 items. There are five subdimensions in the scale, including psychosocial, nutrition, physical activity, hygiene and substance use (20,21). The total raw points of the scale are between 34 and 170. The total raw points obtained from the scale are converted from the absolute value to 100, and a point scale between 20 and 100 is obtained. Higher total score obtained from the scale and its subdimensions expresses that the risky health behavior score of the individual is high, and lower total score expresses that the risky health behavior score of the individual is low (21). The Cronbach alpha value of the RHBS is 0.86. The third data collection tool was the "LSS". Diener et al. (22) developed the scale in 1985. The scale aims to identify the satisfaction that individuals in all age groups generally get from life. The original format of the scale is a seven-point likert-type and is composed of five items. The scale is unidimensional. Dağlı and Baysal (23) conducted the validity-reliability study of the scale in our country in 2016 and determined the Cronbach alpha value to be 0.88. In the Turkish adaptation of the scale, Dağlı and Baysal (23) reduced the number of steps to five by specifying that the answer options in the original form with seven steps were not suitable to Turkish culture, and a five-point likert-type scale was obtained. Low scores obtained from the scale (the lowest is 5) indicate low satisfaction with life, and high scores (the highest is 25) indicate high satisfaction with life (23).

Application of the research

The researchers conducted the study at the start of a class, after receiving permission from the course faculty of the relevant department. The data were collected in approximately 15 minutes.

Ethical Dimension

Institutional ethics committee approved the study (serial number: 2018/11), and written informed consents were obtained from the students who agreed to participate in the study.

Evaluation of the Data

The SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) program was used in the analysis of the data. The significance level in the statistical analyses was taken as $p<0.05$. Frequency, mean, standard deviation, t-test, ANOVA and the Pearson correlation analysis were used in the analyses.

Results

The mean age of the students was 21.5±3.03 years. It was found that the mean of academic grade point average (GPA) of the students was 2.61±0.61 and that the mean of LSS was 16.75±4.57. It was determined that 69.3% of the students who participated in the study were in the age group of ≤21 years, 63.9% were female, and 56.8% had a GPA of 2.51 and above and that the educational status of the mothers (62.4%) and fathers (50.3%) of more than half of the students was at the secondary school or below. While 74.1% of the students were living with their families, 59.6% expressed that they had incomes that were equal to or greater than their expenses. It was reported that 83.7% of the students had expectations for the future, 70.6% expressed their general health status as "good", 61.2% expressed their interpersonal communication level as "good", 69.6% expressed their quality of life as "high", and 66.1% expressed their loneliness level as "low".

The average RHBS scores for the university students were found to be 58.68±7.53. It was determined that the highest average scores that the students received from the RHBS were in the nutrition (69.60±12.13), hygiene (65.88±9.43), physical activity (52.23±14.43), and psychosocial (48.95±10.84) subdimensions while the lowest average score was in the substance abuse (28.94±13.94) subdimension.

Table 1 summarizes the comparisons of RHBS, sub-dimension and LSS, sub-dimension outcomes according to some characteristics of university students. It was reported in our study that the mean scores of female students taken from the RHBS, psychosocial, physical activity, hygiene, and substance use subdimensions were significantly lower than the scores of male students, and it was reported that this difference was statistically significant ($p<0.001$; Table 1). The mean RHBS scores of the students whose GPAs were 2.50 or below were found to be significantly higher than the average scores of students whose GPAs were 2.51 or above, and it was reported that the difference between the two groups was statistically significant ($p<0.001$). It was determined that the mean scores taken from the subdimensions of RHBS, nutrition, hygiene, and substance use of the group of university students whose parents level of education was secondary education or lower, were higher than the group of students whose parents level of education was high school or higher, and that the difference between the two groups was found to be statistically significant ($p<0.001$). The mean scores taken from the RHBS, nutrition, hygiene, and substance use subdimensions of the students living with their families were found to be significantly lower than the scores of those living away from their families (e.g. dormitory, friends), and it was reported that this difference was statistically significant ($p<0.001$). It was determined that mean scores taken from the RHBS, psychosocial, nutrition, hygiene, and substance use subdimensions of the students whose incomes were equal to or greater than their expenses, were lower than those of the students whose incomes were lower than the expenses; and this difference was found to be statistically significant ($p<0.001$, Table 1). The mean RHBS scores of the college students who expressed that they had no expectations for the future were found in the study to be higher than the average scores of those who expressed that they had expectations for the future, and it was reported that this difference was statistically significant ($p<0.001$). The mean RHBS scores of the college students who expressed their general health condition as "poor" were found in the study to be considerably higher than the average scores of those who expressed their general health condition as "good", and it was reported that this difference was statistically significant ($p<0.001$). The mean RHBS scores of the students who expressed their interpersonal communication levels as "good" were found

in the study to be significantly lower than the average scores of those who expressed their interpersonal communication levels as "poor", and it was reported that this difference was statistically significant (p<0.001).

It was reported in our study that the mean RHBS and subdimension scores were not affected based on the quality of life variable and that there was no statistically significant difference (p>0.05).

The mean LSS points of the female students included in the study were higher than the average scores of the male students, and the difference between the two groups was found to be statistically significant (p<0.001, Table 1). It was determined that the mean life satisfaction scores of the students whose GPAs were 2.51 or above were considerably higher than the mean scores of students whose GPAs were 2.50 or below, and it was reported that the difference between the two groups was statistically significant (p<0.001). The mean life satisfaction scores were higher for the students who lived together with their families, whose incomes were equal to or greater than their expenses, and who had expectations for the future than for those who lived away from their families, whose incomes were less than their expenses, and who had no expectations for the future, respectively; and this difference was found to be statistically significant (p<0.001, Table 1). The mean life satisfaction scores of the students who expressed their general health status and levels of interpersonal communication as "good" were higher than the students who expressed their general health status and levels of interpersonal communication as "poor", and it was determined that this difference was statistically significant (p<0.001). The average life satisfaction scores of students with a "high" quality of life were found to be significantly higher than the scores of those with a "low" quality of life, and it was determined that the difference between the groups was statistically significant (p<0.001). The average life satisfaction scores of students with "low" levels of loneliness were significantly higher than the scores of those with "high" levels of loneliness, and it was determined that the difference between the groups was statistically significant (p<0.001).

Table 1. Comparisons of RHBS-subdimension and LSS- subdimension outcomes according to some characteristics of university students (n=856)

Characteristics	n (%)	Psycho-social	Nutrition	Physical activity	Hygiene	Substance use	RHBS	LSS
Gender								
Female	547 (63.9)	46.27±8.99	67.31±12.00	50.02±13.69	62.99±7.52	25.93±12.17	57.12±6.35	18.01±4.44
Male	309 (36.1)	53.69±12.15	70.10±12.36	56.14±14.90	66.90±11.85	34.27±15.23	61.43±8.60	16.29±4.77
p		0.001	0.359	0.001	0.001	0.001	0.001	0.001
Education status of mother								
Secondary school and below	534 (62.4)	48.06±10.61	72.96±11.90	51.38±14.26	64.42±9.60	33.13±12.46	60.53±7.29	16.59±4.63
High school and above	322 (37.6)	48.42±11.09	67.31±12.05	53.63±14.62	61.97±9.08	26.61±14.85	57.57±7.53	17.00±4.45
p		0.272	0.001	0.069	0.019	0.001	0.001	0.200
Education status of father								
Secondary school and below.	431 (50.3)	48.63±11.19	70.62±11.51	51.33±14.15	67.04±8.67	30.20±13.70	59.73±7.25	16.72±4.69
High School and above	425 (49.7)	49.17±10.48	68.59±12.67	53.14±14.67	64.73±10.12	27.72±13.97	56.63±7.69	16.77±4.55
p		0.389	0.017	0.067	0.009	0.001	0.001	0.872
Who they live with								
Family	635 (74.1)	49.08±10.66	65.01±11.24	52.49±14.16	64.45±9.71	27.76±13.25	57.05±7.33	17.10±4.57
Away from the family (dorm, friend etc.)	221 (25.9)	48.57±11.37	71.54±13.62	51.47±14.36	67.21±8.37	32.36±15.27	59.60±7.99	15.74±4.42
p		0.542	0.001	0.365	0.003	0.001	0.013	0.001
Income Level								
Equal to or greater	511 (59.6)	48.71±10.56	64.86±11.97	50.32±14.32	60.16±9.34	28.53±13.75	52.67±7.39	16.86±4.48
Equal to less	345 (40.4)	53.24±14.56	69.80±14.11	51.60±16.31	66.77±7.50	36.44±15.30	58.84±9.73	14.64±5.52
p		0.001	0.001	0.436	0.001	0.001	0.001	0.001

RHBS: Risky Health Behaviors scale, LSS: Life Satisfaction scale

Table 2. The correlation between the mean scores of RHBS and LSS of students

RHBS	Total LSS
Psycho-social	r=-0.586
	p=0.001
Nutrition	r=-0.485
	p=0.001
Physical activity	r=0.191
	p=0.794
Hygiene	r=0.208
	p=0.152
Substance use	r=0.102
	p=0.631
Total RHBS	r=-0.603
	p=0.001

r: Correlation coefficient, RHBS: Risky Health Behaviors scale, LSS: Life Satisfaction scale

Table 2 provides the correlation between the risky health behaviors and satisfaction with life of the students. Highly negative and moderate correlations were found between the average life satisfaction score and the mean scores in the RHBS, psychosocial and nutritional subdimensions, respectively, for the students. This correlation was statistically significant (r=-0.603, p=0.001; r=-0.586, p=0.001; r=-0.485, p=0.001). Based on this, as the average RHBS psychosocial and nutrition subdimension scores of the students increased, their satisfaction with life decreased.

Discussion

The personal, developmental, and social changes and progressions of students in their college years can lead to stress, and these stress factors that students experience can lead to risky health behaviors that endanger their lives in many areas and can decrease satisfaction with life (2). The average risky health behaviors scores of the students were found to be high and the average life satisfaction scores were found to be moderate in our study. In the studies that Muslu and Aygün (9) and Kalkım and Toraman Uysal (24) conducted, the average risky health behavior scores of university students were found to be high. Özgür et al. (19) found in the study they conducted that the satisfaction with life of students living in dormitories was considerably lower compared with students who live at home. The pressure of societal gender molds the shaping of the identity of students, dilemmas and conflicts experienced within family-peer groups, and individual independence and responsibility in college years can increase the inclination to risky health behaviors (4,5). It was determined in the study that for risky health behaviors, university students received the highest average scores from the nutrition and hygiene subdimensions and the lowest average scores from the substance use subdimension. Previous studies have found that risky behaviors relating to nutrition and hygiene are considerably higher among university students (12,24,25). Change in nutritional behaviors based on changes in lifestyle, inadequacies in social and environmental surroundings (e.g. dormitory environment), body image, and the inability to provide the care necessary for cleanliness and outer appearance increases risk in the nutrition and hygiene subdimensions in university students. The scores of the substance use subdimension were found to be low in our study. However, it is reported in the literature that college students can easily access substances like cigarettes, alcohol, and narcotics and that their substance use subdimension scores were high (2,4,26). Risky health behaviors such as driving while intoxicated, tendency to engage in fights, and smoking cigarettes increase the possibility of disease and injury in adolescent students. This is why, although the average substance use score is low, it should be handled with care.

The average RHBS, psychosocial, physical activity, hygiene, and substance use scores of the male students was found to be higher than the average scores for the female students. As per the societal gender roles in traditionally patriarchal societies, males move more freely and independently. This situation can lead males more easily to attaining harmful substances like cigarettes and alcohol, driving while intoxicated, carrying sharp objects-weapons, and getting injured (13,14). Previous studies have reported that 65% of males have drank alcohol at least once, 7% have a substance addiction, and 10-30% tend to engage in fights (10,11). The tendencies towards risky health behaviors in females are fewer relative to males. Females being raised as individuals responsible for housework, who look after children, who are affectionate, submissive, calm, and dependent on their spouse can partially decrease the tendency towards risky health behaviors (3). Previous studies have reported that males have habits of spending time on the computer and watching television more than females and that this situation increases sedentary living and inclination to obesity (1,4). Adolescent females who are receiving a university education providing more importance to cleanliness, outer appearance, and body image relative to males lowers their risk scores in the physical activity and hygiene subdimensions (10,25). The results of our study are consistent with the literature.

It was determined in our study that the inclination of students whose GPA was 2.50 or below towards risky health behaviors was higher than the average scores of those whose GPA was 2.51 or above. The rate of development of risky health behaviors of students who have low academic success and irregular class attendance and who drop out of school is high (5,10,14). Along with this, antisocial behavior disorders and insufficient social environment support in university students can increase school drop outs, failure in classes, and can lead to an inclination to risky health behaviors (2,26). This is why the low academic success of university students can increase inclination to risky health behaviors.

It was found in our study that the average RHBS, nutrition, hygiene, and substance use scores of students whose parents' level of education was secondary education or below were higher than the students whose parents' education level was high

school or above. A previous study reported that, as the parents' educational level decreased, awareness and recognition of risky health behaviors diminished, effective communications and expectations between the parents and adolescents could not be ensured, and deficiencies emerged in taking protective measures against risks (6). The results of our study are consistent with the literature. The educational level of parents being high will result in increase in opportunities provided to university students, provision of parental support, and provision of psychosocial support in the necessary conditions by consciously using current opportunities and decrease in the disposition to risky behaviors.

The average scores takenfrom the RHBS, nutrition, hygiene, and substance use subdimensions of the students living with their families were found to be significantly lower than the scores of those living away from their families (e.g. dormitory, friends). Living away from family, having more unfavorable physical opportunities (living in small, crowded environments), encountering the limitations that the operation of shared living spaces brings (inadequacies on the topic of nutrition and hygiene), and experiencing communication problems with the students with whom they live together can trigger predispositions to risky health behaviors in students (3). It was reported that students who received love, interest, and support from their families and who established effective and healthy communication found more effective solutions to the problems (3,20). Especially parental support carries considerable importance in terms of preventing behaviors aimed at substance use and crime in college-aged adolescents. This is why positive support, interest, understanding, and love provided from the family and social environment can inhibit tendency towards risky health behaviors that may develop in students.

In university-aged adolescents with low incomes, unhealthy living conditions relative to those with higher incomes (nutrition, housing, hygiene), deprioritizing health (economic insufficiency and lack of awareness), and being excessively exposed to psychosocial stress factors can increase the predisposition to risky health behaviors (1,14). The average scores taken from the RHBS, psychosocial, nutrition, hygiene, and substance use subdimensions of the students whose incomes were equal to or greater than their expenses were found to be significantly lower than the scores of those whose incomes were less than their expenses. The results of our study are consistent with the literature. On the other hand, it is a notable, significant finding in previous studies that the sedentary living, substance use, and consumption of ready-made foods are much more in students with greater income levels (4,7,11). Income level is a significant variable that affects inclination towards risky health behaviors in university students.

The average risky health behavior scores of the students whose general health condition and interpersonal communication level were "good" and who had expectations for the future were low in our study. Previous studies report that the health status that adolescents perceive, having positive communication with the people they take as role models (e.g. parents, teachers), the inherent support and proximity of their role models, and positive peer relationships far from being pretentious prevent risky health behaviors (1,3,15). Therefore, it is thought that the tendencies towards risky health behaviors may be less in students who positively assess their general perceptions of health, asset goals and expectations on the road to gain knowledge, skill and to have a profession in the university process, and can establish healthy communication in their social environment.

It was determined in our study that satisfaction with life was higher in female students who had high grade point averages, lived with their families, whose incomes were equal to or greater than their expenses, who had expectations for the future, and whose general health status and interpersonal communication level were good. It was reported in previous studies that gender, sociol economic and cultural level, parental behaviors, satisfaction with academic life, getting adequate support from friends and family, having a positive outlook for the future, and subjective status of wellbeing affect satisfaction with life (15-17,19,20). The results of our study are consistent with the literature.

Loneliness finds its foundation from inadequate social relationships and low levels of satisfaction obtained from these relationships (27). Feelings of loneliness carry great importance for students in their college years and can lead to decrease in self-respect, inadequate social skills, anxiety, substance abuse, obesity, and suicide attempts in university students (20,23). Loneliness and a person's degree of satisfaction with life are directly related to each other. In university students with a high satisfaction with life; loneliness, anxiety, and depression levels were found to be low, and self-respect, level of hope, and academic success were found to be high (27,28). It was determined in our study that the satisfaction with life was low in students who had low level of loneliness. The results of our study are consistent with the literature.

It was also reported in the study that satisfaction with life was high in students with high quality of life. Quality of life includes the relationship with the environment, interactions, and beliefs of people (16). Satisfaction with life is a cognitive assessment that includes quality of life (18). Quality of life and satisfaction with life are in a positive relationship with each other, and, just as in every age group, carry great importance in college. Özgür et al. (19) reported in their study that students with high quality of life had high satisfaction with life. The conclusion of that study shows analogy with the results of our study. Based on the results obtained, it is seen that satisfaction with life increases as quality of life increases.

Highly negative and moderate correlations were found in our study between life satisfaction and the RHBS psychosocial and nutritional subdimensions, respectively, in the students. It was determined that as the risky health behaviors of students increased, their satisfaction with life decreased. Living in a social environment suitable to insight inadequacy in college years (feelings of insignificance and helplessness, depression, aggression, nutrition-housing problems, etc.) can lead students gain risky behaviors (25,26). Therefore, students unable to sufficiently meet their physical, social, and mental needs and

experiencing helplessness in the solution of their problems, will have increased risky behaviors while significantly decreased satisfaction with life.

Conclusion

The mean risky health behaviors scores of students in our study were found to be high, and their mean satisfaction with life scores were found to be moderate. Negative good and moderate correlations were found between life satisfaction and the RHBS psychosocial and nutritional subdimensions, respectively, in the students. It was determined that as the mean total RHBS, psychosocial and nutrition sub-dimension scores of the students increased, their satisfaction with life decreased. The mean risky health behavior scores were high and mean life satisfaction scores were low in male students whose GPAs were 2.51 or below, whose parents' education level was secondary education or below, who lived away from their family (e.g. dormitory), whose incomes were less than their expenses, and whose general health status and interpersonal relationship level were "poor". Satisfaction with life was found to be low in students who had "low" quality of life and "high" level of loneliness.

For the prevention of risky health behaviors and increasing the satisfaction with life in students receiving an education at a university; psychosocial educational programs should be organized (sexuality should be considered); effective social support and communication should be strengthened by providing family, academic advising, and student collaboration; and activities should be provided aimed at gaining health life skills (athletic activities, volunteering at aid associations, etc.). The implementation of all these activities aimed at student participation will prevent risky health behaviors and increase satisfaction with life by contributing to the cognitive evaluation of individuals with regard to them being satisfied with their own lives.

Ethic

Ethics Committee Approval: This study was approved by the Ethics Committee of Çankırı University (serial number: 2018/11).

Informed Consent: Written consent was obtained from the students who agreed to participate in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: D.A., D.P.K., F.B.Ö., Design: D.A., D.P.K., F.B.Ö., Data Collection or Processing: D.A., D.P.K., F.B.Ö., Analysis or Interpretation: D.A., D.P.K., F.B.Ö., Literature Search: D.A., D.P.K., F.B.Ö., Writing: D.A., D.P.K., F.B.Ö.

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Original Article

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Evaluation of The Effects of Raisins and Hazelnuts Added To the Diet on Lipid Profiles and Anthropometric Measurements in Women with Hyperlipidemia

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ABSTRACT

Objective: The positive effects of nuts and grape products on lipid profiles have been proved by epidemiological and clinical studies. However, studies investigating the effect of raisins on lipid profiles are limited. The aim of this study was to compare the effects of consuming a cardioprotective control diet, and the cardioprotective diet containing either raisins, hazelnuts, or a combination of raisins and hazelnuts in hyperlipidemic obese women in terms of lipid profiles and anthropometric measurements.

Methods: Thirty-seven hyperlipidemic obese women were involved in a parallel controlled randomized clinical trial. Participants were randomly divided into four groups. The control group consumed a cardioprotective diet for six weeks while the other participants consumed 50 g/day hazelnut, 50 g/day raisins or 50 g/day hazelnut +50 g/day raisins in a cardioprotective diet. Blood lipids, blood glucose levels, blood pressure, and anthropometric measurements were measured at the beginning and at the end of the study.

Results: There was not any significant difference between groups in terms of lipid profiles, blood glucose, blood pressure and anthropometric measurements (all $p>0.05$). Compared with initial measurements, total cholesterol, low density lipoprotein cholesterol, and body mass index levels decreased statistically significantly (all $p<0.05$) in all groups at the end of the study.

Conclusion: Because of the improvement on lipid profile and anthropometric measurements in four groups at the end of the study, it was concluded that consumption of hazelnut, raisins or combination of hazelnut andraisins can be recommended to hyperlipidemic individuals in addition to an appropriate diet program.

Keywords: Hazelnut, Raisins, cardioprotective diet, hyperlipidemia, lipid profile, anthropometric measurements

Introduction

Among chronic diseases, cardiovascular diseases (CVD) play an important role with high mortality and morbidity rates (1,2). For CVD, the most important risk factors are high level of blood lipids and high blood pressure, unhealthy eating habits, physical inactivity and tobacco use (2). The total cholesterol level of 200-239 mg/dL and the low density lipoprotein (LDL) cholesterol level of 130-160 mg/dL are defined as borderline high values regarding hyperlipidemia and therapeutic lifestyle changes are recommended. These changes include; regulation of diet content, weight control, and increased physical activity (3). Only a single diet pattern is insufficient to regulate the diet content. Studies using the Mediterranean diet as a cardioprotective and healthy diet have shown that it reduces CVD risk. There is not a single

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type of Mediterranean diet. The common characteristics of Mediterranean diets are as follows: High consumption of vegetables, fruits, whole grain products, nuts, legumes, fish, olive oil; low consumption of red meat, moderate consumption of wine, dairy products, poultry, and eggs (4).

Nuts are high-energy foods which are rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Besides the appropriate fatty acid composition, nuts contain a variety of bioactive substances: Plant protein, minerals, alpha-tocopherol, phenolic compounds and fiber (5). In many clinical and epidemiological studies, it has been shown that all nuts have cholesterol-lowering efficacy due to their appropriate fatty acid composition and bioactive components, and frequent consumption of them reduces the incidence of CVD (6,7). Hazelnut has the highest unsaturated/saturated fatty acid ratio among nuts. Its oil pattern consists of 83.2% MUFA, 9% PUFA, while saturated fatty acid content is lower than 8% (8,9). MUFA are mainly oleic acid (82.7%) and PUFA are omega-6 (8.9%) and omega-3 (0.1%) (8). In addition to its fatty acid content, hazelnut is a cardioprotective food (9-11) which involves nutrients such as soluble fiber, arginine, β-sitosterol, vitamin E, folate, vitamin B6, potassium, and magnesium (10,11). Clinical studies with hazelnuts have proven positive effects on lipid profiles (9,12-14).

Raisins, containing fructose and glucose equally are also rich in vitamins, minerals, fiber, and antioxidants (15). The results of several studies have provided that the consumption of grape and grape products has protective effects against chronic diseases. This effect is attributed to the antioxidant compounds in the grape (16,17). Studies on the effect of grape-based products on lipid profiles in humans or animals have used products made from grapes (grape seed extract, grape juice, and wine) (18-24). Studies using raisins have generally been carried out on the glycemic index and blood glucose (15,25). Raisin has a high antioxidant capacity but the studies investigating its effect on lipid profile in humans are limited. According to our research hypothesis, a cardioprotective diet containing raisins is expected to lower blood lipids more than just a cardioprotective diet and raisins with hazelnuts more than just a hazelnut containing diet. The aim of this study was to compare the effects of consuming a cardioprotective control diet, and the cardioprotective diet containing either raisins, hazelnuts, or a combination of raisins and hazelnuts in hyperlipidemic obese women in terms of lipid profile, and anthropometric measurements.

Methods

Subjects

The study was carried out according to the principles of the Helsinki Declaration. All the research related procedures were approved by the İstanbul No 6 Ethics Committee of Clinical investigations in Bakırköy Dr. Sadi Konuk Training and Research Hospital with the decision dated 29.04.2013 and numbered 2013/05/01. A protocol was signed with the approval of the İstanbul Public Health Directorate for the research conducted in a Family Health Centre, located in the Pendik Community Health Centre area.

The research was carried out with volunteer participants who registered to a family health center, attended for routine health check-ups between 11.11.2013-01.03.2014 and met the inclusion criteria of the survey. Non-pregnant women aged 40 years or older who had no accompanying chronic disease and did not use medication due to hyperlipidemia with body mass index (BMI) >30 kg/m² with total cholesterol >200 mg/dL, LDL cholesterol level >130 mg/dL, fasting blood glucose <110 mg/dL and systolic/diastolic blood pressure <140/90 mmHg were included in the study. The exclusion criteria were having a chronic illness (diabetes, hypertension, cancer, chronic kidney failure), using drugs due to hyperlipidemia and having allergies to specific foods (nuts, raisins). The health information of the participants was approved by the family physicians. The research was conducted with only women to prevent sex differences affecting the results of the research. According to the results of a study of cardiovascular disease and risk factors in Turkey (26), mean cholesterol concentrations in women aged 40-59 years were found to be 204 mg/dL in the research area. Because this result was above the hyperlipidemia limit value of 200 mg/dL, the age of the participants was determined to be over 40 years.

For the study, 138 women who attended the Family Health Center for routine check-ups between 11.11.2013 and 01.03.2014, and who were over 40 years old, had BMI >30 kg/m² and had no accompanying chronic disease, were informed about the study and then blood lipids were examined from capillary blood after 12 hours of fasting. Ninety-four women were not included because their lipid profiles were normal (total cholesterol <200 mg/dL, LDL cholesterol level <130 mg/dL). Only 44 of these individuals fit the inclusion criteria of the survey. The participants were informed about the research and the experimental period was started after signing the informed consent form.

Sample Size

The sample size was determined to be 9 per group. In power analysis, when the probability of Type 1 error is 0.05, the power of test is 82%, Type 2 error is 17%, SD of means is 15.16, SD is 25.00, and effect size is 0.61. It was found that at least 9 individuals should be in each group in order to compare four groups (27,28). The study was started with 11 individuals per group.

Study Design

For this parallel controlled randomized clinical trial, participants who fit the inclusion criteria were divided randomly into four groups according to registration number respectively: 1 hazelnut group (HG), 2 raisin group (RG), 3 hazelnut+RG (HRG), and 4 control group (CG).

At the beginning of the study, some socio-demographic information (age, marital status, working status, education

status) and health condition (chronic diseases diagnosed by a doctor, drug and supplement usage, food allergy) of the participants were investigated with a questionnaire prepared by the researcher. The accuracy of health information was confirmed by the participants' family physicians.

Blood lipids are known to stabilize within 3 weeks (29). Clinical studies researching the effect of nuts on blood lipids lasted 4-8 weeks (11). Therefore, the experimental period of the research lasted 6 weeks.

HG consumed a cardioprotective diet with 50 g of hazelnuts added to their diet (HD), RG consumed a cardioprotective diet with 50 g of raisins added to their diet (RD), HRG consumed a cardioprotective diet with 50 g of hazelnuts and 50 g of raisins added to their diet (HRD) and the CG consumed a cardioprotective controlled diet. The American Food and Drug Administration recommend consuming 42.8 g/day of nuts to reduce the risk of CVD due to their positive effects on blood lipids (30). In clinical trials, 40-100 g of nuts were used daily to investigate the effects on blood lipids (11). In our study, 50 g of hazelnuts and the same amount of raisins were given to the participants per day.

According to the cardioprotective diet recommended in the context of lifestyle changes in the prevention of CVD, daily energy should be adjusted to maintain ideal body weight. The energy distribution taken from macronutrients should be as follows: 50-60% carbohydrates, 15% proteins, and 25-35% fats (3,31-33). In addition to these recommendations, whole grains, vegetables, fruits, fish, legumes, nuts are important nutrients to be consumed in terms of cardiovascular disease control (32). The diets of the research groups were prepared in accordance with this information. Daily diet menus designed by the researcher and consumed by the participants during the research are given in Table 1. Energy and nutritional values of the diets were calculated using the BeBIS7.2 program (Entwickeltan der Universitat Hohenheim, Stuttgart Copyright 2010 Dr. J. Erhardt, Stuttgart, Germany). Energy and nutritional values of the diets and the percentage of Recommended Dietary Allowance coverage (34) are given in Table 2. When diets are designed, it is aimed to be as equal as possible regarding energy and nutrients for all four groups. As shown in Table 2, diets are very close to each other in terms of energy, carbohydrate, protein, total fat and fiber content. Because hazelnuts are rich in total fat and MUFA, the diets of other groups are designed to be equal in terms of fat. Due to the differences in eating habits of the groups (such as using sunflower oil while cooking), the diets had differences in their PUFA and MUFA contents.For this reason, PUFA was higher in diets of the CG and RG, and MUFA was higher in diets containing hazelnut.

At the beginning of the study, the researcher gave the nutrition guide and daily diet menu to the participants according to their group; and explained the diet. Participants consumed the diets which were recommended to them according to their group. Only hazelnuts and raisins were provided by the researcher. In order to prevent cross-contamination between the groups, the participants were told not to consume the products of the other groups (the CG was told not to consume hazelnuts and raisins to; the HG was told not to consume raisins; and the RG was told not to consume hazelnuts) and they were followed up weekly. Any change in physical activity was not recommended for the participants so as not to influence the result of the diet.

During the research, subjects were followed up weekly and their weekly required hazelnuts and raisins were provided by the researcher in daily 50 g packages, free of charge. Individuals were asked to fill out weekly product consumption schedules. In weekly checks, the researcher investigated product consumptions and dietary compliance. If the participants did not comply with the diets, they reported that to the researcher. Instructions about product consumption and diets were repeated in weekly checks.

Biochemical Measurements

At the beginning and at the end of the 6-week experimental period, after 12 hours of fasting and 10 minutes of rest, capillary blood was taken by the family physician from the participants' fingertips to analyze total cholesterol, LDL, HDL and triglyceride with refractometric photometric measuring system using CardioChek® P. AAnalyzer (PTS Diagnostics, Indianapolis, USA) portable whole blood test system. The use of capillary blood for the measurement of blood lipids is valid and reliable (35). CardioCheck PA system uses a single reagent strip to measure the lipid profile. It separates plasma from red blood cells, then some of the plasma is directed to analyte-specific reaction pads and lipid concentrations are determined by reflectance photometry. HDL is initially separated from LDL and VLDL fractions following precipitation by phosphotungstic acid. Total cholesterol and HDL cholesterol are both converted enzymatically to cholest-4-en-3-one and hydrogen peroxide. The peroxide then reacts with disubstituted aniline to form quinoneimine dyes. Triglyceride also undergoes enzymatic conversion to dihydroxyacetone phosphate and hydrogen peroxide. Its concentration is determined using the same color reaction as cholesterol and HDL. LDL is calculated using the Friedewald formula for samples with triglyceride concentrations. Fasting blood glucose is analyzed with GlukoDr (AGM-2100, China) blood glucose test meter. The GlucoDR reference device is the YSI 2300 Analyzer. Measurements were done five minutes after the blood was taken.

Blood Pressure Measurements

Individuals systolic and diastolic blood pressure were measured by the family nurse at the beginning and at the end of the 6 weeks after 10-minute rest with Omron Q142 Hem-1040-e Full Automatic Blood Pressure Monitor from each arm. Blood pressure measured in duplicate, the higher value was recorded.

Anthropometric Measurements

At the beginning and at the end of the 6-week experimental period weight, height, waist and hip circumference and skinfold thickness measurements were taken by the researcher. Measurements were repeated 3 times, mean values were recorded.

Table 1. Daily diet menus designed by the researcher and consumed by the participants during the research

Daily HD menu	Daily RD menu	Daily HRD menu	Daily CD menu
Breakfast	**Breakfast**	**Breakfast**	**Breakfast**
Tea 2 slice low fat white cheese (60 g) 3 slices whole grain bread (75 g) Tomatoes (100 g) Cucumber (100 g)	Tea 2 slice low fat white cheese (60 g) 3 slices whole grain bread (75 g) Tomatoes (100 g) Cucumber (100 g)	Tea 2 slice low fat white cheese (60 g) 3 slices whole grain bread (75 g) Tomatoes (100 g) Cucumber (100 g)	Tea 1 slice low fat white cheese (30 g) 2 slices whole grain bread (50 g) Tomatoes (100 g) Cucumber (100 g)
Snack	**Snack**	**Snack**	**Snack**
Apple (120 g)	Apple (120 g)	Apple (120 g)	Apple (120 g)
Lunch	Lunch	Lunch	Lunch
4 tablespoons vegetable (200 g) 4 tablespoon bulgur or pasta (40 g) (oil not added) Salad (oil not added) Half cup of yogurt (100 g) 2 slices whole grain bread (50 g)	1 cup of soup (200 g) 4 tablespoons vegetable (200 g) 2 tablespoon bulgur or pasta (20 g) Salad (with olive oil) Half cup of yogurt (100 g) 2 slices whole grain bread (50 g)	4 tablespoons vegetable (200 g) 4 tablespoon bulgur or pasta (40 g) (oil not added) Salad (oil not added) Half cup of yogurt (100 g) 2 slices whole grain bread (50 g)	1 cup of soup (200 g) 4 tablespoons vegetable (200 g) 2 tablespoon bulgur or pasta (20 g) Salad (with olive oil) Half cup of yogurt (100 g) 2 slices whole grain bread (50 g)
Snack	**Snack**	**Snack**	**Snack**
1 packet of hazelnut (50 g)	1 packet of raisin (50 g)	1 packet of hazelnut (50 g) and 1 packet of raisin (50 g)	1 slice low fat white cheese (30 g) 2 slices whole grain bread (50 g) Mandarin (125 g)
Dinner	**Dinner**	**Dinner**	**Dinner**
Chicken or fish (60 g) 4 tablespoons vegetable (200 g) Salad (oil not added) Half cup of yogurt (100 g) 3 slices whole grain bread (75 g)	Chicken or fish (60 g) 4 tablespoons vegetable (200 g) 4 tablespoon bulgur or pasta (40 g) Salad (oil not added) Half cup of yogurt (100 g) 2 slices whole grain bread (50 g)	Chicken or fish (60 g) 4 tablespoons vegetable (200 g) Salad (oil not added) Half cup of yogurt (100 g) 3 slices whole grain bread (75 g)	Chicken or fish (60 g) 4 tablespoons vegetable (200 g) 2 tablespoon bulgur or pasta (20 g) Salad (with olive oil) Half cup of yogurt (100 g) 2 slices whole grain bread (50 g)
Snack	**Snack**	**Snack**	**Snack**
Apple (120 g) Orange (200 g) Half cup of milk (100 g)	Orange (200 g) Half cup of milk (100 g)	Orange (200 g) Half cup of milk (100 g)	Orange (200 g) Half cup of milk (100 g)

HD: Hazelnut diet, RD: Raisin diet, HRD: Hazelnut + raisins diet, CD: Control diet

Tanita BC 601 body analysis scale for weight measurement, Holtain Skinfold Caliper for skinfold thickness, and standard tape measure for waist and hip circumference were used. Weight and height measurements were used for calculating BMI, waist and hip measurements were used for calculating waist/hip ratio. Body fat mass and fat-free tissue mass were calculated by measuring the skinfold thicknesses from 4 regions (triceps, biceps, subscapular and suprailiac).

Table 2. The energy and nutrients of the diets designed by the researcher and consumed by the participants during the research

	HD	HD RDA%	RD	RD RDA%	HRD	HRD RDA%	CD	CD RDA%	RDA
Energy (kcal)	1996.5	103	2015.2	104	1998.7	103	2013.5	104	1934.1
Protein (g)	70.1 g	123	65.8	115	69.8	122	68.0	119	57.1
Protein (%)	14	-	13	-	14	-	14	-	-
CHO (g)	255.8	93	266.7	97	256.9	93	264.4	96	276.1
CHO (%)	51	-	53	-	52	-	53	-	-
Total fat (g)	78.7 g	120	77.5	118	78.1	119	77.7	118	65.6
Total fat (%)	34	-	34	-	34	-	34	-	-
Total SFA (g)	22.4	-	24.1	-	22.4	-	24.0	-	-
Total SFA (%)	10.1	-	10.7		10.1		10.7	-	
Total MUFA (g)	40.0	-	30.1	-	40.0	-	30.2	-	-
Total MUFA (%)	18.0	-	13.5	-	18.0	-	13.5	-	-
Total PUFA (g)	11.0	110	18.1	181	10.6	106	18.3	183	10.0
Total PUFA (%)	4.9	-	8.1	-	4.8	-	8.2	-	-
Omega 3 (g)	1.2	109	1.2	109	1.1	100	1.3	118	1.1
Omega 6 (g)	9.8	89	16.9	153	9.5	86	17.0	154	11.0
Cholesterol (mg)	117.6	-	117.6	-	117.6	-	117.6	-	-
Fiber (g)	39.8	133	31.0	103	34.1	114	34.9	116	30.0
Vitamin A (µg)	1034.1	129	929.1	116	907.5	113	1095.2	137	801.0
Vitamin E (mg)	26.9	224	24.0	200	25.9	216	24.2	202	12.0
Vitamin K (µg)	150.3	251	153.2	255	147.2	245	151.9	252	60.0
Thiamine (mg)	1.4	139	1.2	115	1.3	131	1.3	125	1.0
Riboflavin (mg)	1.9	156	1.7	143	1.8	149	1.8	150	1.2
Niacin (mg)	25.7	197	23.4	180	24.8	191	24.6	189	13.0
Pantothenic acid (mg)	6.5	109	5.6	92	6.0	100	6.2	103	6.0
Vitamin B6 (mg)	2.0	167	1.7	144	1.9	154	1.8	151	1.2
Biotin (µg)	79.9	178	55.2	123	73.8	164	59.6	133	45.0
Folic acid (µg)	360.1	90	318.5	80	339.6	85	354.4	86	400.0
Vitamin B12 (µg)	5.3	176	5.3	176	5.3	176	5.3	176	3.0
Vitamin C (mg)	154.0	154	122.0	122	123.5	123	154.9	155	100.1
Sodium (mg)	2193.6	110	2316.5	116	2182.2	109	2456.4	123	2001.0
Potassium (mg)	3999.9	114	3460.5	99	3740.4	107	3637.5	104	3500.0
Calcium (mg)	1259.9	126	1106.6	111	1213.4	121	1176.4	118	1001.0
Magnesium (mg)	438.3	146	331.6	111	405.0	135	364.8	122	300.0
Phosphor (mg)	1504.2	215	1330.2	190	1466.2	209	1394.4	199	701.0
Iron (mg)	13.8	92	11.6	77	13.0	87	11.9	79	15.0
Zinc (mg)	12.9	184	11.7	167	12.3	176	12.4	177	7.0
Copper (mg)	2.0	158	1.3	106	1.9	152	1.3	106	1.3
Manganese (mg)	9.1	260	5.8	166	8.5	242	6.5	186	3.5
Iodine (µg)	66.4	33	62.2	31	61.7	31	64.5	32	200.1

HD: Hazelnut diet, RD: Raisin diet, HRD: Hazelnut + raisins diet, CD: Control diet, SFA: Saturated fatty acids, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, RDA: Recommended dietary allowance, %: Ratio of energy from nutrients

Table 3. The baseline characteristics of the four groups

	HG (n=9)		RG (n=9)		HRG (n=9)		CG (n=10)		*p* value
	X	SD	X	SD	X	SD	X	SD	
Baseline characteristics									
Age (years)†	52.0	6.4	49.7	7.8	48.1	6.4	52.0	3.5	0.500
Total cholesterol (mg/dL)†	238.7	26.7	232.6	31.0	229.3	19.7	232.5	25.9	0.804
LDL cholesterol (mg/dL)†	158.2	15.7	153.6	17.2	142.7	17.9	155.4	19.1	0.059
HDL cholesterol (mg/dL)‡	49.9	9.4	51.4	11.4	51.3	9.7	49.3	4.9	0.942
Triglyceride (mg/dL)‡	151.8	78.1	137.6	76.9	176.3	76.7	140.5	49.8	0.641
Glucose (mg/dL)‡	94.2	7.8	92.9	6.6	96.1	8.3	94.7	9.8	0.872
Systolic blood pressure (mmHg)†	121.7	6.1	123.3	6.6	123.6	4.5	126.0	4.6	0.402
Diastolic blood pressure (mmHg)†	77.2	7.5	76.7	5.0	79.7	4.4	80.5	1.6	0.331
Weight (kg)‡	78.5	12.5	83.7	13.7	80.0	9.9	81.9	12.8	0.821
BMI (kg/m²)‡	35.7	4.7	35.8	6.4	34.6	4.3	36.0	6.1	0.945
Waist circumference (cm)‡	104.1	11.2	106.1	9.4	98.3	10.7	108.1	16.5	0.376
Waist/Hip ratio‡	0.88	0.07	0.89	0.05	0.85	0.05	0.91	0.07	0.196
Fat mass (kg)‡	34.0	6.7	35.4	7.9	33.3	6.5	35.7	7.0	0.868
Fat mass (%)‡	43.1	2.6	41.9	2.6	41.4	3.6	43.4	2.8	0.407

HG: Hazelnut group, RG: Raisin group, HRG: Hazelnut-raisin group, CG: Control group, BMI: Body mass index, X: Mean, SD: Standard deviation, †p-values were assessed using the Kruskal-Wallis tests, ‡p-values were assessed using the OnewayAnova tests

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS version 16.0, Chicago, IL) program was used to statistically evaluate the data obtained from questionnaires, biochemical tests, and anthropometric measurements. Obtained continuous variables were expressed as mean (X) and standard deviation (SD). Normality distribution of variables was analyzed with Kolmogorov-Smirnov. Oneway-ANOVA was used for the data with normal distribution and Kruskal-Wallis test was used for the data without normal distribution. The General Linear Model-Repeated Measures analysis was used for the evaluation of repeated measurements between the groups. For the evaluation of changes in measurement over time within groups (changes occurring in itself), General Linear Model-Repeated Measures analysis was used for data with normal distribution and the Wilcoxon test was used for data without normal distribution. To determine the significance of the study, statistical analyzes focused on comparing the changes that occurred in biochemical and anthropometric measurements between the four groups at the end of 6 weeks and comparing the self-generated changes in the groups compared with baseline measurements. The statistical significance level in the tests was evaluated as $p<0.05$.

Results

Forty-four hyperlipidemic women participated in the study. The research was completed with a total of 37 individuals. Data from 9 individuals in the HG, 9 individuals in the RG, 9 individuals

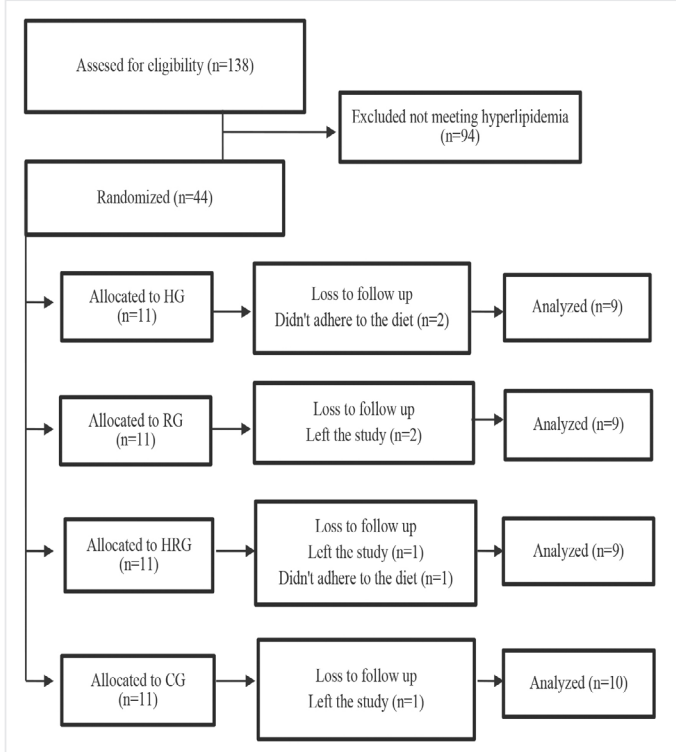

Figure 1. CONSORT 2010 flow diagram for the randomized controlled clinical tri

HG: Hazelnut group, RG: Raisin group, HRG: Hazelnut-raisin group, CG: Control group

Table 4. Evaluation of changes in biochemical measurements, blood pressure and anthropometric measurements among groups

	HG (n=9)		RG (n=9)		HRG (n=9)		CG (n=10)		F	*p* value*
	X	SD	X	SD	X	SD	X	SD		
Biochemical indicate										
Total cholesterol (mg/dL)										
Baseline	238.7	26.7	232.6	31.1	229.3	19.7	232.5	25.9	0.391	0.760
After intervention	216.9	29.7	210.0	18.5	204.7	34.4	216.8	35.1		
LDL cholesterol (mg/dL)										
Baseline	158.2	15.7	153.6	17.2	142.7	17.9	155.4	19.1	0,007	0.999
After intervention	139.7	27.2	134.8	16.2	123.0	22.3	136.4	31.2		
HDL cholesterol (mg/dL)										
Baseline	49.9	9.4	51.4	11.4	51.3	9.7	49.3	4.9	0.784	0.511
After intervention	52.6	11.3	49.7	8.9	52.4	11.3	47.4	6.6		
Triglycerides (mg/dL)										
Baseline	151.8	78.1	137.6	76.9	176.3	76.7	140.5	49.8	2.442	0.082
After intervention	123.9	42.1	128.1	55.6	145.8	53.8	165.4	79.9		
Glucose (mg/dL)										
Baseline	94.2	7.8	92.9	6.6	96.1	8.3	94.7	9.8	2.427	0.083
After intervention	93.3	11.7	101.6	6.9	93.1	10.4	98.5	8.2		
Blood pressure										
Systolic										
Baseline	121.7	6.1	123.3	6.6	123.6	4.5	126.0	4.6	1.236	0.312
After intervention	121.1	5.5	119.4	7.3	115.6	8.5	122.0	7.9		
Diastolic										
Baseline	77.2	7.5	76.7	5.0	79.7	4.4	80.5	1.6	0.663	0.581
After intervention	75.6	5.3	76.7	5.6	75.6	7.3	77.5	5.4		
Anthropometric measurements										
Weight (kg)										
Baseline	78.5	12.5	83.7	13.7	80.0	9.9	81.9	12.8	0.558	0.647
After intervention	76.0	12.5	82.2	13.5	77.9	9.6	79.6	12.3		
BMI (kg/m²)										
Baseline	35.7	4.7	35.8	6.4	34.6	4.3	36.0	6.1	0.560	0.645
After intervention	34.5	4.7	35.1	6.2	33.6	4.0	34.9	5.7		
Waist circumference (cm)										
Baseline	104.1	11.2	106.1	9.4	98.3	10.7	108.1	16.5	1.714	0.183
After intervention	98.3	10.9	101.2	9.9	95.1	9.0	99.9	13.9		
Waist/Hip ratio										
Baseline	0.88	0.07	0.89	0.05	0.85	0.05	0.91	0.07	1.852	0.157
After intervention	0.85	0.04	0.86	0.04	0.85	0.05	0.87	0.04		
Fat mass (kg)										
Baseline	34.0	6.7	35.4	7.9	33.3	6.5	35.7	7.0	1.232	0.314
After intervention	31.8	6.2	34.1	7.9	31.1	6.0	33.5	6.6		
Fat mass (%)										
Baseline	43.1	2.6	41.9	2.6	41.4	3.6	43.4	2.8	1.036	0.389
After intervention	41.7	2.5	41.1	3.3	39.6	3.7	41.9	2.9		

HG: Hazelnut group, RG: Raisin group, HRG: Hazelnut-raisin group, CG: Control group, BMI: Body mass index, X: Mean, SD: Standard deviation, *p values were assessed using the general linear model of variance for repeated measures

in the HRG and 10 individuals in the CG were finally analyzed (see CONSORT flow-diagram in Figure 1).

Generally, the compliance rate was high. All of hazelnuts and raisins were consumed during the study in requested groups. No serious complications were reported during the study.

According to surveys conducted at the beginning of the research, it was determined that 94.6% (n=35) of the participants were educated for five years or less, 91.9% (n=34) did not work and 89.2% (n=33) were married. None of the participants had chronic illness, used a drug, a supportive product such as vitamin-mineral usage, or had food allergy. The baseline characteristics (age, blood lipids, blood glucose, blood pressure, and anthropometric measurements) of the four groups are given in Table 3.

At the end of the study, changes in blood lipids, blood glucose, blood pressure and anthropometric measurements between groups were compared with general linear model repeated measures analysis. As shown in Table 4, there was no statistically significant difference between groups regarding the changes in blood lipids, blood glucose, blood pressure and anthropometric measurements at the end of the study (all $p>0.05$).

The evaluation of initial and final blood lipids, blood glucose, blood pressure levels and anthropometric measurements by groups are given in Table 5. At the end of the study, total cholesterol and LDL cholesterol values were found to be statistically significantly (all $p<0.05$) lower in all groups compared to the baseline measurements. The decrease in total cholesterol was -21.8±13.9 mg/dL for the HG, -22.6±27.7 mg/dL for the RG, -24.7±20.4 mg/dL for the HRG, and-15.7±10.9 mg/dL for the CG. There was an increase in HDL cholesterol values in HG and HRG (2.7±3.8 mg/dL, 1.1±11.3 mg/dL respectively), and a decrease in RG and CG (-1.86.5 mg/dL, -1.9±7.3 mg/dL respectively). Triglycerides decreased in HG, RG and HRG (-27.9±48.7 mg/dL, 9.4±43.5 mg/dL, -30.6±55.3 mg/dl respectively) and triglycerides increased in CG (24.9±54.1 mg/dL).

As shown in Table 4, at the end of the study there was no statistically significant difference between groups regarding the changes in weight, BMI, waist circumference, waist/hip ratio, and fat mass (all $p>0.05$). As shown in Table 5, there was a statistically significant decrease in the BMI levels and fat masses (kg) of all groups ($p<0.05$) at the end of the study. While there was a statistically significant decrease in terms of waist circumference in CG, HG and RG ($p<0.05$) compared to baseline measurements, there was not found a significant difference in HRG ($p>0.05$) (Table 5).

Discussion

The aim of the study was to evaluate the effects of raisin consumption in hyperlipidemic obese women regarding lipid profile and anthropometric measurements and compare it with hazelnut consumption and cardioprotective diet which are known to have beneficial effects on the lipid profile. As a result of the research, no significant difference was found between the four groups regarding blood lipids and anthropometric measurements (Table 4).

Nuts are rich in unsaturated fatty acids and most nuts include high MUFA. Because of having healthy fats, frequent nut intake lowers cholesterol, increases LDL resistance to oxidation, improves endothelial function and prevents CVD (36). Besides having appropriate fatty acid composition, nuts contain a variety of bioactive substances: plant protein, minerals, alpha-tocopherol, phenolic compounds and fiber (5). These bioactive components reduce oxidation, have anti-inflammatory effect, and improve endothelial function (6). More than 40 interventional studies on healthy or moderately hyperlipidemic subjects have shown that nuts raise HDL, lowers total cholesterol and LDL (37). An important result of the studies conducted on nuts is that the cholesterol-lowering effects of these products depend on the amount of consumption and they are especially effective in patients with high baseline LDL levels (7).

Among nuts, hazelnut is important for nutrition and health because of its MUFA content. Besides MUFA, other components including PUFA, phytosterols, and soluble dietary fiber, present in hazelnut, decrease plasma total cholesterol, and LDL. Hazelnut also contains fat-soluble bioactive components (tocopherols and phytosterols), minerals (magnesium, selenium), amino acids, antioxidants, and phytochemicals (8,9). Moreover, vitamin E, found in hazelnut, has a cardioprotective effect (by inhibition of LDL oxidation) (9).

In a study, moderately hyperlipidemic subjects consumed daily 30 g hazelnut, 5 days a week for 4 weeks. Compared to baseline measurements, total cholesterol and LDL statistically significantly reduced, and HDL raised after hazelnut consumption (38). In this study, similar to study by Tey et al. (45), daily 50 g hazelnut consumption in HG statistically significantly decreased total cholesterol and LDL compared to initial measurements. Despite the increase in HDL and a decrease in triglyceride compared to baseline measurements, there was no statistically significant difference (Table 5).

The results of various studies have determined that the consumption of grape and grape products has a protective effect against CVD (15,16,18-21). This effect is attributed to the antioxidant compounds found in the grape (17). Anthocyanin is an important component of raisin. A study comparing the effect of anthocyanin supplementation with the placebo in hyperlipidemic subjects revealed that the anthocyanin supplements reduced LDL cholesterol levels statistically significantly ($p<0.001$) compared to baseline measurements. LDL cholesterol reduced in a much more extent in the anthocyanin group ($p=0.001$). Supplementation of anthocyanin increased HDL cholesterol levels compared to baseline measurements. HDL raised in a much more extent in the anthocyanin group compared with the placebo group after the intervention ($p=0.001$) (39). Without changing the daily diet of healthy individuals, the effect of 6-week raisin consumption and walking on blood lipids was investigated in a study and at the end, the total cholesterol and LDL statistically significantly decreased compared to initial measurements and no difference was found between the groups (40). In this study, similar to the study by Puglisi et al. (40), a statistically significant reduction was detected in the total cholesterol and LDL levels

Table 5. Effect of 6 weeks of diet intervention on biochemical indices, blood pressure and anthropometric measurements based on groups

	Baseline		After intervention		Δ		*p* value
Biochemical indicate	X	SD	X	SD	X	SD	
Total cholesterol (mg/dL)							
HG (n=9)†	238.7	26.7	216.9	29.7	-21.8	13.9	0.008
RG (n=9)†	232.6	31.1	210.0	18.5	-22.6	27.7	0.021
HRG (n=9)‡	229.3	19.7	204.7	34.4	-24.7	20.4	0.020
CG (n=10)‡	232.5	25.9	216.8	35.1	-15.7	10.9	0.004
LDL cholesterol (mg/dL)							
HG (n=9)†	158.2	15.7	139.7	27.2	-18.6	14.9	0.008
RG (n=9)†	153.6	17.2	134.8	16.2	-18.8	19.6	0.008
HRG (n=9)†	142.7	17.9	123.0	22.3	-19.7	16.3	0.008
CG (n=10)†	155.4	19.1	136.4	31.2	-19.0	17.2	0.019
HDL cholesterol (mg/dL)							
HG (n=9)‡	49.9	9.4	52.6	11.3	2.7	3.8	0.207
RG (n=9)‡	51.4	11.4	49.7	8.9	-1.8	6.5	1.000
HRG (n=9)‡	51.3	9.7	52.4	11.3	1.1	11.3	1.000
CG (n=10)‡	49.3	4.9	47.4	6.6	-1.9	7.3	1.000
Triglycerides (mg/dL)							
HG (n=9)‡	151.8	78.1	123.9	42.1	-27.9	48.7	0.373
RG (n=9)‡	137.6	76.9	128.1	55.6	-9.4	43.5	1.000
HRG (n=9)‡	176.3	76.7	145.8	53.8	-30.6	55.3	0.408
CG (n=10)†	140.5	49.8	165.4	79.9	24.9	54.1	0.202
Glucose (mg/dL)							
HG (n=9)‡	94.2	7.8	93.3	11.7	-0.9	12.2	1.000
RG (n=9)‡	92.9	6.6	101.6	6.9	8.7	9.2	0.068
HRG (n=9)‡	96.1	8.3	93.1	10.4	-3.0	9.1	1.000
CG (n=10)‡	94.7	9.8	98.5	8.2	3.8	9.3	0.683
Blood pressure							
Systolic (mmHg)							
HG (n=9)†	121.7	6.1	121.1	5.5	-0.6	6.3	0.705
RG (n=9)‡	123.3	6.6	119.4	7.3	-3.9	8.2	0.579
HRG (n=9)†	123.6	4.5	115.6	8.5	-8.0	9.2	0.040
CG (n=10)†	126.0	4.6	122.0	7.9	-4.0	8.8	0.168
Diastolic (mmHg)							
HG (n=9)†	77.2	7.5	75.6	5.3	-1.7	6.1	0.450
RG (n=9)†	76.7	5.0	76.7	5.6	0.0	7.5	1.000
HRG (n=9)†	79.7	4.4	75.6	7.3	-4.1	7.1	0.078
CG (n=10)†	80.5	1.6	77.5	5.4	-3.0	5.4	0.098
Anthropometric measurements							
Weight (kg)							
HG (n=9)‡	78.5	12.5	76.0	12.5	-2.5	2.3	0.030
RG (n=9)‡	83.7	13.7	82.2	13.5	-1.5	1.6	0.074
HRG (n=9)‡	80.0	9.9	77.9	9.6	-2.1	1.2	0.002
CG (n=10)‡	81.9	12.8	79.6	12.3	-2.4	2.1	0.017

Table 5 contiuned

BMI (kg/m²)							
HG (n=9)‡	35.7	4.7	34.5	4.7	-1.2	1.1	0.031
RG (n=9)‡	35.8	6.4	35.1	6.2	-0.7	0.7	0.046
HRG (n=9)‡	34.6	4.3	33.6	4.0	-0.9	0.6	0.004
CG (n=10)‡	36.0	6.1	34.9	5.7	-1.1	0.9	0.020
Waist circumference (cm)							
HG (n=9)‡	104.1	11.2	98.3	10.9	-5.7	6.9	0.113
RG (n=9)‡	106.1	9.4	101.2	9.9	-4.9	1.4	0.0001
HRG (n=9)‡	98.3	10.7	95.1	9.0	-3.2	3.9	0.122
CG (n=10)‡	108.1	16.5	99.9	13.9	-8.2	5.2	0.002
Waist/Hip ratio							
HG (n=9)‡	0.88	0.07	0.85	0.04	-0.03	0.06	0.650
RG (n=9)‡	0.89	0.05	0.86	0.04	-0.03	0.02	0.009
HRG (n=9)‡	0.85	0.05	0.85	0.05	-0.01	0.03	1.000
CG (n=10)†	0.91	0.07	0.87	0.04	-0.05	0.04	0.009
Fat mass (kg)							
HG (n=9)‡	34.0	6.7	31.8	6.2	-2.21	1.42	0.005
RG (n=9)‡	35.4	7.9	34.1	7.9	-1.32	1.10	0.021
HRG (n=9)‡	33.3	6.5	31.1	6.0	-2.26	0.96	0.0001
CG (n=10)‡	35.7	7.0	33.5	6.6	-2.17	1.31	0.002
Fat mass (%)							
HG (n=9)‡	43.1	2.6	41.7	2.5	-1.41	0.68	0.001
RG (n=9)‡	41.9	2.6	41.1	3.3	-0.90	1.35	0.241
HRG (n=9)‡	41.4	3.6	39.6	3.7	-1.72	0.94	0.002
CG (n=10)‡	43.4	2.8	41.9	2.9	-1.42	0.95	0.003

HG: Hazelnut group, RG: Raisin group, HRG: Hazelnut-raisin group, CG: Control group, BMI: Body mass index, X: Mean, SD: Standard deviation, Δ: Difference after intervention-baseline, p values show the general differences among baseline and diet periods for each group, †p-values were assessed using the Wilcoxon tests, ‡p-values were assessed using the general linear model of variance for repeated measures

of the RG compared to the baseline measurements and there was no statistically significant difference in terms of blood lipids between groups.

Studies using nut and grape products have shown that these products have beneficial effects on lipid profiles and reduce CVD risk (6,7,10,19,38,41). As a result of these positive results, it was expected that the consumption of hazelnut and raisins together would decrease the risk of CVD. As a result of this research, positive changes in blood lipids were determined in HRG compared to initial measurements. As shown in Table 5, the total cholesterol and triglyceride levels of HRG decreased more than those of CG and the increase in HDL levels was found to be higher. However, when compared with the CG, the changes in the HRG were not statistically significant ($p>0.05$). Nevertheless, these results are thought to be important in decreasing CVD risk.

As a result of the research, positive changes were found compared to the initial measurements in lipid profiles and anthropometric measurements of the four groups, which consumed cardioprotective diets with similar energy content, containing either raisins, hazelnuts, or a combination of raisins and hazelnuts. The difference in blood lipids between dietary groups was not statistically significant. This may be due to the cardioprotective diet consumption in all groups.

Epidemiological studies have shown a negative relation between regular nut consumption and type 2 diabetes in women (42). Raisin is rich in fiber and fructose , and it belongs to the low-middle glycemic index nutrient class (15). It has been shown in some clinical trials that the consumption of raisins does not have any adverse effect on blood glucose (15,43,44). As a result of this study, there was no statistically significant difference in fasting blood glucose compared to initial measurements. This result is also supporting previous studies.

Epidemiological and clinical studies have found a negative relation between the consumption of nuts (5,45), dry fruits (15,44), and blood pressure. There was no statistically significant change in systolic/diastolic blood pressures measured at baseline compared to the values measured after 6 weeks on the diet. Therefore, it was concluded that hazelnuts and raisins have no adverse effect on blood pressure.

At the end of our study, positive changes were found in the anthropometric measurements of all four groups. Long-term

epidemiological and clinical studies have shown that there is no significant relationship between consumption of nuts and weight gain (46). In some studies conducted by adding nuts such as hazelnuts (12,38), pistachios (47), and peanuts (48) to daily diets, no significant difference was found in anthropometric measurements compared to baseline. At the end of our study, there was a statistically significant decrease in weight, BMI levels, waist circumference and fat mass in all groups as expected because all the individuals were obese and their diet contained recommended daily energy values. The decrease in waist circumference, an important risk factor for CVD, was also observed in all groups, but there was no significant difference in terms of waist circumference between the groups.

As shown in Table 4, there were no statistically significant differences in weight, BMI, and body fat mass changes between groups. It was concluded that the reason for not finding any difference between groups regarding the anthropometric measurements was the isocaloric diet consumption of all groups during the study.

Study Limitations

The sample size was considered among the limitations of research. Reasons such as stricted inclusion criteria for research, abandonment of research and individuals not complying with the diet limited the sample size negatively. There were some limitations for diets in this research: Participants consumed diet menus given to them by the researcher for 6 weeks. The dietary assessment was made only with the participants' declaration. The dietary record was not received. Individual compliance with diets and consumption of nuts and grapes given to them were checked with the product consumption schedule given to them at weekly controls, and no individual observations were made. Another limitation was that the amount of MUFA in diets of hazelnut-consuming groups was higher than the amount of PUFA in other groups. In order to demonstrate the effects of the products on blood lipids and anthropometric measurements clinically, it is suggested to increase the amount of the products, to have a larger study group and to have a longer study period

Conclusion

As a result of the research, there was no significant change in lipid profile and anthropometric measurements between the four groups. This may be due to consumption similar cardioprotective diet with similar energy content in all groups. Because of the improvement on lipid profile and anthropometric measurements of four groups at the end of the study, it was concluded that hazelnut and raisins consumption can be recommended in addition to an appropriate diet program to hyperlipidemic individuals.

Ethics

Ethics Committee Approval: All the research related procedures were approved by the İstanbul No 6 Ethics Committee of Clinical investigations in Bakırköy Dr. Sadi Konuk Training and Research Hospital with the decision dated 29.04.2013 and numbered 2013/05/01.

Informed Consent: The participants were informed about the research and the experimental period was started after signing the informed consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article

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Venous Insufficiency in Pediatric Patients

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ABSTRACT

Objective: The diagnosis and treatment of chronic venous disease has been well standardized in adults. However, the diagnosis of chronic venous insufficiency in pediatric patients, except for Klippel-Trénaunay syndrome and post-thrombotic syndrome, could not be established.

In this retrospective study, we planned to present pediatric patients diagnosed with venous insufficiency by our clinic.

Methods: Between January 2016 and May 2018, patients under 18 years of age who were referred to our clinic were included in this study. Venous Doppler ultrasonography was performed in patients and reflux in deep and superficial veins was evaluated. The reflux time is simply duration of inverse flow. A reflux time of >500 ms was used to define the valve insufficiency of the superficial and perforating veins. A reflux time of >1 s was used to define the valve insufficiency of the deep venous system. Venous reflux parameters were compared using t-test.

Results: Twenty-five patients were identified in this study. The mean age of the patients was 12 (4-17) years. As a result of clinical examination, 11 patients had varicose venous or venous malformation, 9 patients had swelling (pedal edema), and 6 patients had edema or venous ulcer. Two patients had normal examination findings. Chronic inflammation, hyperpigmentation was not present. No venous thrombomboemboli story was available in any patient.

Conclusion: The uncertainty of the factors that cause venous reflux in children still maintains itself today. However, this uncertainty will continue for a long time as the methods used in the diagnosis and treatment of chronic venous disease are the same in both adults and children.

Keywords: Child patients, venous insufficiency, Doppler ultrason, tromboemboli

Introduction

The diagnosis and treatment of chronic venous disease are well standardized in adults. However, the diagnosis of chronic venous insufficiency has not been established except in Klippel-Trénaunay syndrome (KTS) and post-thrombotic syndrome in pediatric patients (1).

Because venous Doppler ultrasonography (RDUS) is used less in children than in adults and it is performed or interpreted according to adult protocol, it may cause less data on venous disease in pediatric patients (2).

In this retrospective study, we planned to present pediatric patients diagnosed with venous insufficiency by our clinic.

Methods

Patients under 18 years of age who were consulted to our clinic between January 2016 and May 2018 were included in this study. Demographic history, clinical examination and RDUS results of the patients were the indications for our study. Since our study was a retrospective study, informed consent was not obtained from the patients. As it was a retrospective study, ethics committee was not applied.

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RDUS was performed in the upright position as allowed by the patient (mobility, difficulty in positioning). Alternatively, patients with difficulty in that position were given supine position. All proximal veins were examined, including femoral vein and popliteal vein. Superficial vessels, including great saphenous veins, were evaluated at 3 to 5 cm intervals in a similar manner to accessory saphenous veins with small saphenous vein and perforating veins.

Reflux was evaluated in deep and superficial veins by venous Doppler ultrasonography. Reflux was achieved using a Valsalva maneuver for the common femoral vein or saphenofemoral junction by manual compression and release of the extremity until examination.

The reflux time was considered as the reverse flow time of the blood flow. A reflux time of >500 ms was used to identify valve failure of the superficial and perforating veins. A reverse flow time of >1 second was used to define valve failure of the deep venous system (eg. common femoral, femoral, and popliteal veins).

Venous reflux parameters were compared by using variance analysis and unpaired t-test.

Results

A total of 25 patients were identified in this study. The mean age of the patients was 12 (4-17) years. Twelve of our patients had swelling in the foot, 9 had varicose veins, and 4 had redness/venous ulcers in the foot (Table 1).

As a result of clinical examination performed on the patients, it was detected that 10 patients had lesions showing varicose vein or venous malformation, 9 patients had swelling (cases of pedal edema), and 4 patients had edema or venous ulcer accompanying to edema (one of which was pedal wounds consistent with advanced complex regional pain syndrome). Two patients had normal examination findings. There was no chronic inflammation or hyperpigmentation. None of the patients had a history of venous thromboembolism (Table 2).

In 11 of 18 patients who had venous reflux detected via venous Doppler ultrasonography, atypical triad such as capillary malformations (port wine stain), bone hypertrophy or lower extremity hypertrophy was observed to occur in addition to the diagnosis of chronic venous disease, and KTS was diagnosed.

Four of the patients with chronic venous insufficiency (n=6) specific to KTS had C2 disease (varicosity) and two had C3 disease (edema). One of the patients with chronic venous disease (CVD) and KTS (n=3) had spider telangiectasia (C1), one patient had isolated varices (C2) and one had swelling with varicoses (C3). There were no patients with advanced venous disease (ie. C4-C6). One patient was classified as normal (C0) except very mild deep venous reflux (Table 3).

Postural orthostatic hypotension syndrome was found in 2 patients, vascular malformation affecting the lateral foot and posterolateral proximal calf and distal thigh in 2 patients, grade

Table 1. Demostrative data of patients

Number of patients	25
Age (mean)	12 (4-17) years
Symptoms	
Swelling on foot	12
Varicose veins	9
Redness/venous ulcer	4

Table 3. CEAP classification of patients

Patients with chronic venous insufficiency specific to Klippel-Trénaunay syndrome	6	100%
C2	4	6636%
C3	2	33,3%
Patients with chronic venous insufficiency and Klippel-Trénaunay Syndrome	3	100%
C1	1	33,3%
C2	1	33, 3%
C3	1	33,3%
C4-C6	0	0
C0	0	0

C0: Mild deep venous reflux, C1: Spider telangiectasia, C2: Varicosity, C3: Edema, C4-C6: Advanced venous disease, CEAP: Clinical-Etiological-Anatomical Pathophysiological

Table 2. Clinical examination results of patients

Total number of patients	25	100%
Varicose vein or venous malformation	10	40%
Edema (pedal edema cases)	9	36%
Edema or accompanying venous ulcer	4	16%
Normal examination findings	2	8%
Chronic inflammation, hyperpigmentation	0	0
Venous thromboembolism	0	0

I congenital lymphedema in 2 patients, complex regional pain syndrome in 1 patient, and acrocyanosis in 1 patient, and alternative diagnostic methods such as magnetic resorance, venography or lymphography were used.

The maximum great saphenous vein (GSV) diameter, reflux time, and valve closure rate were then compared to isolated patients, such as CVD, KTS, or an alternative diagnosis. The first observed mean GSV diameter was 0.52 cm and there was no significant difference in maximum GSV diameter between the groups.

Discussion

In this study, ten patients had varicose vein or lesions showing venous malformation, nine patients had swelling (pedal edema cases), and four patients had edema or edema accompanied by venous ulcers (one of which was consistent with advanced complex regional pain syndrome). Two patients had normal

examination findings. There was no chronic inflammation or hyperpigmentation. None of the patients had a history of venous thromboembolism.

Although the diagnosis and treatment of CVD are well standardized in adults, no enough regulation could be made in children due to lack of data (1).

The Bochum Study (3) was initially performed for venous reflux on the development of venous disease in children aged between 10 and 12 years. According to this study, Although the incidence of venous disease in school-age children is 0.2-2.9% and the incidence of physiological venous reflux is approximately 13% in children aged between 14 and 16 years, RDUS has been used less in children than in adults (3,4).

RDUS is the most important diagnostic method that can help us to answer all questions in patients with suspected venous insufficiency and Van Bemmelen et al. (5) showed in the late 1980s that it could be safely used for the diagnosis of varices and venous insufficiency. RDUS evaluates both anatomical detail and hemodynamic changes in blood flow.

With RDUS, the presence and severity of reflux, vein diameters and the presence of obstruction in all three components of the venous system can be determined (6). We performed RDUS on our patients and evaluated the presence and severity of reflux, vein diameters and the existence of obstruction in the deep and superficial veins.

In our study, venous reflux was achieved by establishing a valsalva maneuver for the saphenofemoral junction by manual compression and release of the extremity until examination, as long as the patient's age or cooperation allowed.

The reflux time was considered as the reverse flow time of the blood flow. A reflux time of >500 ms was used to identify valve failure of the superficial and perforating veins. A reverse flow time of >1 second was used to define the valve insufficiency of the deep venous system (eg. common femoral, femoral, and popliteal veins).

According to a conducted study, in spite of the presence of venous reflux in the duplex ultrasound imaging, signs and symptoms, 44% of children were diagnosed with an alternative disease consistent with venous disease. Our study was also consistent with this study. In another study, they found a 2.5% incidence of prevaginal saphenous reflux in school-age children (10-12 years) without evidence of venous varices by clinical examination and found that these children had a 30% risk for the development of truncal varicose veins during their follow-ups (7). Truncal saphenous reflux was not observed in our patients.

Venous thromboembolism is a condition that can have serious consequences such as death in children (8). Complications of deep vein thrombosis (DVT), a form of venous thromboembolism, such as post-thrombotic syndrome and pulmonary embolism, may have significant long-term results although they occur less frequently in pediatric patients than in adult patients (9).

While 40-60% of children with venous thromboembolism may develop mild to moderate venous insufficiency symptoms, severe symptoms such as ulcers are very rare (10). Thrombosis in both iliofemoral veins and failure of venous recanalization have been identified as risk factors for post-thrombotic syndrome in children (10). None of our patients had thromboembolism.

KTS is a rare, sporadic, complex malformation characterized by (1) capillary malformations (port wine stain), (3) soft tissue and bone hypertrophy, or sometimes hypotrophy of a lower extremity and (4) atypical clinical triad. Mostly, lateral venous varices (11). Varicose veins or venous malformations are present in 72% of CTS patients (12). In our study, in 11 of the 18 patients who had venous reflux detected by venous doppler ultrasonography, atypical triad such as capillary malformations (port wine stain), bone hypertrophy or lower extremity hypertrophy was observed to coexist in addition to the diagnosis of chronic venous disease.

In recent screening studies, 10-15% of high school children have been found to have venous insufficiency symptoms. In our study, 3 of the patients diagnosed with venous insufficiency were in high school age. In a study conducted by Studennikova et al. (13), they found that the prevalence of chronic venous insufficiency increased in childhood and adolescence due to the relationship among genomic changes. In our study, genotypes of the patients could not be examined.

It has been suffered from the lack of diagnostic accuracy in chronic venous diseases for a long time, which has led to complex conclusions about the same disease in different studies. In 1994, at the American Venous Forum, classification and staging was performed in the CVD called CEAP (14). With this classification, clinical symptoms (C), etiologic (E) cause, anatomical features (A) and underlying pathophysiological events (P) are defined (15). Clinical findings are divided into 6 stages according to the severity of the event: C0=normal, C1=spider/reticular veins, C2=varicose veins, C3=edema, C4=skin changes, C5=healed ulcer and C6=active ulcer (16,17). We also divided the patients into groups using the CEAP classification.

In our study, 4 of the patients with chronic venous insufficiency (n=6) specific to KTS had C2 disease (varicosity) and two had C3 disease (edema). One of the patients with CVD and KTS (n=3) had spider telangiectasia (C1), one patient had isolated varices (C2) and one had swelling with varicoses (C3). There were no patients with advanced venous disease (ie. C4-C6). We classified one patient as normal (C0) except for very mild deep venous reflux.

Nurmeev et al. (18) used the CEAP classification in their study and they reported that they encountered C1 class more frequently in children. Also, in our study, we found that C1 class was more common in children.

Conclusion

The uncertainty of the causative agents of venous reflux in children is still present. In addition, uncertainty will continue for a long time due to the determinative methods and treatment protocol applied to adult individuals.

Ethics

Ethics Committee Approval: As it was a retrospective study, no ethics committee was applied.

Patient consent: Since our study was a retrospective study, consent was not obtained from the patients.

Reviewer evaluation: The study was evaluated by people outside the editorial board.

Authors Contributions

Concept: M.E.T.A., B.İ., Design: B.İ., Data collecting and processing: M.E.T.A., Analysis or interpretation: M.E.T.A., B.İ., Literature review: B.İ., Written by: M.E.T.A.

Conflict of Interest: Authors declare no conflict of interest.

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Original Article

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The Effect of Cinnamon on Microbiological, Chemical and Sensory Analyses of Probiotic Yogurt

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ABSTRACT

Objective: This study was performed with the aim of determining the effects of different levels of cinnamon which were added to probiotic yogurts on the microbiological, chemical and sensory properties of these yogurts.

Methods: In this study, probiotic yogurt was produced using yogurt and probiotic yogurt cultures. Yogurts are divided into four groups; control, cinnamon 1, cinnamon 2 and cinnamon 3 groups. To the groups, 0%, 0.3%, 1% and 2.5% of powdered cinnamon were added, respectively. Content analysis of cinnamon used in the study was conducted by gas chromatography- mass spectrometry method.

Results: The addition of cinnamon to probiotic yogurt showed antibacterial activity on *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* while cinnamon depending on its concentration was found to support bacterial growth of *Lactobacillus delbrueckii* subsp. *bulgaricus*. The pH values and the fat-free dry matter ratios varied depending on the proportion of cinnamon added to the yogurts. When the sensory characteristics of yogurt were compared, the score of the control group was higher than the score of cinnamon groups. On the other hand, cinnamon (1%) group had the closest score to the sensory analysis score of the control group.

Conclusion: These results have shown that cinnamon added at different ratios in probiotic yogurt has a limited positive effect on the microbial, chemical and sensory properties of this food.

Keywords: Cinnamon, functional food, probiotic yogurt, probiotic bacteria, yogurt bacteria

Introduction

Probiotics are available in the market as food and nutritional supplements. Probiotics, as functional food group, play a role in protection and development of health, and treatment of diseases. Probiotics are recommended especially in cases of gastrointestinal disorders, atopic dermatitis and food intolerance (1). Probiotic has been described as a "living microbial dietary supplement" that positively affects the host's intestinal pathways (2). Probiotic bacteria boost the immunity in individuals as well as reduce fecal enzymes and mutagenicity. The nutritional sources of probiotics include fermented foods such as yogurt, kefir, cheese, pickles and raw sausage, which contain probiotic bacteria and/or yeasts (3). The probiotic property of a food is based on its medical activity, the number of active cells or the total number of living cells per mL (4). A probiotic food must contain at least $1.0x10^6$ colony-forming units (cfu)/g of living probiotic microorganisms (5).

Yogurt has an important role in our nutrition but also has a therapeutic role in the protection and development of health (6). Yogurt is briefly defined as a clot formed by fermentation and precipitation of milk proteins (7). Yogurt is traditionally produced by fermentation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* starter culture (8). Most of the classic

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yogurt cultures used in the fermentation of yogurt are unable to survive in the intestines after individuals consume the yogurt due to its low pH value (9). Therefore, prophylactic use of yogurt is limited. However, on the other hand, this limitation encourages the production of different types of yogurts with different starter culture formulations. In particular, the use of probiotic cultures consisting of *Lactobacillus acidophilus* and bifidobacteria in yogurt production is increasing (10). Thus, yogurt gains prophylactic and therapeutic value. Vitamins and enzymes produced by lactic acid bacteria contribute to host metabolism, while antimicrobial agents produced control the proliferation of undesired pathogens in the host (6). It has been reported in several studies that these bacteria and other probiotic bacteria possess tumor suppression properties by reducing mutagenic, carcinogenic and genotoxic components in colorectal cancer, which is the third most common cancer in the world (11).

Cinnamon (*Cinnamomum*) is derived from a tree belonging to the family *Lauraceae*. In studies, antioxidant, insecticide, anti-diabetic, anti-inflammatory, anti-thrombosis and analgesic effects of cinnamon have been reported (12). Many studies have shown that the components of cinnamon oil or its extract has an inhibitory effect on pathogenic microorganisms by 75-100% (13). Cinnamon as the main component of , cinnamaldehyde, has antibacterial properties by inhibiting the synthesis and function of bacteria cell wall, the synthesis of nucleic acid and protein. The shelf life of the product was investigated in a study conducted by adding different amounts of cinnamon powder (0.3%, 1% and 2.5%) to pasteurized milk (14). In this study, it was determined that the number of lactic acid bacteria in milk, in which cinnamon was added, was low compared to the control group in which cinnamon was not added. In another study, powder cinnamon in different concentrations did not have an antibacterial effect on *Staphylococcus aureus*, because the buffering capacity of milk and yogurt neutralized the acidity of cinnamon (15). But powdered cinnamon has had a positive effect on the sensory quality of milk and yogurt.

In this study, probiotic yogurts with cinnamon added in different proportions were produced and the effect of cinnamon on microbiological, chemical and sensory properties of these yogurts was investigated.

Method

GC-MS Analysis of Cinnamon

Cinnamon was obtained from Bezmialem Vakif University, Phytotherapy Center (Istanbul, Turkey) and the chemical components of cinnamon were analyzed using gas chromatography-mass spectrometry (GC-MS) (Agilent 5977A, USA) method. The GC-MS system used provided these conditions: Injection volume (5 µL), inlet temperature (250 °C), split ratio (50:1), column (DB-WAX 60 m x 0.25x0.25), flow (1 mL/min He) and temperature program (10 ºC/min temperature increase, 5 min incubated and 70 ºC, 29 min incubated and 230 ºC).

Preparation of probiotic yoghurts added in different ratios of Cinnamon

Pasteurized cow's milk (3% fat) used in the production of yogurts was purchased from the market (Dost milk, Turkey). Regular yogurt and probiotic yogurt starter cultures (*Streptocus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis*) were obtained commercially (Maysa food, Adana, Turkey). Starter culture and cinnamon at 0% (control group), 0.3% (cinnamon 1), 1% (cinnamon 2) and 2.5% (cinnamon 3) ratios were added to pasteurized milk heated at 45 ºC. Each experimental group was repeated 3 times. The amount of culture used in yogurt production and the incubation temperature were provided in according to company recommendation that produced the culture. After incubation, probiotic yogurts were matured at + starter 4 ºC for 24 hours and microbiological, chemical and sensory analyses of yogurts were performed.

Microbiological Analysis

Microbiological analyses of experimental probiotic yogurts were performed according to IDF and ISO standards (16). Pepton powder, M17, MRS, MRS-CC and TOS-MUP agar plates used in the study were commercially obtained (Merck, Turkey). In aseptic conditions, 10 g of each experimental yogurt was weighed in sterile bags (Sterile Stomacher Bag, VWR, Turkey) with 90 mL 0.1% peptone water. Homogenization of the first and dilution (10^{-1}) was achieved in Stomacher (VWR, Italy). The adilution series were prepared up to 10^{-7}. One hundred microliters were taken from each dilution and inoculated on agar plates mentioned above (17). Inoculated MRS, MRS-CC and TOS-MUP media were incubated at 37 ºC in anaerobic conditions for 72 hours (Memmert, IN 110, Germany). Anaerobic conditions were provided using the anaerobic jar and related kits (Merck, Darmstadt, Germany). M17 agar plates were incubated for 24 hours at 45 °C in aerobic conditions after inoculation. Colony counter (Interscience, Scan 100, France) was used to evaluate agar plates after incubation. *Streptococcus thermophilus* in M17 agar plate, *Lactobacillus delbrueckii* subsp. *bulgaricus* in MRS agar plate, *Bifidobacterium animalis* ssp. *lactis* in TOS-MUP agar plate and *Lactobacillus acidophilus* bacteria in MRS-CC agar plates were identified. Only 25-250 colonies presented agar plates were evaluated (17). The determined bacterial numbers were calculated as colony-forming unit (cfu)/g yogurt with the cfu formula.

Chemical Analysis

For quantitative analysis of the oil, 50 g of each yogurt sample was weighed (Ohaus Company, USA) and 5 mL ammonia (Merck 28-30%) was added and they were mixed together. Each specimen was studied twice. For this reason, 2 butyrometers (Funke Gerber, 0.1% sensitivity) were taken and 10 mL of sulfuric acid (Merck 90%, d=1.82) and 11 mL of prepared yogurt-ammonia mixture were added to each butyrometer. One mL of N-amyl alcohol (Merck 100%, d=0.815) was added to

each. Butyrometers were centrifuged in the Gerber centrifuge (Funke Gerber Germany, T=65 ºC, 1350 rpm) for 5 min. After centrifugation process, butyrometers were kept in water bath (J. P. Selecta, Spain) at 63 ºC for 10 minutes and centrifuged for another 5 minutes. The results were read from the butyrometer and fat ratio was calculated in percentage (18).

Some sea sand (Merck, Turkey) was added into the petri dishes for oil-free dry matter analysis and dried at 103 ºC in drying-oven (Binder, ED 115, Germany). It was then cooled in a desicator and weighed on the precision scale (Ohaus, USA). Three grams from homogenized yogurt sample were transferred to the Petri box and dried in drying-oven at 103 ºC for 2 hours and then cooled in a decicator. The resulting sample was weighed at the precise scale and the percentage amount was calculated by mass as m/m (19).

Protein content analysis of experimental yogurt groups was done by formol titration method (19). Fifty g of yoghurt sample was weighed (OHAUS, USA) and 50 mL of destile water was added. Then 0.5 mL of 2% phenolphthalein (Merck, Turkey) and 2 mL of saturated potassium oxalate (Merck, Turkey) were added and mixed. The mixture was titrated 2 minutes later with 0.1 N of sodium hydroxide (NaOH) (Merck, Turkey) until it slightly got pink. Then 10 mL of formaldehyde (Merck, Turkey) was added to the mixture and mixed. After waiting 1 min, the mixture was titrated with 0.1 N NaOH until the pink color was obtained again. Finally, the amount of protein in yogurts and the amount of 0.1 N NaOH solution titrated were calculated.

For titration acidity determination, 10 g of samples from experimental yogurt groups were weighed and 10 mL of distilled water was added. 0.5 mL phenolphthaline (Merck, Turkey) was added to the mixture and titrated with 0.1 N NaOH until it received a pink color that did not disappear for about 30 seconds. Titration acidity was calculated with the amount of 0.1 N NaOH used (18).

The pH values of experimental yogurt groups were measured with a calibrated heat-sensor pH meter (Mettler-Toledo, Switzerland) containing a xerolyt polymer electrolite filled electrode (20).

Sensory Analysis

The probiotic yogurts produced were evaluated by the project researchers as blind according to the Turkish Standards (19,21). In general, yogurt must comply with TS 1330 Yogurt Standard in terms of appearance, consistency, smell and taste and must receive at least 4 points from each feature and 16 points in total.

Statistical Analysis

Data from microbiological and chemical analyses of experimental probiotic yogurts were evaluated using the SPSS 16.0 statistical package program. The Mann-Whithney U test was performed to compare the result of each cinnamon group with the control group and to determine whether there was a statistical difference between them. P value <0.05 was considered statistically significant. The data obtained from the sensory analysis were calculated as total points for each group.

Results

The volatile oils found in powdered cinnamon are listed as follows from high to low amounts, respectively: cinnamaldehyde, alpha-Coapene, caryophyllene, eucalyptol and others. Cinnamon powder volatile oil GC-MS chromatogram is shown in Figure 1.

The number of *Streptococcus thermophilus*, a yogurt culture bacterium, was higher in the control group than in cinnamon-doped yogurt (1% and 2.5%) groups (Figure 2a). On the other hand, the number of these bacteria decreased in yogurts due to the increased cinnamon rate.The number of *Lactobacillus delbrueckii* subsp. *bulgaricus*, a yogurt culture bacterium, was similar in the control group and in 1% and 2.5% cinnamon-doped yogurt groups (Figure 2b). Only a statistically significant difference was found between the control group and cinnamon 1 (0.3%) group ($p<0.05$).The number of *Bifidobacterium animalis* ssp. *lactis* was significantly higher in the control group than the cinnamon groups ($p<0.05$) (Figure 2c). There was no statistically significant difference between the cinnamon groups. *Lactobacillus acidophilus*, another probiotic bacterium used in the study, was statistically significantly higher in the control group compared to the cinnamon-added yogurts ($p<0.05$) (Figure 2d).

The results of the chemical analyses of probiotic yogurt groups are presented in Table 1 with average amounts and standard deviations of % fat, % non-fat dry matter, % protein, % lactic acid and pH values together. The fat ratio of the control group (3.3±0.1%) was found to be higher than all cinnamon groups. There was no statistically significant difference between the results. Cinnamon-added (0.3%) yogurts had the lowest fat-free dry matter ratio, while the fat-free dry matter ratio also increased due to the increased cinnamon ratio. Statistically significant difference was found between all groups ($p<0.05$). The protein ratios of all yogurt groups, including the control group, were the

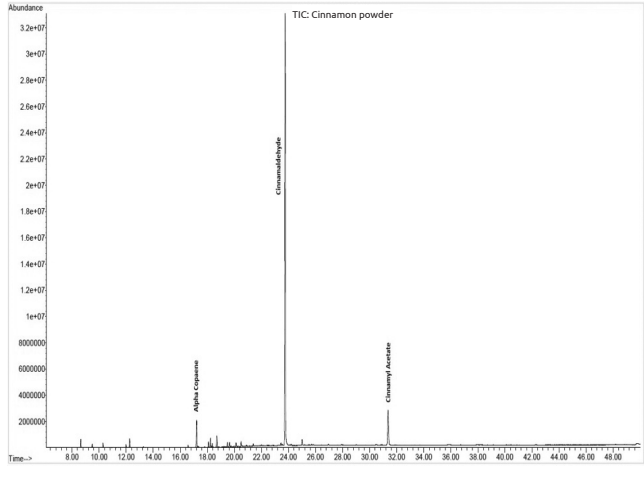

Figure 1. GC-MS chromatogram of volatile oil of cinnamon powder

same. Titration acidity of the control group was significantly higher than all cinnamon groups ($p<0.05$). On the other hand, titration acidity decreased due to the increased cinnamon rate. The pH value of the control group was significantly lower than the cinnamon groups ($p<0.05$). The pH value of yogurts increased due to the increased cinnamon ratio (4.06±0.08, 4.07±0.06 and 4.27±0.01 respectively).

When the sensory analysis of yogurts was scored, the control group received the highest score (160 points) in total, while the closest value to this score was in 0.3% cinnamon-added yogurt. Sensory analysis scores of yogurts with 1% and 2.5% cinnamon addition were found to be close to each other.

Discussion

Probiotics are microorganisms that are alive and can produce substances that encourage the reproduction of other microorganisms (22). However, the effects of these microorganisms depend on the strain to which they belong and the dose in the product in which they are found. On the other hand, the survival, growth and viability of these microorganisms are affected by the stages of production of probiotic food (23). It has been reported in various *in vitro, in vivo* and human studies that probiotic microorganisms in the product must be more than 1.0 x 10^6 cfu/g in order for a probiotic food to have therapeutic effect (24). In our study, the number of probiotic bacteria was more than 1.0x10^6 cfu in each gram of all produced probiotic yogurts.

Cinnamon is widely used in the food industry because of its antioxidant effects, colour and flavoring properties, as well as in the medical, cosmetic, perfumery and nutraceutical industries (13). Scientific studies also report that cinnamon has antibacterial effect besides its many bioactivities (12,13). On the other hand, cinnamon increases the safety of food products against foodborne pathogens and food-spoiling bacteria due to its antimicrobial

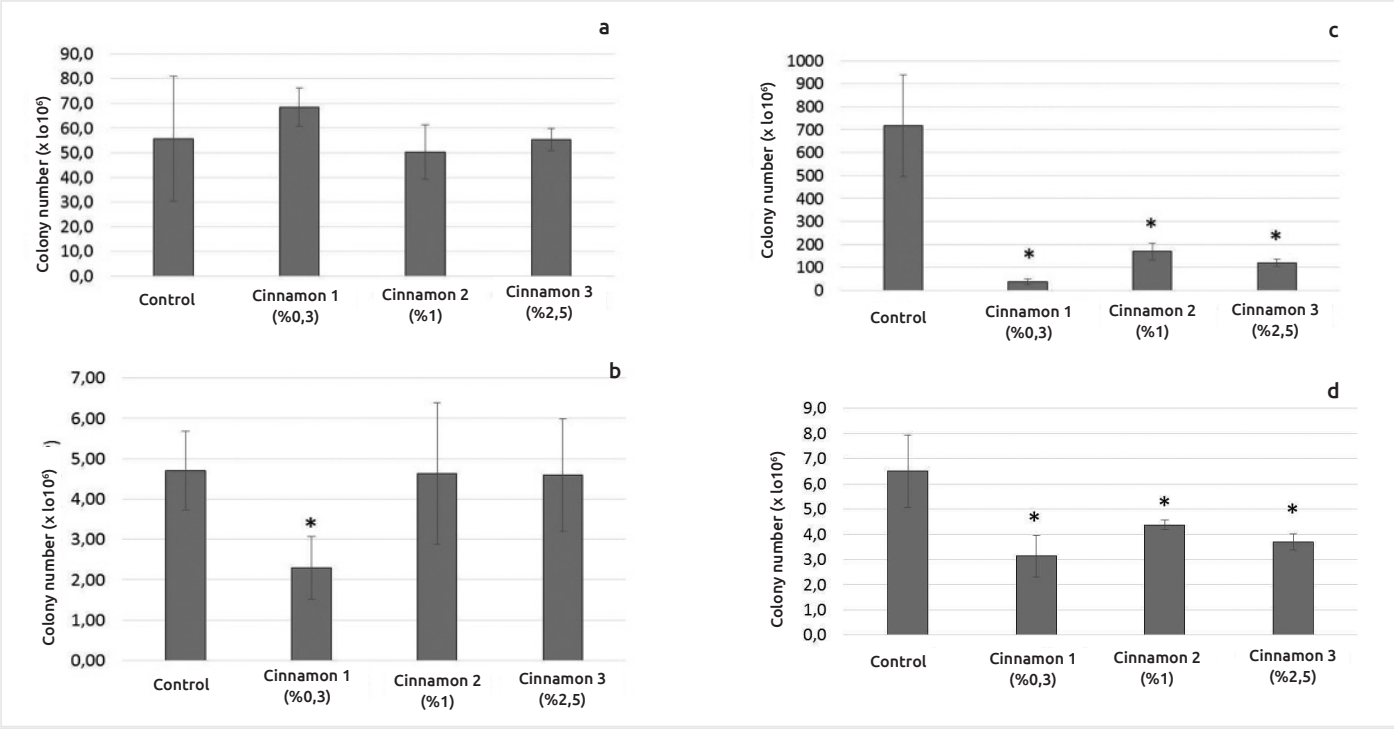

Figure 2. The bacteria counts in probiotic yogurt groups are determined as colony forming unit. a) *Streptococcus thermophilus*, b) *Lactobacillus delbrueckii* subp. *bulgaricus*, c) *Bifidobacterium animalis* ssp. *lactis* and d) *Lactobacillus acidophilus*. * Differences between control group and cinnamon groups are statistically significant ($p<0.05$)

Table 1. Fat, fat-free dry matter and protein percentages and pH value of control and cinnamon added probiotic yogurt groups are shown as mean ± SD.

	Control (0%)	Cinnamon (0.3%)	Cinnamon 2 (1%)	Cinnamon 3 (2.5%)
Fat (m/v %)	3.3±0.1	3.2±0.05	3.2±0.05	3.2±0.05
Fat-free dry matter (m/m %)	8.15±0.01	8.08±0.02	8.24±0.02	9.53±0.02
Protein (m/m)	3.54±0.02	3.54±0.02	3.54±0.01	3.54±0.02
Titration Acidity (m/m %)(acidity in terms of lactic acid)	0.97±0.05	0.87±0.05	0.86±0.02	0.74±0.01
pH	3.98±0.08	4.06±0.06	4.07±0.05	4.20±0.01

SD: Standard deviation

and antibacterial properties and extends shelf life (12). This is why cinnamon is an effective food preservative. The inhibiting effect of cinnamon on the development of *Staphylococcus aureus*, which is particularly contagious to food, is due to its various chemicals contained in cinnamon (25). The most important chemical component in cinnamon is cinnamaldehyde, which has been reported to inhibit *Staphylococcus aureus*. As a result of GC-MS analysis of the cinnamon used in our study, cinnamaldehyde was found highest.

The antibacterial effect of cinnamon on gram-positive and gram-negative bacteria that cause infectious diseases in humans and on *Salmonella typhimurium*, *S. aureus*, *E. coli*, *Arcobacter butzeiri* and *Arcobacter skirrowii* that are food and cosmetic disrupting agents and lead to major public health problems has been shown in studies (12). In our study, for the first time, the effect of cinnamon spice on yogurt and probiotic yogurt starter cultures was investigated. In all cinnamon groups, the number of *B. animalis* ssp. *lactis* and *L. acidophilus* probiotic bacteria decreased statistically significantly compared to the control group. The results of a study performed with *L. rhamnosus,* a probiotic bacterium, supports our findings. The number of *L. rhamnosus* was found to be lower in the cinnamon volatile oil-added (0.04%) yogurts compared with the control group (26). In our study, the number of *L. delbrueckii* subsp. *bulgaricus*, a yogurt culture bacterium, increased as the ratio of cinnamon increased and the number of this bacterium was found to be lowest in the yogurt containing the lowest level of cinnamon (0.3%). Also, the ratio of cinnamon added affected the sensory analysis result of probiotic yogurts. Sensory analysis evaluation scores and number of *Sc. thermophilus* were similar in the control group and cinnamon 1 (0.3%) group. In a study, a positive correlation was found between sensory quality of yoghurts and the number of *Sc. thermophilus*, which supports our findings (27). In the same study, was reported that the number of *B. bifidum* had a positive effect on the sensory quality of yogurt, and in our study, the sensory quality of the control group and the number of *B. animalis* ssp. *lactis* in the control group was found to be higher than other groups.

The fat ratio of probiotic yogurts produced in the study was higher than the fat ratio of milk used (3%). The average fat ratio of all groups was %3.2±0.5%. It has been observed that the fat ratios of yogurt groups to which cinnamon was added was not affected by cinnamon. A study by Lindasari (28) found that the fat ratio decreased as the rate of cinnamon extract added increased in probiotic yogurts made from goat's milk with 3%, 4%, 5% cinnamon extract added (fat ratios were 6.40±1.22%, 6.30±0.54% and 5.70±1.49%, respectively). The fat-free dry matter ratios of cinnamon-added probiotic yogurts were higher than the control group and the average of all groups was 8.62±0.69%. A study of probiotic yogurts produced by adding prebiotics also supports our results (29). The protein ratios of the yogurts we produced were the same (3.54%). This can be attributed to very low protein content in cinnamon (30). On the other hand, the study of probiotic yogurts with cinnamon extract added showed that there was no correlation between the groups in terms of protein ratio (4.03±0.32%, 3.84±0.38% and 4.16±0.69%, respectively) (28). Titration acidity was lower in cinnamon groups compared to the control group and a decrease was found due to cinnamon ratio (%0.97±0.05 in control group, %0.87±0.05 in cinnamon 1, %0.86±0.02 in cinnamon 2 and %0.74±0.01 in cinnamon 3). In a study, it was reported that no change in acidity and pH of probiotic yogurts produced from cow and camel milk with cinnamon added was observed (31). In another study, titration acidity and pH values confirmed each other (1.66±0.15%, 1.69±0.01% and 1.87±0.12%) (28). In our study, the pH value of the control group (3.98±0.08) was significantly lower than the cinnamon groups. In addition, the pH value of cinnamon groups increased due to the increased rate of cinnamon. On the other hand, in probiotic yogurts made from goat's milk with cinnamon extract (3%, 4% and 5%), pH values were lower compared to the control group, regardless of the rate of cinnamon extract (28).

Cinnamon 1 and 2 groups with similar acidity values were also found to be close in total acceptability scores as a result of sensory analysis. In a study of probiotic yogurts with banana additives, it was reported that there was an association between acceptability of yogurts as a result of analysis of sensory properties of yogurts and acidity of yogurts (27).

Conclusion

As a result, cinnamon added to probiotic yogurt has been shown to improve the microbial and chemical quality of the product and to have limited positive effects on sensory properties.

Ethics

Ethics Committee Approval: The study was approved from the Non-Interventional Research Ethics Committee (20.03.2018, number: 54022451-050.05.04).

Peer-review: Externally peer-reviewed.

Authorship Contributions

Consept: A.G.B., Design: M.G.B., A.G.B., Data Collection or Processing: A.G.B., M.G.B., Analysis or Interpretation: A.G.B., M.G.B., Literature Search: A.G.B., Writing: A.G.B., M.G.B.

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

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Original Article

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Effects of 18-month Vildagliptin Treatment on Portal Vein Pressure and Hepatosteatosis

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ABSTRACT

Objective: Patients with type 2 diabetes have an increased tendency to develop hepatosteatosis. The effects of drugs used to treat diabetes on the liver, regardless of the disease, are unknown.The aim of this study was to investigate the effects of vildagliptin, a dipeptidyl peptidase-4 inhibitor, on the portal vein pressure and hepatosteatosis in patients with type 2 diabetes in the 18 months of follow-up.

Methods: Patients to whose treatment vildagliptin was added while they were on therapy with metformin and gliclazide for type 2 DM the vildagliptin group were included. As the control group, 49 patients with type 2 DM treated with metformin and gliclazide were included. These patients were followed up for 18 months. These patients were followed for 18 months and their pre-treatment and post-treatment examinations were repeated. Portal vein diameter, portal vein flow and portal vein velocity were calculated to evaluate portal vein pressure with the same Doppler ultrasonography (US) by the same radiologist. In the same session, the liver steatosis stage of all patients was evaluated with US and recorded. The data before treatment and the data 18 months after treatment were compared.

Results: Nineteen patients completed the study in the study group, while 10 patients completed the study in the control group. A significant increase in portal vein flow velocity and vein diameter was found in the study group when portal vein parameters were compared before and after treatment (p=<0.001, p=0.035, respectively). There was no significant difference in portal vein flow volume. In the control group, no significant changes in flow velocity and flow volume were detected, although there was a significant increase in portal vein diameter (p=0.04, p=0.07, p=0.14, respectively). There were no significant changes in vildagliptin group before and after treatment in terms of hepatosteatosis (p=0.41). There were no significant changes between control and study groups in terms of hepatosteatosis after 18 months of treatment.

Conclusion: As a result, we did not find any significant changes in the parameters of portal vein pressure with vildagliptin use. We think that vildagliptin has no effect on hepatosteatosis.

Keywords: Di-peptidyl peptidase 4 inhibitors, vildagliptin, portal vein pressure, hepatosteatosis, tip 2 diabetes mellitus

Introduction

Incretin-based therapies are agents that have been developed for the treatment of type 2 diabetes mellitus (DM). Improved glucoregulation has been observed following decreased break down or exogenous administration of an incretin hormone, glucagon-like peptide-1 (GLP-1). The advent of dipeptidyl peptidase-4 (DPP-4) inhibitors allows prevention of GLP-1 break down, thus increases its plasma concentration. Although these DPP-4 inhibitors act primarily on the pancreatic gland, they also exert effects on non-pancreatic organs (1,2). The gastrointestinal system in particular, as well as the central nervous system, bone, fatty tissue and the cardiovascular system are the most affected organs (3,4). This includes portal vein (PV) pressure and hepatic steatosis. In dog cell culture studies, there is an increased nitric

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oxide (NO) release from endothelial cells associated with the increased levels of an incretin hormone, gastrointestinal peptide (GIP), and increased PV flow (5). NO is a short-living vasodilator with a significant role in the regulation of the vascular tonus (6). The effects of vildagliptin on PV pressure and hepatosteatosis were investigated previously (7). However, a long-term prospective study was not performed. In our study, we aimed to investigate the effects vildagliptin (DPP-4 inhibitor) on portal system pressure and hepatic steatosis by means of laboratory tests and portal system Doppler ultrasonography (US) in patients with type 2 DM following 18-month therapy.

Method

The study was designed as a prospective observational study. The study included patients to whose treatment vildagliptin (2*50 mg) was added while they were on therapy with metformin (2*1000mg), gliclazide (1*60 mg) for type 2 DM (the vildagliptin group). As the control group, patients treated with metformin (2*1000 mg) and gliclazide (1*60 mg) were included (the control group). Complete blood counts (CBCs) and biochemistry tests were performed in all subjects and their demographics were recorded. Each subject's PV diameter, PV flow volumeand PV flow velocity were measured with the same doppler US device and by the same radiologist to evaluate PV pressure . Hepatic steatosis was also graded with US during the same session. Both the vildagliptin group and the control group were followed up for a period of 18 months and subjects who were still on the same treatment at the end of 18 months and who did not meet the exclusion criteria underwent repeat PV doppler US. These subjects' CBCs and biochemistry tests were also repeated and demographics were recorded again. Of these subjects, vildagliptin users were evaluated in the pre-treatment and post- treatment periods. The subjects in the control group who did not receive vildagliptin were evaluated in the pre-treatment and post- treatment periods. The obtained results were compared at the end of the study.

The study included patients who were at least 18 years of age, treated with gliclazide and metformin only or with vildagliptin added to gliclazide and metformin therapy. Patients with chronic liver disease which may increase portal pressure, congestive heart failure, chronic renal failure, chronic obstructive pulmonary disease, vasculitis and active infectious disease were excluded. In addition, patients receiving agents that are known to act on portal pressure including beta-blockers and isosorbide mononitrate were also excluded. Further exclusion criteria included patients treated with agents that are known to induce NO release including angiotensin converting enzyme blockers, angiotensin receptor blockers, calcium channel blockers. The study was initiated after it was approved by both the Ministry of Health and Ethics Board of Bezmialem Vakif University.

Doppler US method: The patients were instructed to fast after midnight and the analysis was performed between 09-12 a.m. Logiq 9 (GE, Milwaukee, USA) US and 3.5 mHz convex probe were used for the analysis when the patient was lying on left lateral decubitus position. First, all liver segments were examined on gray scale and presence and degree of hepatosteatosis were recorded. PV measurements were performed on portal confluence. Doppler degree was maintained at 30°-60°. Appropriate Doppler gain and filter adjustments were made. PV spectrum was recorded for at least 5 seconds mid-inspiration and measurements were made over this wave pattern. PV diameter, flow pattern, flow velocity and flow volume were assessed. These parameters were measured three times for each patient and the mean of measurements was taken.

Venous blood samples were obtained from the patients at least 10-12 hours of fasting. Patients' blood samples were used to measure the following laboratory values: HbA_{1c} (turbidimetric inhibition immunoassay; Roche), low-density lipoprotein cholesterol (LDL-C), triglyceride, hemogram, and alanine aminotransferase (ALT) and creatinine values (immunoassay chemiluminescent method; Beckman Coulter, Pasadena, CA, USA).

Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 15.0 software was used in statistical analyses.

During the assessment of study findings, Student's t-test and paired-samples t test were used to compare parameters with normal distribution. Mann-Whitney U test and Wilcoxon were used to compare parameters without normal distribution. Chi-square and marginal homogeneity tests were employed to compare the proportional data. A two-sided p value of <0.05 was considered significant.

Results

The study was initiated with 48 patients with type 2 DM in the vildagliptin group. After 18 months of follow-up, and the study was completed with 19 participants, 5 of whom were male. On the other hand, the control group including the subjects who did not receive vildagliptin started the study with 49 patients with type 2 DM. This group completed the study with 10 subjects, 5 of whom were male. Demographics and laboratory values of subjects who were treated with vildagliptin did not differ significantly from those of the subjects in the control group. Pre- and post-treatment demographics and laboratory values of subjects in the vildagliptin group were mostly comparable. There was, however, a significant decrease in the creatinine level ($p=0.02$) and a significant increase in ALT level ($p=0.02$) (Table 1). Pre- and post-treatment values in the control group did not differ significantly (Table 2).

PV flow velocity, PV flow volume and PV diameter were measured with doppler US in the control and the vildagliptin groups. Comparison of PV parameters in the vildagliptin group demonstrated significant increases in terms of PV flow velocity and PV diameter ($p=<0.001$, $p=0.035$, respectively). No significant difference was observed for PV flow volume. In the control group, no significant differences were seen for PV flow velocity or PV flow volume, although there was a significant increase in the PV diameter (Table 3). Comparison of 18-month therapy results between the vildagliptin group and the control

group demonstrated no significant differences in the PV flow velocity, PV flow volume or diameter (p=0.66, p=0.2, p=0.67, respectively).

Assessment of hepatosteatosis grades did not demonstrate any significant increases in terms of steatosis (p=0.157). There was no significant increase between the control and vildagliptin groups in terms of hepatosteatosis after 18 months of treatment (Table 4).

Vildagliptin and control groups' pre- and post-treatment white blood cell, hemoglobin, hemotocrit, mean corpuscular volume and platelet values did not differ significantly (p=0.98, p=0.53, p=0.35, p=0.38, p=0,29, p=39, p=0.94, p=0.7, p=0.27, p=0.7, respectively).

Power analysis calculation of the study based on PV flow velocity yielded a value of 98% in the vildagliptin group.

Discussion

DPP-4 inhibitors are demonstrated to reduce glucose and glucagon levels by increasing serum levels of GLP-1 and GIP hormones. These hormones are known to potentiate NO release by acting on the endothelia in several tissues (6). It is recognized that elevated levels of NO induce vasodilation in the PV, whereas decreased levels of glucagon are known to cause vasodilation in the splenic vein. In addition to these data, DPP-4 activity was found to be increased in patients with non-alcoholic hepatosteatosis, and hepatosteatosis grade was found to be higher with increasing levels of DPP-4 (8). Vildagliptin is a DPP-4 inhibitor which is recommended rarely in patients at risk of asymptomatic hepatitis, because its prescribing information advices hepatic function monitoring four times yearly. Based on these data, a decrease in PV pressure and increase in hepatic steatosis may be expected with short- and long-term vildagliptin use. In our study, we intended to investigate these potential effects of vildagliptin using non-invasive methods.

Table 1. The demographic and laboratory characteristics of the subjects by groups

	Pre vildagliptin (n=19)	Post vildagliptin (n=19)	*p*
Age (years)	52.16±7.18		
BMI (Kg/m²)	33.3±5.5	33.16±4.9	0.72
Glucose (mg/dL)	160.5±37	155.5±33.8	0.58
HbA1c (%)	7.16±1.08	7.02±1	0.8
Creatinine (mg/dL)	0.75±0.14	0.62±0.14	0.02
ALT (U/L)	23.6±8.5	31.9±13.9	0.02
LDL-C (mg/dL)	127.8±40.3	121.8±61	0.39
Triglyceride (mg/dL)	153±60	148±24.4	0.98

Vildagliptin group: Subjects on metformin, gliclazide and vildagliptin, HbA1c: Hemoglobin A1C, ALT: Alanine aminotransferase, LDL-C: Light-density lipoprotein-cholesterol, BMI: Body mass index

Table 2. The demographic and laboratory characteristics of the subjects by groups

	Pre control (n=10)	Post control (n=10)	*p*
Age (years)	55.4±9.2		
BMI (Kg/m²)	30.8±6.3	30.6±4.8	0.8
Glucose (mg/dL)	149.8±19.6	156.8±35.3	0.7
HbA1c (%)	6.75±0.5	6.95±0.39	0.58
Creatinine (mg/dL)	0.83±0.15	0.68±0.08	0.07
ALT (U/L)	16.5±5.3	27.7±4.3	0.09
LDL-C (mg/dL)	124.3±42.2	134.3±38.9	0.08
Triglyceride (mg/dL)	106.3±41	130.3±116	0.6

Control group: Subjects on metformin and gliclazide, HbA1c: Hemoglobin A1C, ALT: Alanine aminotransferase, LDL-C: Low-density lipoprotein cholesterol, BMI: Body mass index

Table 3. Portal vein flow velocity, portal vein flow volume, and portal vein diameter by group

	Pre vildagliptin (n=19)	Post vildagliptin (n=19)	*p*	Pre control (n=10)	Post control (n=10)	*p*
Portal vein flow velocity (L/min)	7.8±1.2	15.4±5.6	<0.001	8.4±2.3	9.5±2.25	0.07
Portal vein flow volume (cm/s)	475±145	428±182	0.44	488±95	599±139	0.14
Portal vein diameter (mm)	11.77±1.8	12.83±1.9	0.035	11.04±1.37	12.5±1.14	0.04

Vildagliptin group: Subjects on metformin, gliclazide and vildagliptin, Control group: Subjects on metformin and gliclazide

Table 4. Comparison of steatosis grades with ultrasonography between groups in pre- and post-treatment periods

Grade	Pre vildagliptin (n=19)	Post vildagliptin (n=19)	p	Pre control (n=10)	Post control (n=10)	p
Grade 0-1	10	8	0.157	6	8	0.157
Grade 2-3	9	11		4	2	

Vildagliptin group: Subjects on metformin, gliclazide, and vildagliptin, Control group: Subjects on metformin and gliclazide

Significant increase in PV flow velocity and PV diameter was observed in subjects using vildagliptin in our study. These may be interpreted as a non-invasive indicator of reduced PV pressure (9). In vildagliptin users, increased PV diameter may be expected as a consequence of increased NO levels. There was no significant decrease in PV flow volume.

Reduced PV flow volume is recognized as an indicator of reduced PV pressure. Cell culture studies showed that exogenous GIP given to dog fetal cell culture increased NO release from PV (4). We could not identify any studies investigating the effect of DPP-4 inhibitors on NO in portal veins. There are, however, many studies on other tissues and organs. In one of these, obese rats were treated with saxagliptin and aortic and glomerular endothelial NO levels were measured. The authors showed significantly elevated endothelial NO levels in animals treated with saxagliptin (10). Another study demonstrated decreased levels of serum acetyl di-methyl arginine, which is recognized as an indirect indictor of NO elevations, in subjects receiving vildagliptin (11). These results may be supportive of the results obtained in our study. On the other hand, significantly increased PV diameter in the control group was not, in fact, an expected outcome. Absence of a significant difference in PV flow volume and PV flow velocity may indicate that PV pressure was not significantly affected. Vildagliptin seems to cause a reduction in PV pressure when the results obtained from the vildagliptin group and the control group were taken separately. However, when we compared the 18-month differences of the vildagliptin and control groups, we determined that PV parameters were all comparable between the groups. This contradiction may be explained as follows: although these parameters seemed to differ significantly when assessed in individual groups, the difference, in fact, was very smalland when the whole sample was evaluated, the difference was not significant. These results may suggest that the parameters which allowed indirect estimation of PV pressure were not altered to a significant extent with vildagliptin use.

Patients who completed the 18-month vildagliptin treatment did not differ significantly with regards to hepatosteatosis grade. In the control group, there were no significant differences in hepatosteatosis or serum ALT levels. A published review on the effects of DPP-4s on the liver included studies which reported that DPP-4 inhibitors corrected hepatic steatosis as well as those which described a close association with hepatic steatosis (12). A study investigating the effects of sitagliptin, a DPP-4 inhibitor, in patients with moderate hepatic impairment found that the drug was safe and did not cause clinical deterioration (13). Another study reported increased hepatosteatosis with increasing levels of DPP-4 (8). Our results did not indicate a significant increase in hepatic steatosis.

Another important outcome of the study was that there was a statistically significant decrease in serum creatinine levels in patients receiving vildagliptin versus no significant difference in the creatinine levels in the control group. A previous study found significantly reduced levels of urinary albumin creatinine in subjects who used vildagliptin for 8 weeks compared to controls (14). Another study demonstrated a significant decrease in creatinine levels in rats treated with vildagliptin (15). The results of these studies are consistent with the results of our study.

The limitation of our study was that we did not measure the hepatic venous pressure gradient with the invasive angiography, the golden standard of assessing PV pressure. Instead, we preferred portal doppler US which is a non-invasive method and is efficient in determining the severity of portal hypertension. PV flow velocity, diameter and flow volume measured with PV doppler US was reported to have a sensitivity and specificity of 80% in reflecting PV pressure (16,17). Another limitation was that a low number of patients completed 18-month treatment in the study.

In conclusion, satisfactory evidence to suggest that vildagliptin use results in a significant decrease in PV pressure was not obtained. However, these results may suggest that vildagliptin does not increase PV pressure. This may be particularly important when selecting an agent for patients with PV pressure. It should also be kept in mind that increased NO release can be dangerous in patients who have esophageal varices or are at risk of having bleeding in esophageal varices. As the second outcome of the study, vildagliptin was not shown to have an effect on hepatosteatosis. This requires larger studies. Further studies are also needed to elucidate whether the obtained results are associated with vildagliptin or are class effects of DPP-4 inhibitors.

Ethics

Ethics Committee Approval: The study was initiated after it was approved by both the Ministry of Health and Ethics Board of Bezmialem Vakif University.

Informed Consent: Written consent was obtained from the students who agreed to participate in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: C.K., Design: C.K., Data Collection or Processing: M.Z., M.K., Analysis or Interpretation: C.K., R.K., M.G., S.A., T.Ö., Writing: C.K.

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Review

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Development of Dentin Bonding Systems from Past to Present

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ABSTRACT

Providing a stable and long lasting bonding to the tooth is an ideal requirement for the success of restorations. Therefore dentin bonding systems are important clinical contributions to recent composite technology. The first, second and third generations are classified under historical title while; etch&rinse, self-etch and multi-mode adhesives are classified under the title of current clinical practice procedures strategies. The preferred properties of adhesive systems are biocompatibility with dental tissues, improved bond strength to dental tissues, and better resistance to chewing stresses. In recent years, the success rate of restorative treatment of teeth that have suffered from caries or loss of supporting tissue has increased considerably due to the developments in dentin donding systems.

Keywords: Dental enamel, dentin, dentin-bonding agents, dental adhesives, classification

Introduction

Adhesive systems are one of the most important factors affecting the success of restorative dentistry. First, adhesive dentistry was born out of the idea of Buonocore (1) to provide retention with micromechanical retention by roughening the enamel tissue with 85% phosphoric acid for 30 seconds. At the end of the 1970s, dentin bonding systems have made a major breakthrough with the introduction of the view that phosphoric acid can be applied on dentin tissue.The mechanism of adhesion of the dentin bonding systems used today to the tissues of the teeth is defined as a natural micromechanical adhesion with the penetration of adhesive resin to collagen which is exposed on the surface of the dentin roughened with acid. "Hybrid Layer" (2) in other words' interdiffusion layer' (3) is one of the basic mechanisms of adhesion. The terms and their meanings used to understand adhesion mechanism are very important.

Adhesion

The word "adhesion" (attachment) originates from the Latin word "adhaere". The adhesion of the two materials can be expressed as the contact of their interfaces with each other decisively. In adhesive terminology, adhesion is the bonding of one substance to another. This substance or surface is called "adherent", whereas the substance that creates the adhesion is called "adhesive". Three different mechanisms of adhesion are mentioned in the dentistry literature (4).

Chemical Adhesion: It is based on primary bond values (joining forces) such as covalent, ionic and metallic bonds. Chemical adhesion is the limited and weak bonding between atoms of surfaces which are different in structure (5).

Physical Adhesion: It is a weak type of bonding between surfaces which are different in structure, resulting fromVan der Walls forces and hydrogen bonds (5).

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Mechanical Adhesion: At the microscopic level, it is based on the penetration of a material into a different material. It is the strong locking that occurs between rough surfaces. In this strong locking, both geometric and rheological factors are engaged. Mechanical adhesion caused by surface roughness or microscopic porosity is an example of geometric factors, while the flow of the material around a bulge due to its fluid properties and its hanging on with shrinkage is an example of rheological factors (5).

In order to achieve adhesion effectively, the distance between adhesive and adherent must be minimal. It is possible for the adhesive to wet the surface when the free surface energy of the adherent is greater than the surface tension energy of adhesive. It is possible for a liquid to wet a surface when the angle between the surface and the liquid is close to zero degrees. In other words, if this angle is zero degrees, it is assumed that the adhesive completely wets the relevant surface.

These basic criteria, which are necessary to establish adhesion forces, are realized by acid application. The enamel and dentin tissues are subjected to acidification process and the previously mentioned criteria of adhesion are fulfilled. After the acid process is applied, the enamel and dentin surfaces are ready for first primer and then bonding agent applications. The area between the surfaces of these tissues and the surface on which the binding agent comes into contact is called the "interface", and this is where adhesion occurs.

Important Terms Related to Adhesive Systems

Hybrid Layer

After demineralization of the dentin surface by acidification process, collagen fibrils are released. Low-viscosity monomers fill the nano-cavities formed by demineralized hydroxyapatite crystals by penetrating into this region and surround the collagen. By polymerization process, adhesive resin is micromechanically bonded with dentin collagens. This resin-reinforced, acid-resistant layer is called "hybrid layer" (2). The main binding mechanism of adhesive restorative materials is based on the formation of the hybrid layer. The hybrid layer was first identified by Nakabayashi (2) in 1982 and expressed as a mixture of demineralized dentin compounds and polymerized adhesive resin at molecular-level.

Resin Tags

Adhesive resin extensions directed/flowing into open dentin tubules are called "resin tags" (6). The structure of these tags varies according to the application technique of acid, the thickness of the remaining dentin, the surface moisture and structure of the dentin. When the peritubular dentin is removed from the tubule wall by the acidification process, the adhesive resin diffuses into the demineralized matrix. After polymerization, resin tags are attached to the tubule wall by hybridization. "Submicron resin tags" are formed when the adhesive resin infiltrates the lateral tubule arms (7).

Primer

Primers are used as binding-enhancing agents and consist of hydrophilic monomers dissolved in organic solvents such as water, acetone and ethanol. They facilitate the infiltration of the monomer into the nano cavities in the resulting collagen network by replacing with water on the dentin surface and in the moist collagen network with their volatile characters (7). Applying primers on the dentin tissue which are acidified restores the shrunken collagen, allowing the resin to be better diffused into the dentin tissue. Thus, the quality and binding resistance of the hybrid layer is increased (8). In other words, primers harmonize hydrophilic dentin with hydrophobic adhesive resin. The primer-applied surface contains non-polymerized binding-enhancing molecules. These molecules polymerize together with the bonding agent applied to the demineralized surface. The ideal binding is completed after polymer intertwins with collagen fibrils and hydroxyapatite crystals and wraps them.

Adhesive Resin

Adhesive resins, also called bonding agents, have both hydrophilic and hydrophobic properties. These systems consist of hydrophobic monomers such as bisphenol A-glycidyl methacrylate (Bis-GMA) and urethane dimethacrylate (UDMA), viscosity regulators such as triethylene glycol dimethacrylate (TEGDMA) and wetting agents such as hydroxyethyl methacrylate (HEMA) (2). Hydrophobic monomers interact with restorative materials and copolymerize, while hydrophilic monomers increase the wettability of dental hard tissues (9). The biggest difference between hydrophilic and hydrophobic adhesives is the chemistry of their monomers and solvents. The most commonly used monomers in adhesive systems are HEMA and Bis-GMA. HEMA can be fully mixed in water and acts as a polymerizable wetting agent perfect for dental adhesives. Bis-GMA, on the other hand, is much more hydrophobic and, when polymerized, absorbs only about 3% water by weight into its structure (10). The mixture of the two is like an intermediate and serves as a useful adhesive for dental hard tissues. The chemical composition of adhesive systems also includes initiators, inhibitors or stabilizers, solvents and, in some cases, inorganic fillers (11). In 1982, Nakabayashi (12) was the first to demonstrate the formation of a true hybrid layer, and named this new biocomposite structure, the hybrid layer. This layer has been considered the main binding mechanism of binding agents. The most important tasks of binding agents are to fill the nano cavities formed in collagen after acidification, to ensure the formation of resin tags by infiltrating into the dentin tubules, to provide the formation of a uniform and stable hybrid layer (13). Proper wetting of the surface with the binding agent is based on proper selection ofprimer and ideally application of the primer. The hybrid layer obtained after the application of primer is polymerized together with the bonding agent (7).

Development, Use and Classification of Dentin Adhesive Systems

Dentin Adhesives

They are intermediate materials that can be connected with both dentin tissue and composite resin and are developed to help ensure the connection between dentin tissue and composite resin surfaces and the retention of restoration, to prevent microleakage and to prevent dentin sensitivity that may occur after restoration by covering dentin tubules (13).

Adhesive systems consist of dentin conditioner, dentin primer, and dentin adhesives applied at different stages. There are also systems where the primer and conditioner are combined or primer and adhesive are combined (14).

Properties of Dentin Adhesives

Enamel/dentin bonding systems used to perform adhesive bonding are today called "adhesive systems". Properties sought in adhesive systems:

• Prevention of microleakage and secondary caries,

• To be able to withstand stresses caused by polymerization shrinkage and under chewing forces,

• Micromechanical and chemical bonding to enamel and dentin tissue,

• To be able to connect to enamel and dentin tissue as well as to be able to connect to metal and porcelain,

• Easy application on moist surfaces (Wet-bonding),

• Easy clinical application without technical precision,

• Preventing post operative sensitivity by closing all or part of the dentin channels,

• Being biologically acceptable (15).

As a result of the tissue removal procedures performed during cavity preparation by using milling tools, the dentine surface is covered by a smear layer consisting of blood, saliva, bacteria, hydroxyapatite crystals and denatured collagen (16). The smear layer, which protects the dentin and pulp tissue against irritation, is about 0.5-2 μm thick and is porous and amorphous in appearance. The different thickness of the smear layer also causes differences in the permeability of dentin tissue. Dentin tubule mouths are clogged with smear plugs, which reach a depth of 1 to 10 μm of tubules. These smear plugs are a continuation of the smear layer consisting of fragmented and denatured hydroxyapatite (16). There are various opinions about the removal or modification of this layer, which is effective in adhesion binding. Some of the researchers have argued that the smear layer creates a barrier for rnicroorganisms to reach the pulp, and have reported that with removal of this layer, dentin permeability will increase 5-10 times. Another group of researchers have shown that the smear layer is a shelter for bacteria to settle and multiply (12).

Classification of Dentin Adhesives

Over the years, dentin adhesive systems have been classified numerous times by authorities. Researchers have made this classification based on the stages of clinical practice and modern adhesive strategies, sometimes through generation.

With changes in adhesive dentistry, adhesive systems have developed from the stage where no acid is applied to total-etch (4. and 5. generation) and later self-etch (SE) (6., 7. and 8. generation) (17). Each generation has tried to minimize procedure steps, allowing clinicians to finish restorations in less time with less technical precision. In addition, improved chemical structures of dentin adhesives have resulted in better bonding (18).

Dentin adhesives can be classified in three main groups (Table 1) (19-22):

The first group is called the historical classification. It consists of 1st., 2. and 3. generation dentin adhesive systems.

Second group: Dentin adhesives are classified according to their effect on the smear layer. They can be examined in 4 groups: The adhesive systems which are applied on the smear layer, modify the smear layer, remove the smear layer completely and dissolve the smear layer (22).

The third group is the current classification, in other words, the classification of dentin adhesives according to the type of application in the clinic:

1. Etch&rinse (ER)

a-3-stage

b-2-stage

1. Self-etch

a-2) Stage

b-1) Stage

1. Universal (Multi-mode) (17).

According to Historical Classification;

First Generation Adhesive Systems (Adhesive Systems Applied on Smear Layer)

In 1955, Buonocore demonstrated that glycerophosphoric acid dimethacrylate can bind with hydrochloric acid to the surface

Table 1. Classification of dentin adhesives

Chronological classification
1. Historical classification
First generation
Second generation
Third generation
2. Classification according to their effect on smear layer
-Smear tabakasının üzerine uygulanan
-Modifying the smear layer
-Completely removes the smear layer
-Dissolves the smear layer
3. Current classification (according to clinical application types)
Etch&rinse (ER) adhesives
Three-stage etch & rinse (ER) adhesives (Fourth generation)
Two-stage etch & rinse (ER) adhesives (Fifth generation)Self-etch adezivler
Two-component self-etch adhesives (Sixth generation)
One-component and one-stage self-etch adhesives (All-in-one)
Eighth generation
Universal (Multi-mode)

of roughened enamel (1). Later, Bowen and Rodriguez theorized that N-phenyl glycine glycidyl methacrylate (NPG-GMA) forms a chemical bond with dentin (21). As a result of these studies, in 1962, manufacturers produced NPG-GMA origin dentin bonding agents, also called first generation dentin adhesives, but dentin bondings in this generation had a hydrophobic structure, so their attachment strength to dental tissues was low (2-6 MPa) (23).

Second Generation Adhesive Systems (Adhesive Systems Applied on Smear Layer)

These systems, which are halophosphate esters of resin monomers such as Bis-GMA or HEMA, were developed in the early 1980s. The second generation adhesive systems did not have sufficient binding strength to resist the polymerization shrinkage of composite resin (1-10 MPa). Although the first and second generation adhesive systems were developed to bind to the inorganic structure of the dentin, the desired clinical success was not achieved (23).

Third Generation Adhesive Systems (Adhesive Systems that Modify the Smear Layer)

In this system, the smear layer is modified and the penetration of the resin monomer to the dentine is provided (20). The idea of roughening dentin tissue with phosphoric acid before this bonding agent containing phosphonate ester is applied was put forward by Fusayama et al. (23). However, due to the hydrophobic nature of the bonding agent, the desired success in terms of binding strength was not achieved again (23).

Fourth Generation Adhesive Systems (Adhesive Systems that Completely Eliminate the Smear Layer)

The fourth generation dentin bonding systems are the first to completely eliminate the smear layer and are recognized as the gold standard. They are still used in current clinical applications (20). In these systems, which reduce dentin permeability and eliminate the smear layer, which is considered to be a diffusion barrier, orthophosphoric acid is applied to both enamel and dentin tissue at the same time (Total-etch) at a concentration of 30-40%. Etch & rinse adhesive systems can be either three-stage or two-stage.

Fourth Generation: Three-stage Etch&Rinse Adhesive Systems

These adhesive systems with proven binding strength values toenamel (20-50 MPa) and dentin (13-80 MPa) are applied in three stages following each other.

1st Stage: Changing the surface conditions of enamel and dentin (roughening with acid),

2nd Stage: Application of adhesion enhancing agents (application of primer),

3rd Stage: Infiltration of bonding agent to demineralized enamel/dentin surface.

1st Stage: Change Of Enamel/Dentin Surface Conditions

The reason for changing the enamel/dentin surface conditions is to create a suitable enamel/dentin surface that can provide chemical and micro-mechanical bonding of bonding agents. At this stage, different concentrations of phosphoric acids are applied to the enamel and dentin tissue simultaneously (Total-etch) for a certain period of time (minimum=15 sec, maximum=30 sec) and then the acid is washed away from the tooth surface as far as the duration in which acid is applied. As a result of acid roughening process, the smear layer in the enamel is removed and the aprismatic layer is removed about 10 μm from the surface of the enamel.By acidification, a large number of microscopic indentations and protrusions are created at a depth of approximately 5-50 μm, increasing the surface area and increasing the critical surface tension value (CST) of the enamel tissue to 72 dynes/cm. All these factors provide an alignment between composite resin and cavity wall, increasing the retention of restoration and greatly reducing edge leaks.

Generally, 37% orthophosphoric acid is used for roughening with acid. Silverstone et al. (24) showed with Scanning Electron Micrograph (SEM) studies that three types of roughening occur in orthophosphoric acid-roughened enamel tissue, depending on the concentration of the acid and the duration of application. In the first type of roughening, the inner parts of the mine prisms are dissolved and removed, resulting in the appearance of honeycomb. In the second type of roughening, the periphery of the mine prisms is dissolved and removed, resulting in a paving stone image. The third type of roughening is a dissolution that does not conform to the morphology of the prisms, and a more faint appearance is observed (23). Hydroxyapatite crystals show a regular distribution in enamel tissue, while in dentin tissue they are randomly distributed in organic matrix. In addition, the hydroxyapatite crystals in the dentin tissue are smaller than the crystals in the enamel tissue and contain less calcium and carbonate. For this reason; the mineralization of the dentine tissue is less than the enamel tissue, more than the cement tissue and bone.

Dentin has a large number of tubules/ducts filled with fluid. These begin from the pulp tissue and pass through the dentine tissue, reaching the mine-dentin border. The tubules are coiled with well mineralized peritubular dentin. Among the tubules is intertubular dentin, whose mineralization is less than peritubular dentin. The liquid inside the tubules is pushed from the pulp tissue towards the outer surfaces with a approximately 25-30 mmHg (34-40 cm water pressure). This is why dentin tissue is always moist (23). There is a continuous fluid exchange in the dentin tissue which is dynamic. The protein ratio of dentin tissue is high and therefore the critical surface tension value (CST, 44.8 dynes/cm) is lower than enamel tissue. The low surface energy of the dentin tissue also reduces the wettability of this tissue and makes bonding difficult.

The main factors involved in dentin adhesion are the content of dentin tissue (density, diameter, peritubular and intertubular dentin ratio), dentin thickness and structure (demineralized/sclerotic), smear layer and age. The diameter and number of dentin tubules in deep or superficial cavities affect the adhesion strength.The tubules make up 28% of the dentin near the pulp by volume and 4% of the dentin at the enamel-dentin border.

Furthermore, the number of tubules near the pulp (45,000 in mm^2) and its diameter (25 μm) is greater than the number of tubules at the enamel-dentine border (20,000 in mm^2) and its diameter (0.8 μm). Accordingly, adhesive bonding strength is lower on deep dentin surfaces closer to pulp tissue (23).

By applying acid to dentin tissue, smear layer is removed as a result of removal of tissues using milling tools or cutting hand tools during cavity preparation, smear plugs (plug) are eliminated, there is a flow of fluid from the dentin tubules towards the dentin surface and the permeability of dentin tissue increases 5-20 times.In addition, a drop in the critical surface tension value of dentin tissue (44.8 dynes/cm) is observed with acidification (29.48 dynes/cm) (23). This decrease in the value of CST negatively affects adhesion. The purpose of the primer applied in the second phase of adhesive systems is to increase this value.

2nd Stage: Use of Adhesion Enhancing Agents

The primer containing the HEMA monomer is applied to the surface of the enamel/dentin, where the surface conditions have been changed, in order to increase the surface energy due to its wettability. Instead of HEMA, many monomers are also used as primers. The primer molecules are bipolar and contain two different functional groups. Of these, the hydrophilic one interacts with moist dentin, while the hydrophobic one interacts with adhesives. Primers are binding-enhancing materials that dissolve in solvents such as water, ethanol, or acetone (23).

The primer is applied to the dentin surface with a microbrush until a bright surface is obtained in two or more layers according to the case after the roughening stage with acid, and dried with air for 5-10 seconds. During the drying process, care is taken to fully vaporize the solvents (acetone, ethanol) in the adhesive content.The primer prepares the surface for adhesive bonding by altering the sequences of collagen fibrils, and then helps make the penetration of the monomer more effective. The primer, which passes through the residual smear base in the acidified dentin tissue, is replaced by water on the dentin surface due to the volatile property of acetone and/or ethanol and fills the nano-cavities left by hydroxyapatite crystals that melt between the collagen fibrils.

3rd Stage: Infiltration of Bonding Agent to Demineralized Enamel/Dentin Surface

Bonding agents attached to enamel/dentin and resin are applied to enamel/dentin surfaces where surface conditions have been changed by acidification and then primer has been applied. Resin tags formed between the outer surfaces of enamel prisms as a result of penetration of bonding agents into interprismatic spaces are called “macrotag”, while many resin tags formed in the form of network by penetration into intraprismatic spaces are called “mikrotag” (25,26). Macro and microtags are responsible for micromechanical bonding in enamel tissue. Due to having large number and width of the fields of touch, the contribution of microtags to bonding is greater than that of macrotags. As a result of the studies, acceptable tag length is determined as 10-30 μm and it is shown that longer tags can break from the neck regions and tag length does not have an important role in bonding (27). After the primer applied, bonding agent is applied to the surface with a microbrush, a thin film layer is created by gently squeezing air and then it is polymerized. Thus, the hybrid layer formed after the primer application is polymerized with the binding agent.

Three-stage etch&rinse (ER) adhesive systems (fourth generation) are still recognized as the gold standard. However, these systems can be time consuming because they require multiple application steps and technical precision. Due to the difficulty of the process steps, clinicians have begun to demand more simple and technical precision-free adhesive systems (18).

Fifth Generation: Two-stage Etch&Rinse Adhesive Systems

By switching from three-stage systems to two-stage systems, the practice was simplified and began to be widely used by clinicians. In two-stage ER adhesive systems; following the stage of changing surface conditions of enamel/dentin (1. stage), the primer and bonding agent (adhesive resin) application stages (2. and 3. stage) are combined, hydrophilic and hydrophobic monomers are collected in the same bottle (2. stage). The binding strength values of enamel tissue (35-45 MPa) are the same as the three-stage adhesive systems, but the binding strength values of dentin tissue (30-35 Mpa) are found to be lower than enamel (28).

In two-stage ERadhesive systems, in the first stage,enameland dentin tissues (30 seconds) (15 seconds) are simultaneously roughened with 30-40% orthophosphoric acid (total-etch) for 30 and 15 seconds, respectively and then washed with water. While the enamel is air-dried (15-10 seconds) until a matte image is obtained, excessive moisture on the dentin surface is taken with mild air squeezing (15-10 seconds) or a dry cotton pellet (2-5 seconds) to create moist dentin.In the second stage, primer and bonding (adhesive) agent,combined in a single bottle,are applied in two or more layersdepending on the casewith a brush until a bright surface is obtained. Then, by squeezing light air (5-10 seconds), a thin layer is formed and it is spread to the cavity. Then, the polymerization is carried out. Moist dentin tissue is important in terms of moist attachment strength (29). When the demineralized dentin tissue is over-dried, the water supporting the collagen fibrils evaporates and shrinkage occurs about 1/3 of the original volume of the collagen web. There is a contraction in the spaces between the fibers, and then the formation of the hybrid layer is prevented. In the studies, it was determined that the binding strengths of acetone based adhesive systems were affected by the amount of moisture on the dentin surface (23).

Today, polyalcenoic acid in the primer forms a bond with calcium in the dentin tissue and the presence of moisture facilitates ion exchange. It was determined that moisture provides flexibility by reducing the increased elasticity modules value of collagen fibers after acidification, that moisture supports collagen fibers, and that moisture facilitates the infiltration of the monomer by widening the nanoscale gaps between fibers.In moist bonding, the dentin surface is washed (10-15 seconds) after being roughened with acid (15 seconds), the excess moisture on

the surface is removed with mild air squeezing (5-10 seconds) or a dry cotton pellet (2-5seconds). Re-wetting of over-dried dentin ensures that the shrunken Type 1 collagen returns to its former volume. As a result, the collagen network expands. Instead of an over-wer (over-wet-20 pL) or over-dry (over-dry-4 pL) dentine surface, a uniform glossy dentine surface is a clinically accepted moist dentin surface (4).

Sixth and seventh generation adhesive systems: SE adhesive systems (Adhesive systems that solve the smear layer)

Sixth Generation: Two-stage Self-etch Adhesive Systems

In this system, adhesiveand acidic primer are applied in two separate stages. In the first stage,after weakly acidic primer is applied intwo layers to dentin covered with smear layer, it is waited for the solute smear layer to join the binding and for adequate penetration of the primer into the intercollogenous cavities. After making it spread into the cavity by squeezing mildair, it is polymerized with a light source. If necessary, this process can be repeated several times in accordance with the recommendations of the manufacturer. In the second stage, the primer is polymerized with light by applying adhesives to the applied surfaces.

SE adhesive systems have been developed to protect the dentin and pulp tissue of the smear layer against bacterial irritation, to reduce fluid movements in the tubules and dentin permeability. Therefore, the working principle of SE adhesive systems is based not on removing the smear layer but on solving this layer.In these systems, the absence of roughening with separate acid, washing and drying processes found in ER adhesive systems reduces the stages of clinical application and saves clinicians' time. In this system; wet-bonding method is not applied and this system is not sensitive to changes in dentin moisture (over-wet, over-dry) thus, the possibility of error during the application decreases (4).

Weakly acidic primers used in SE adhesive systems are classified as ultra-light (pH >2.5), light (pH ≥2), medium (pH~1.5) and strong (pH ≥1) SE adhesives according to their pH (30). They cause minimal dissolution of the smear tags without eliminating the smear layer and partially demineralize the dentin tissue, allowing the monomer to infiltrate the dentine. SE adhesive systems do not produce as effective roughening of enamel as ER adhesives. For this reason, it is recommended to apply acid to enamel to increase the binding with enamel (31).

The thickness of the hybrid layer formed in these systems, which is not affected by regional differences and pulpal pressures, is thinner than the thickness of the hybrid layer formed by ER Adhesive Systems (0.5-1.5 μm) but the layer formed is uniform in structure. The adhesion mechanism is based on "hybridization", in which a uniform hybrid layer is formed intertwined with the smear layer. The upper part of the hybrid layer, in other words, hybridized smear layer, is formed as a result of infiltrationof resin monomers into the demineralized smear layer. The lower part of the hybrid layer, which is a real hybrid layer and is thinner isformed as a result of infiltrationof resin monomers into the collagen network (23).

Although SE adhesive systems form a thinner hybrid layer than total-etch systems, it has been reported that some of the monomers in their content make chemical bonding with the remaining hydroxyapatite crystals thus, these systems have good attachment strength (11).Because these systems do not completely eliminate the smear layer containing bacteria they may contain MDPB-12-methacryloyloxydode cylpridinium bromide, a quaternary ammonium analogue, which shows a strong antibacterial activity against to bacteria in the mouth, especially to S. Mutans andwhich also continues its effect after polymerization (32).

Seventh Generation: Single Stage Self-etch Adhesive Systems

Self etch adhesive materials that combine acid, primary and bonding steps in a solution, without washing and with reduced clinical application stages are gaining increasing popularity. The reduction of stages has made this system an easier system for physicians (33). Whereas there is a mixing process prior to application in type 1 single-stage adhesive systems with two-components,the mixing process is not necessary in type 2 single-stage (all-in-one) adhesive systems with one component (34). Compared to classical adhesive systems, single-stage adhesive systems contain non-polymerized ionic monomers that come in direct contact with the composite (35).This unreacted acidic monomers are partly responsible for the mismatch between single-stage adhesive systems with self-cure composites (35). Furthermore, these systems tend to act as semi-permeable membranes (34), resulting in hydrolytic degradation of the resin-dentin interface (36). These adhesives usually contain resin monomers with organophosphate and carboxylate structure, as they have to have sufficient acidity to demineralize the enamel and dissolve the smear layer (37). They also contain highly acidic hydrophilic monomers and water (5 to 50%) which allows acidic monomers to be ionized.However, due to their content, they are prone to hydrolysis, hydrolytic degradation and chemical degradation (33,36). The permeability of polymerized adhesive allows water passage from the dentin, creating water bubbles along the composite-adhesive interface that cause hydrolytic degradation (34). Water bubbles are an indication of free water that has not evaporated sufficiently, remaining in the adhesive which is not polymerized during the drying process. Tay and Pashley indicated that these microscopic formations, which Sano et al. (37) first showed in regions where there is not fully resin infiltration before polymerization, are masses of water in the view of the water tree, clinging to adhesive-dentin interface, which vary depending on the ionic structure, hydrophilicity and thickness of adhesive (38). The TEM (Transmission Electron microscopy) microscope showed that these masses of water starting from the hybrid layer and reaching the adhesive-composite interface, allowing the movement of water in the adesiv and hybrid layer, causing nanoleakage formation,formed spotted or reticular images (39). Water masses form obstacles for the polymerization reaction and for completion of hydrogel formation of the HEMA in the adhesive content (38). No such problems are encountered in two-stage SE adhesives, which are less permeable and more hydrophobic than single-stage SE adhesives (23).

Eighth Generation Adhesive Systems

Eighth generation adhesive systems containing nanoscale particles were first introduced in 2010 (36).These systems have single component and have nano fillers with an average particle size of 12 nm in their structure. These nano fillers increase the thickness of the hybrid layer which improves the penetration of resin monomers and the mechanical properties of adhesive systems (31,40). Nano-binding agents are nano-filling solutions with a longer shelf life, which demonstrate better enamel and dentin binding strength and stress absorption (17). Acidic hydrophilic monomers are involved in the structure of eighth generation adhesive systems. The main advantages of these systems are that they can be easily applied to acidified enamel surface even if they are subsequently contaminated with saliva or moisture (41). The manufacturer claims that nanoparticles acting as crucibles will reduce dimensional changes (42,43).The type and manner in which nano fillers are incorporated into the structure affect the viscosity of the adhesive and the ability of resin monomers to penetrate collagen fiber cavities. With dimensions greater than 15-20 nm or with content greater than 1.0% by weight, these systems can increase the viscosity of adhesives. It can also result in clustering of fillers on the moist surface. These clusters can cause cracks and decrease in binding force (43).

Universal (Multi-mode) Adhesive Systems

Universal adhesives have been used in clinics since 2011. These systems are also known as multi-mode or multi-purpose adhesives. Because these adhesive systems can be used as SE adhesives, the ER adhesives or SE adhesives in dentin tissue and ER adhesives on enamel tissue (a technique known as selective acidification of enamel) (31,40,44). These systems, enabling the implementation of the total-etch or selective-etch approaches, have been developed to improve weakness of the previous generation single-step SE adhesives and to obtain a strong bonding in enamel tissue. As a result of the studies, it was revealed that good results were obtained regarding the binding strengths of universal adhesives (31,45).

The majority of universal adhesives are designed based on the all-in-one concept of existing single-stage SE adhesives. Water is needed to ionize hydrophilic acidic monomers in formulations of these new adhesive systems (31). The pH value of current universal adhesives varies between 2.2 and 3.2, although it varies by product (46, 47). There is a concern that universal adhesives in this pH range may be very effective when evaluated for attachment with dentin tissue, but they may not be effective when it comes to attachment to enamel tissue, especially to prepared enamel (36,48). Universal adhesives have, in fact, similar content with conventional single-stage SE adhesives and contain carboxylate or phosphate monomer that binds to calcium in hydroxyapatite. Monomers such as methacryloyloxydecyl dihydrogen phosphate (10-MDP), silane, polyacrylic acid are often added to their structures. 10-MDP monomer is included in universal adhesive systems due to its chemical binding to hydroxyapatite, which exists in both mine and dentin tissue (11). Additionally, these systems include BPDM, PENTA (49) and polyalkenoic acid copolymers which can increase attachment to dental tissues (50,51). Furthermore, the matrix structure of universal adhesive systems consists of a combination of hydrophilic HEMA, hydrophobic UDMA and Bis-GMA monomers. As a result of the combination of these properties, universal adhesives provide a bridge between hydrophilic dental tissue and hydrophobic composite resin under various surface conditions. Silane, which is included in the formulations of universal adhesives, eliminates silanization during the binding phase of composite resins to glass ceramics (17). The multifaceted uses of these systems primarily include the advantages of the traditional ER technique. In addition, clinicians using this bonding system have the opportunity to apply both selective etch and SE techniques. Therefore, universal adhesives have a much wider application area than 7th generation dentin bonding systems. In addition, the manufacturer states that universal adhesives can be used for the application of both direct and indirect restorations, and they are also compatible with self-cure, light-cure and dual-cure resin-based simans, metal, zirconia, porcelain and composites (18). However, the main disadvantage of universal adhesives is that they contain water like other single stage SE adhesives, resulting in hydrolytic destruction. Therefore, it is recommended to apply hydrophobic resin on the polymerized universal adhesive. Since the presence of water is a problem for all single-stage adhesives, ethanol is being studied (31).

Glass İonomer Based Adhesives

These systems are resin-modified glass ionomer adhesives that connect resin composites to dental tissue as a result of combining resin and glass ionomer technology. Glass ionomer material is the only self-adhesive material that can hold on to the tooth tissue without any surface treatment (15).

Places Where Adhesive Systems are used in Dentistry

a) In composite restorations,

b) In compomer (Dyract) restorations,

c) In adhesion of indirect restorations

d) As cavity varnish under Amalgam restorations,

e) In the protection of root surfaces revealed after gingival removal and removal of dentin sensitivity,

f) In repair of crown-bridge restorations obtained from Porcelain, hybrid ceramic, and composite materials in the mouth and in the construction of core together with composites polymerized by light or dual-cure.

Properties of Adhesive Systems that are Widely used in Dentistry Clinical Applications

a. They should be biocompatible and should not damage the pulp tissue of the tooth in particular,

b. They should be able to connect micromechanically and chemically to hard tissues of teeth (enamel and dentin),

c. Apart from the hard tissues of the teeth, they should also be connected to metal and porcelain,

d. They should prevent post-treatment sensitivity by blocking all/most of the dentin channels,

e. Be able to resist the stresses caused by mastication forces and polymerization shrinkage stresses,

f. They must be resistant to thermal expansion and thermal shrinkage,

g. Should be able to apply easily on moist surfaces,

h. They must be resistant to microleakage and prevent secondary caries,

i. Shelf life should not be short,

j. Clinical applications should not be difficult and application steps should be reduced,

k. The film thickness should be minimum (less than 20μm) (18).

Conclusion

Safe bonding of composite resins to enamel and dentin tissues via adhesive systems allows more conservative cavity preparation instead of cavity prepared for amalgam restorations in operative dentistry. Advances in dentin bonding systems and application techniques make it possible for these systems to be used in many other areas of Dentistry. However, even if better and easier-to-use materials are produced, the clinician must first pay attention to the technique during the application in order to make a successful restoration in the clinic. However, it is also very important that bonding is done under ideal conditions.

The current concept of minimally invasive dentistry has led to a significant development in dental adhesiv technology. The Etch & rinse approach, especially in cases where the enamel is more dominant than the dentin tissue, in other words, in cases in whom a better adhesion on the front teeth is wanted, can be more appropriate. In posterior teeth, active application of 10-MDP based two-stage SE adhesive systems to both enamel and dentin tissue following selective etch process in enamel with phosphoric acid, can be considered a good strategy for achieving optimal restoration durability.

With the new adhesive systems, successful results are achieved in reducing or eliminating sensitivity after restoration, reducing micro leakages and ensuring that the resin can be spread nicely to the tooth tissues. Therefore, the success of adhesive materials in in vitro should be supported by clinical follow-up to evaluate the long-term durability of composite materials within the mouth.

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Review

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The Golgi Apparatus: Morphology and Function with Recent Facts

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ABSTRACT

At the center of the secretory pathway, the Golgi complex ensures correct processing and sorting of cargos towards their final destination sites which are the cytoplasm, plasma membrane, and endosome-lysosome system. Recent technological developments have discovered new morphological and functional charcteristics of the Golgi apparatus. In this review, I emphasize that the Golgi apparatus is not a stable organelle instead a highly dynamic organelle changing its morphological and functional characteristics in different physiological and pathological conditions. It is not composed of a simple discontinuous parallel stacks but is composed of various domains such as cis-Golgi network, trans-Golgi network, and intercisternal network including various coated vesicles, vacuoles and tubules. Furthermore, the Golgi apparatus is not only involved in the secretory pathway, but also involved in the storage of Ca^{2+} very likely to smooth endoplasmic reticulum. Additionally, the Golgi apparatus might serve as a microtubule organizing center which is especially important to participate in Golgi reassembly after cell division.

Keywords: Golgi apparatus, secretory pathway, ER-Golgi intermediate compartment, microtubule organizing center

Introduction

The Importance of the Golgi Apparatus

The eukaryotic cells have various membraneous and non-membranous organelles within their cytoplasm. According to the scientists, the Golgi apparatus (GA) is commonly considered as the most important organelle. According to my opinion it is also the most suprising and exciting oragenelle. Although it has been detected many years ago, several unexpected morphological or functional features of the GA are still emerging. This is why I am especially interested in the GA in this review.

The Morphology of the Golgi Apparatus

The Arrangement of the Golgi Apparatus: The GA consists of compact stacks of membrane limited sacs or cisternae, dilated at the periphery, along with many vesicles involved in vesicular transport between the sacs (1) (Figure 1). Its main functions are to modify and sort proteins and lipids that are transported through this organelle en route to their final destinations, such as the extracellular medium, plasma membrane and the endosomal/lysosomal compartments (2). In mammalian tissue culture cells, it consists of flattened membrane-bound compartments, called "cisternae". The cisternae form Golgi stacks (one Golgi stack is formed via grouping of a number of cisternae in a parallel manner), themselves interconnected by lateral tubules to form the Golgi ribbon (Figure 2). The Golgi ribbon displays a juxtanuclear localisation next to the microtubule organising center (3). The Golgi ribbon is intact in interphase and dispersed into tubular-reticular and vesicular elements in mitosis (4). Additionally, in the absence of intra-Golgi transport, the Golgi ribbon is partially fragmented (5). Both Golgi stacks and ribbon are polarised with an entry (cis) face, where cargo molecules synthesised in the endoplasmic reticulum (ER) reach the Golgi, and an exit (trans) face, where they leave for their downstream locations (3). Up

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to 100 Golgi stacks, each composed of five to eight flattened cisternae are connected by membranous tubular bridges, called the noncompact zones, and are positioned next to the centrosome (6). Golgins are peripheral membrane proteins that are proposed to maintain this 3D arrangement of Golgi membranes by providing a structural skeleton (7). Golgin-mediated tethering is important for maintaining optimum Golgi function required for correct cargo processing and trafficking, which in the context of a whole organism is important for normal development and physiology (8). Golgi ribbon organization also depends on an intact microtubule and actin cytoskeleton, specialized cytoskeleton-based motors and membrane input from the ER (5,9). The cisternae stacking might be related with the accuracy of protein glycosylation but not with the protein transport (10). The dispersed Golgi cisternae of *S. cerevisiae* are able to secrete efficiently (11). Interestingly, a recent study showed that loss of Golgi stacking leads to an increased rate of protein transport but defects in glycosylation (12). It has been suggested that the Golgi ribbon allows lateral diffusion of glycosylation enzymes between cisternae of adjacent stacks and also facilitating optimal processing of proteins transisting through the GA (13). It is possible that one part of the ribbon is in contact to several restricted organelles or moieties, such as localised mRNAs, and that some of stacks

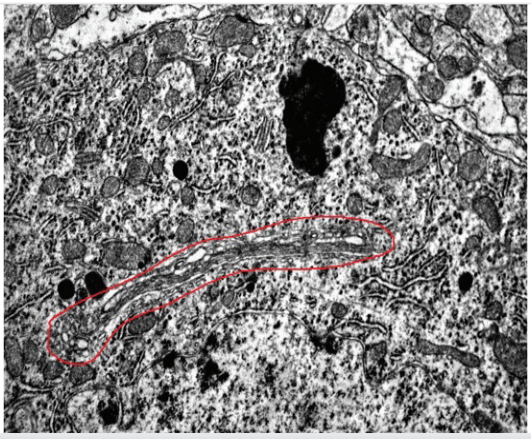

Figure 1. The Golgi apparatus (in red line) within the cytoplasm of a cerebellar neuron is observed. Note the vesicles around the Golgi apparatus. X 10.000

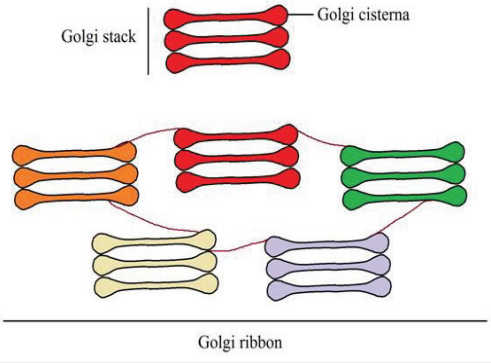

Figure 2. The formation of the Golgi ribbon is shown (drawn by the author herself)

of the ribbon mediate transport of the encoded proteins (14). Taken together, these data show that the integrity of the Golgi stacks and cisternae is not needed for competently carrying the bulk of proteins to the cell membrane.

The Biogenesis of the Golgi Apparatus: Two opposing models have been suggested about the biogenesis of the GA; the Golgi matrix and the *de novo* Golgi formation. According to the first model, the GA is an autonomous organelle built on a pre-existing template which is proposed to be a Golgi matrix containing some Golgi matrix proteins and F-actin (15,16). These proteins including p115, GM130 might take part in building or maintaining Golgi stack architecture and ribbon formation (14,16). In the de novo Golgi formation model, the GA is considered as an ER outgrowth. The membrane at ER exit sites carries all the necessary molecular information to trigger the building of a GA by a mechanism of self-organization (17). In this model, it is the structural integrity of ER exit sites and anterograde transport that are crucial for Golgi stack formation (14). It is not clear whether the membrane flux from the ER is the major determinant of the formation and reorganization of the GA or the GA exhibits considerable anatomy.

It is a fact that the GA is a dynamic organelle. During the cell proliferation, first sign of the Golgi ribbon disintegration is the disconnection to yield seperate Golgi stacks (disruption of the intermembraneous bridges) (18). Then, stacks are transferred into tubular-reticular membranes, called "Golgi blobs", which are dispersed throughout the cytoplasm. The "Golgi blobs" are then broken down to yield the "Golgi haze", which is composed of small dispersed vesicles (19). Postmitotic Golgi reassembly begins in telophase and involves the formation of two smaller Golgi ribbons that eventually coalesce (20).

The Domains of the Golgi Apparatus: The GA has two faces. One of them which is convex one is the forming face (or input face or -cis face; receiving department) which receives the proteins in transport vesicles from the adjacent cisternae of the rough ER (RER). The opposite concave face is the maturing face (or trans face; shipping department) which releases the modified, concentrated, sorted, packaged or labelled product into the cytoplasm (1). The most salient hallmark of Golgi structure is the presence of multiple membranous compartments, differentiated into cis, medial, and trans-Golgi, and organized into flattened stacks, which facilitates many key Golgi functions in mammalian cells (21). Morphological studies suggest that cisternae form at the cis face of the GA, progress through the stack to the trans face, and ultimately dissipate (22). Indeed, detailed studies provided strong evidence that procollagen containing cisternae progress from the cis to the trans side of the stack (23). Because trans cisternae have a different resident protein composition than cis cisternae, the implication is that cis cisternae mature into trans cisternae (11). At the cis face of the Golgi ribbon, proteins and lipids are delivered from the ER via vesicular-tubular clusters (VTCs), which dock at the cis-cisterna (24). Here, they either fuse with a preexisting cis-cisterna, or use the existing cis-cisterna as a template for the formation of a new cisterna (25). VTCs alternatively are referred to as the

ERGIC (ER-Golgi intermediate compartment) or the cis-Golgi network (26). The formation of new cisternae at the cis-Golgi is coordinated with the fragmentation and consumption of the trans-Golgi (27). Trans face [trans Golgi network (TGN)] is the compartment where proteins and lipids are sorted and exit the GA. The proteins delivered from the GA may more or less temporarily remain within the cytoplasmic matrix (secretory proteins, glycoproteins) or integrate in apical and basolateral plasma membrane (integral or peripheral membrane proteins) or enter into the endosomes or lysosomes (lysosomal proteins) (Figure 3). The GA is especially well-developed and active in cells that secrete protein/glycoprotein by exocytosis (both for exocrine and endocrine secretion) and in cells that produce large amounts of membrane proteins (e.g. nerve cell with large membrane surface) (1). Molecules targeted to the endosomal-lysosomal pathway leave from the clathrin-coated, trans-most cisterna. Molecules for the apical and basolateral plasma membranes exit from the preceding trans-cisternae (27).

The Functions of the Golgi Apparatus

The Golgi complex is not only the core structure of the secretory pathway but is also essential to ensure lipid homeostasis, and plays a major role in signaling, autophagy, and apoptosis. As such it is involved in many human diseases and several Golgi-associated factors represent promising therapeutic targets (28).

The Role of the Golgi Apparatus in the Intracellular Transports and the Secretory Pathway: The secretory pathway through the ER to the plasma membrane includes the ER exit sites, the intermediate compartment (between the ER and the Golgi), the GA itself, the TGN, and several post-Golgi compartments. Since the GA is polarized, both morphologically and functionally bi-directional transport of the products is possible. In the course of anterograde transport, coated vesicles carry newly synthesized proteins from the GER to forming face of the GA. Traditionally, it has been accepted that newly synthesized proteins are transported from the GER to GA via COP-II coated vesicles (29). The exit sites of the ER appear as clusters of COP-II-coated buds, small 52-nm vesicles, and some tubules (30,31). When the exit from the ER is blocked, the exit sites contain only one or two COP-II-coated buds. In contrast, the transporting exit sites contain more tubules and buds (30).

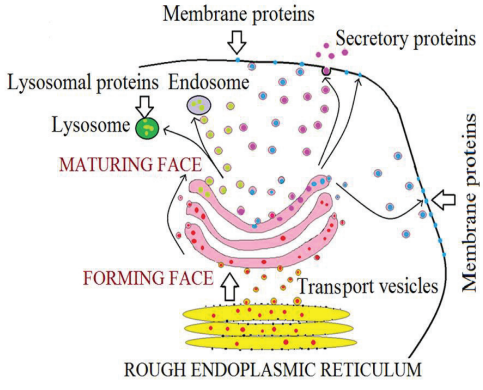

Figure 3. The sorting of the proteins from the Golgi apparatus is shown (drawn by the author herself)

Ever since, a number of new data have added to the complexity of the transport events that take place at the ER-Golgi boundary. Currently, the two models for the GER to Golgi transport in mammalian cells are considered. One of those is the transport complex model. According to this model, from the ER exit sites, COP-II coated vesicles tether and fuse to form pleimorphic transport complexes, which in turn are transported along the microtubules. At the forming face of the GA transport complexes gather and undergo fusion. They may either fuse with the adjacent Golgi cisternae or alternatively form a new Golgi cisternae by homotyping fusion (1,32). The second model is the stable compartment model which involves the anterograde membrane traffic through the ER-Golgi intermediate compartment (ERGIC) (intermediate organelle) (33). ERGIC is the tubulovesicular and vacuolar membrane clusters of the ERGIC (34). The basic difference between these models is that the former emphasizes the dynamic and transient character of this compartment, whereas the latter proposes that the membrane clusters defined by ERGIC-53 represent immobile, stable structures (35). Strikingly, recent results show that the ERGIC is composed of spatially and functionally distinct vacuolar and tubular domains that constitute a dynamic, but at the same time permanent, interconnected membrane system (36,37). According to this model, short-range vesicular transport from the ER to the ERGIC depends on the transport of COP-II coated vesicles in a microtubule independent manner. However, long-range transport from the ERGIC to the forming face of the GA requires microtubules. Fission of anterograde carriers from the ERGIC may involve the COP-I coat, spectrin/anykrin skeleton.

According to the live cell imaging studies two types of mobile ERGIC carriers detach from the ER; one of those corresponds to the large-sized elements, which become elongated as they move, and the other corresponds to the narrow tubules, which bud from the more stationary, large ERGIC structures. The latter which are enriched in cargo proteins, the cargo receptor p58 and COP-I coats likely correspond to ERGIC vacuoles bound to the GA (33,37). The microtubule dependent, long-distance movements of both types of carriers are often directed towards the cell center (35). The first marker protein of the ERGIC, ERGIC-53, functions as a mannose-binding cargo receptor in ER-Golgi trafficking and also involves microtubule dependent, long-distance movement of transport intermediates between widespread transitional ER sites [also called ER exit sites (ERES)] and the Golgi region (34,38). Part of the tubules move in the opposite direction, fusing with or interconnecting nearby, more stationary ERGIC elements, thus creating a dynamic network (36,37). In addition, some of the tubules move towards the cell periphery and establish a Golgi bypass pathway between the ERGIC and the cell cortex (35). It has been shown that the centrally located elements of the ERGIC communicate with the endolysosomal system, so such a connection might play an important role in the transport pathway by allowing newly made molecules to bypass the GA on their way for exocytosis (36).

The initial capture or tethering of transport vesicles at the cis-face of the GA is most likely mediated by golgins, a family of elongated coiled-coil tethering proteins. Golgins are anchored to the Golgi membrane via their carboxy terminus (39). Because of their coiled-coil nature, golgins are thought to extend a considerable distance (predicted to be in the range of 100-600 nm) into the surrounding cytoplasm which is ideal for capturing vesicles at long range. Different types of golgins are localized in different Golgi regions. For instance, the golgins at the cis-Golgi capture ER- and ERGIC derived vesicles, golgins localized to cisternal rims capture intra-Golgi transport vesicles, and trans-Golgi golgins capture endosome-derived carriers (40). Another scenario could be that they tether non-vesicle membranes, which may include Golgi cisternae, or tubular regions of the Golgi such as the TGN (trans-Golgi network), the fenestrated regions located between stacks within the Golgi ribbon, or tubular carriers that have been postulated to mediate intra-Golgi traffic (41). Giantin is able to promote linking of Golgi stacks within the ribbon, suggesting a possible role in membrane tethering that is required to maintain Golgi organization as opposed to vesicle traffic (42). Conversely, Rab binding is likely more important for the steps that follow capture, where the vesicle has to move closer to the target membrane to undergo docking and fusion Rab2 binding acts downstream of tethering (40). Interaction between the vesicle-bound Rab and Golgi-membrane associated golgins could allow the vesicle to hop down the length of a golgin, or even onto an adjacent golgin able to bind the same Rab, helping it navigate its way through the meshwork of golgin "tentacles" to contact the Golgi membrane (7). Following entry to the Golgi system, the transport vesicles bud off dilated ends of the Golgi cisternea mediate transport between the adjacent cisternae. Conserved oligomeric Golgi (COG) is a complex comprised of eight subunits that is present on all Golgi cisternae and functions in intra-Golgi trafficking (43). They are shorter than the golgins, which suggests they would be less efficient at vesicle capture over long distance. In fact both COG and [Golgi-associated retrograde protein (GARP)] are well known to facilitate the transition from tethering to fusion. By interacting with components on both the vesicle and the target membrane, and by acting in conjunction with golgins, both the fidelity of vesicle recognition and the strength of vesicle attachment to the target membrane are likely greatly enhanced by COG and GARP (40). Retrograde transport (for re-processing of any inadequately or insufficiently modified, sorted/packaged product) from the forming face of the GA to GER as well retrograte transport between adjacent Golgi cisternae is mediated by COP-I coated vesicles (1,44). The GA is embedded in a network of microtubules carrying the vesicles (1,25). COP-I vesicles have been proposed to recycle resident Golgi enzymes, thereby keeping these enzymes in the organelle during cisternal maturation (45).

The ERGIC constitutes an independent structure that is not continuous with the ER or the cis-Golgi face. The ERGIC clusters are mobile transport complexes that deliver cargo from the GER membranes to the forming face of the Golgi. Traditionally, it has been accepted that newly synthesized proteins are transported from the GER to GA via COP-II coated vesicles. In summary, transport from the GER to the ERGIC is controlled by COP-II, and subsequent sorting in the ERGIC involves COP-I (1). COPI-dependent vesicles are too small for the transport of large cargo aggregates, the size of which is greater than the size of the lumen of these vesicles (e.g.; chylomicron) (46). COP-I coated vesicles also play a well-established role in retrograde traffic from both the ERGIC and GA. The ERGIC, apart from the trafficking of the proteins, also contributes the concentration, folding, and quality control of newly synthesized proteins.

The role of the Golgi Apparatus in the Sorting Mechanism: Both anterograde and retrograde transport are mediated by the processes of budding and fusing of the vesicles between the Golgi cisternae. Namely, the vesicle bud from one cisterna fuses with the adjacent cisternae. So the continuity of membrane systems of the adjacent cisternae does not seem to be necassery (1). However, in different types of cells intercisternal heterogenous connections have been described (47). Even in some cells the GA forms a single continuous membranous system (but not in the resting stacks) (48,49). The number of intercisternal connections increases after the arrival of cargo at the Golgi cisternae (41). These connections containing soluble cargo proteins might be involved in rapid transport through the Golgi cistarnea (50). Not only proteins but also lipids travel through the Golgi cisternae. They undergo a series of post-translational modifications during this journey. The tubulovesicular array of the maturing face of the GA serves as the sorting station for transporting vesicles that deliver proteins to the plasma membranes, to the apical region of the cell cytoplasm (of secretory cells) where proteins are stored in secretory vesicles, and to the endosomes and lysosomes (Figure 4). The actual sorting of proteins in the maturing face of the GA is primarily based on sorting signals. Most proteins destined for organelles bear specific signal sequences (sorting signals). For instance, mannose-6-phosphate (M-6-P) is attached to the surface of the inactive precursors of lysosomal enzymes (which are prohydrolases) which are destined to travel to late endosomes and lysosomes as recognition signal (1). The majority of soluble acid hydrolases are modified with M-6-P residues, allowing their recognition by M-6-P receptors in the Golgi complex and ensuring their transport to the endosomal/lysosomal system.

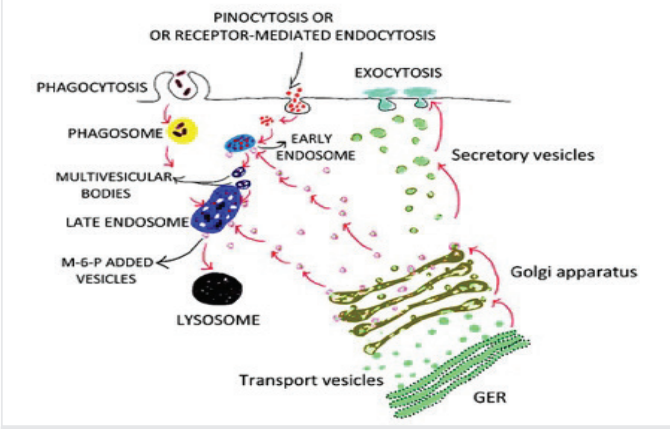

Figure 4. The Golgi apparatus and vesicular trafficking are shown (drawn by the author herself)

M-6-P marker is generetad on specific N-linked oligosaccharides of the glycosylated lysosomal hydrolases in the secretory pathway. Next, the modified proteins are recognized by two independent receptors that bind the M-6-P residue of the newly synthesized lysosomal hydrolases in the trans-Golgi network. Finally, the ligand-receptor complex is packaged into clathrin coated transport vesicles for delivery to endosomes and lysosomes (51). M-6-P receptors are present in early and late endosomes, lysosomes (destination area) and GA (for transport between the Golgi sacs). Once anchored in the N-linked oligosaccharides of newly synthesized hydrolases, the M-6-P recognition marker has to be recognized by specific receptors, so that the labeled hydrolases are correctly transported to the lysosome (52). M-6-P receptors, which are type I transmembrane glycoproteins that bind their specific oligosaccharide at pH 6,5-6,7, in the trans-Golgi network and release it at pH 6, the typical pH inside late endosomes. Inside these compartments, lysosomal hydrolases dissociate from the M-6-P receptors, and when the pH reaches 5 (the typical acidic pH of lysosomes which is maintained by a membrane ATP-driven H+ pump), hydrolases begin to digest the endocytosed material delivered from early endosomes (53). Although M-6-P receptors play a major role in the intracellular transport of newly synthesized lysosomal enzymes in mammalian cells, several lines of evidence suggest the existence of alternative processes of lysosomal targeting. Among them, the two that are mediated by the M-6-P alternative receptors, lysosomal integral membrane protein (LIMP-2) and sortilin, have gained unequivocal support. There are soluble enzymes as well as non-enzymatic proteins that are transported to lysosomes in a M-6-P independent manner (54) (Coutinha, Alves 2012). LIMP-2 transports glucocerebrosidase to the lysosomes. On the other hand, sortilin which is multifunctional protein was proposed to be responsible for the lysosomal transport of some enzymes including sphingomyelinase and cathepsins D and H (55-57). Like M-6-P receptors, sortilin is thought to be an ancient receptor involved in a conserved trafficking mechanism (55). The sorting mechanism sometimes is not perfect, so for instance proteins destined for lysosomes may travel directly to and incorporated into the plasma membrane. These proteins then move back into the endosomal pathway (into early endosomes, to late endosomes and then to lysosomes, as expected) (1). The presence of clathrin-coated buds and tubules on only the trans-most cisterna of the Golgi indicates a unique exit site for cargo destined for the endosomal-lysosomal pathway (53). In contrast, buds and tubules arising from the other trans-cisternae are either nonclathrin-coated or uncoated (27). Long ago, it has been suggested that that newly synthesized lysosomal enzymes bypassed Golgi cisternae and were delivered directly from the ER to the trans-most cisterna, whereas secretory proteins exited the Golgi from the penultimate transcisterna (58).

In summary, the main function of the GA is to modify, sort and package the macromolecules synthesized for secretion or for internal utilization. The Golgi complex has the ability to modify proteins by adding carbonhydrates (thus produce glycoproteins and proteoglycans) and phosphate by the process of glycosylation and phoshphorylation respectively (1). The glycosylation enzymes that reside in the Golgi cisternae and are involved in the early stages of glycosylation are located mostly at the cis-side. Conversely, the late and terminal glycosylation enzymes are situated at the trans-side of the Golgi stacks (59). Additionally, it also involves in the sulfation of some molecules. In fact; it also creates lysosomes by releasing targeted lysosomal enzymes.

The Role of the Golgi Apparatus in Ca^{2+} Store: In the last decade, it became clear that the GA also play an important role as an intracellular Ca^{2+} store behaving similarly to the main intracellular Ca^{2+} store that is SER. The GA is thus equipped with all the molecular components necessary for its function as a Ca^{2+} store: Ca^{2+} pumps, Ca^{2+} channels and luminal Ca^{2+} binding proteins (60). Chandra et al. (61) showed that the GA can store up to 5% of the total cellular Ca^{2+} and that it is substantially more resistant to Ca^{2+} depletion than other cellular organelles. The recent similar evidences showed that the latencies and initial rates of Ca^{2+} release from the GA and the ER were similar. However, Ca^{2+} release from the GA terminated faster resulting in a smaller extent of Ca^{2+} release. Fast reuptake of released Ca^{2+} by the GA may underlie fast termination of Ca^{2+} release from this compartment. It is clear that both the GA and the ER release Ca^{2+} during the generation of cytosolic Ca^{2+} signals, but the time-courses of the Ca^{2+} release are different (62). Luminal Ca^{2+} within the GA might be fundamental in controlling some key processes of the organelle including post-translational modifications, protein sorting and trafficking. The activity of the enzymes located in the GA and trans Golgi network, retrograde membrane traffic from the Golgi to the ER and selective aggregation of regulated secretory proteins in the trans Golgi network seem to be critically dependent on GA Ca^{2+} content (63,64). Therefore, luminal concentration changes of the Ca^{2+} within the GA could affect several cellular functions. Moreover, selective reduction of Ca^{2+} concentration within trans-Golgi compartment results in major alterations of protein trafficking and induces the collapse of the entire GA morphology (65).

The Role of the Golgi Apparatus in Microtubule Nucleation: In recent years, the GA has emerged as a site that can nucleate and stabilize the microtubules (66,67). Traditionally it has been accepted that the microtubule organizing centers which are centrosomes, basal bodies and centromers of the chromosomes are responsible for the microtubule nucleation, stabilization and organization (1). The microtubules derived from the GA participate in Golgi assembly when the cell division is completed. Golgi-derived microtubules participate in Golgi reassembly after cell division by promoting fusion of the Golgi stacks into the Golgi ribbon (67). Furthermore, unlike centrosomal microtubules, Golgi-anchored micrtobule arrays are polarized and can thus promote cell asymmetry and polarized transport of post-Golgi carriers that are important for cell migration (68). Interestingly, in differentiated cells, the membranes of the GA are suggested to serve as microtubule organizing centers controlling the formation of non-centrosomal microtubule arrays, which are for cell morphogenesis and function. A good example of this kind of function of the GA is neurite outgrowth and branching in neuronal cells (69).

Conclusion

As a conclusion, I emphasize that:

- The GA is not composed of a simple discontinuous parallel stacks but is composed of various domains including cis-Golgi network, trans-Golgi network, intercisternal network, ERGIC (we may consider that as a part of the Golgi), various coated vesicles, vacuoles and tubules,

- The GA is not only involved in the secretory pathway, but also involved in the storage of Ca^{2+} very likely to smooth endoplasmic reticulum,

- The GA might serve as a microtubule organizing center which is especially important to participate in Golgi reassembly after cell division,

- The GA is not a stable organelle instead a highyl dynamic organelle changing its morphological and functional characteristics in different phyological and pathological conditions.

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Case Report

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Conservative Management of the Isolated Mandibular Coronoid Process Fracture

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ABSTRACT

Fractures of the coronoid process are uncommon and can easily be missed. Coronoid fracture may manifest as simple, linear line with minimal displacement. If the periosteum is traumatized, the degree of displacement may increase. Restricted mouth opening and mandibular movement, malocclusion, and swelling below the zygomatic arch may be evident. The decision of treatment plan should be based on the fracture pattern, time of the fracture, the presence or absence of concomitant fractures, and clinical symptoms. Coronoid fractures are generally managed conservatively, a few cases require surgical intervention. A rare case of fracture of the coronoid process caused by trauma of the temporalis muscle is described.

Keywords: Coronoid process, mandible, fracture

Introductıon

The incidence of coronoid fractures is reported to be 1-2% in all facial fractures (1). Etiological factors are stated to include traffic accident, fall, attack, third molar tooth extraction and sagittal split osteotomy (2-4).

Coronoid fractures are usually simple and linear and have little displacement. If the periosteum is injured, this displacement rate may increase with the contraction of the temporal muscle (3). Its definite diagnosis is established by considering patient history, clinical examination and radiographic examination. Although conservative approaches are generally used in its treatment, surgical intervention is rarely required (5).

In this report, a case of isolated coronoid process fracture and its treatment was presented with the review of the literature.

Case Report

A systemically healthy 34-year-old male patient presented to our clinic with the complaints of pain in the left temporomandibular joint, trismus, and restricted chewing. It was learned from the patient's history that head trauma occurred as a result of falling 15 days ago. In the oral examination, restricted mouth opening (7 mm) and pain, tenderness in the left retromolar region, ramus and zygomatic arch with palpation were recorded (Figure 1). There was no irregularity in the occlusal relationship of the patient. Extraoral examination revealed no soft tissue irregularity and bone contour disorder.

Radiological examination revealed a unilateral, isolated, linear, anteroposterior oblique and slightly displaced fracture in the left mandibular coronoid process (Figures 2, 3). The presence of mandibular and midface fractures accompanying coronoid process fracture was not detected.

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The patient was informed and informed consent was obtained and conservative treatment was preferred because of the slight displacement of the fractured bone segment and the simple, linear fracture line. The lower and upper jaws were fixed with intermaxillary fixation (IMF) and elastics for 15 days, considering the patient's symptoms and the 15-day period following the trauma. As a result of no change in the position in the fracture fragment, IMF application was completed at the end of 14th day, physical therapy exercises were continued and a soft diet was suggested.

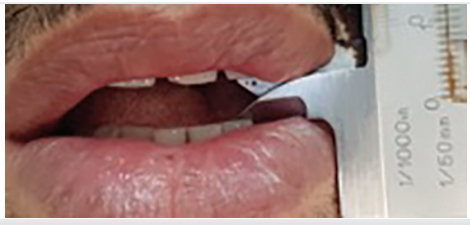

Figure 1. Mouth opening measurement value before the treatment (7 mm)

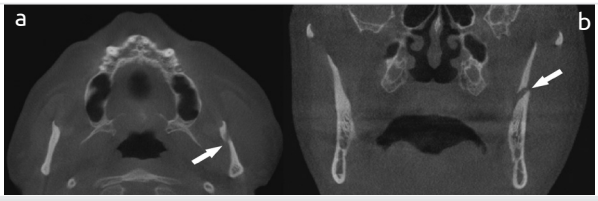

Figure 2. In the axial (a) and coronal (b) sections, the left coronoid fracture line is indicated by the white arrow

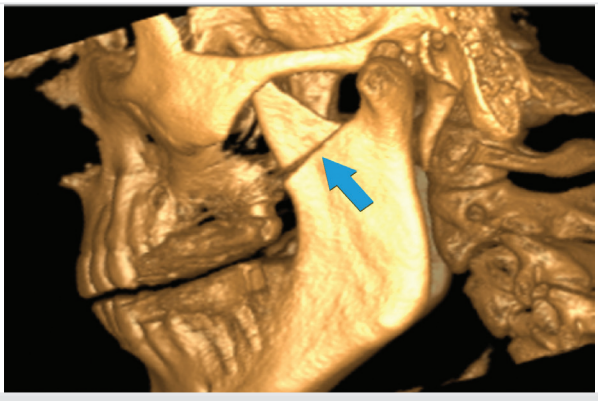

Figure 3. Left coronoid process fracture is observed on a three-dimensional model generated from computerized tomography recordings (blue arrow)

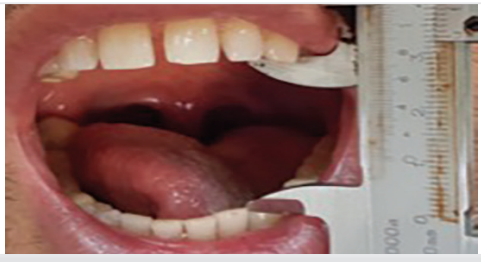

Figure 4. Mouth opening measurement value after the treatment (32 mm)

At the end of four-week follow-up, the position of the fracture fragment was observed to be stable in the tomographic images. The mouth opening value was measured as 35 mm after the treatment (Figure 4). The treatment was completed with complete elimination of the patient's complaints and achievement of an acceptable anatomical, functional and aesthetical result.

Discussion

Isolated coronoid process fractures without an accompanying maxillofacial fracture, which was the diagnosis of our case, are rarely seen and there are limited number of reports on their treatment according to the authors' knowledge (6).

Coronoid process is a structure that is anatomically protected by the zygomatic complex and muscles. Because it is not associated with cranial bones, the etiology of fracture does not involve indirect trauma. Coronoid fractures are often caused by a direct, penetrating trauma or sudden and severe contraction of the temporal muscle during trauma (7). The temporal muscle starts from the temporal fossa and attaches to the medial surface of the coronoid process. Myotatic reflex triggered by tension in a muscle causes excitation of muscle spindles, contraction of muscle skeletal fibers and synergistic muscles. Therefore, a blunt trauma to the temporal region plays a role in initiating temporal muscle contraction. In such a case, if the teeth are not in an occlusal relationship, the coronoid process is exposed to significant stress and fracture may occur (8). Although our case could not confirm the jaw position during injury, it can be said that acute reflex contraction due to trauma in the temporal muscle causes coronoid fracture.

Depending on the severity of the injury, symptoms such as malocclusion, limitation of lower jaw movements, and swelling of the zygomatic arch may occur. Since the fracture is mostly accompanied by zygomatic bone fracture, the main cause of trismus is the zygomatic arch dislocation (2,4). The main cause of isolated fractures is muscle spasm because the coronoid process is the place of the attachment of temporal muscle anteriorly and masseter muscle fibers laterally. In our case, fractured coronoid process segment without displacement and dislocation caused restricted mouth opening due to temporal and masseter muscle spasm associated with trauma.

It is recommended that treatment protocol be determined according to the degree of displacement of the fracture fragment and clinical symptoms (3,9). In patients with severe malocclusion and severe pain, IMF can be applied up to 4 weeks. For simple coronoid fractures without displacement or malocclusion, treatment is not recommended because temporal muscle spasm shows the splint effect and causes the segment to remain in stable position (10). In our case, after 14-day IMF treatment, lower jaw mobilization was provided and soft diet and physical therapy exercises were given to prevent osseous adhesions.

If trismus persists after conservative treatment, coronoidectomy is recommended because of the risk of osseous junction between the coronoid process and the zygomatic arch. In addition, rigid internal fixation can be performed in patients with

severe displacement of the fracture fragment, accompanying midfacial or mandibular fractures, and in patients who cannot undergo IMF for a long time (1,5). It has been reported that coronoidectomy performed by intraoral approach is superior to rigid internal fixation treatment which has disadvantages such as facial scar, facial nerve damage, long operation time, and plate-screw cost (5,9).

In conclusion, although coronoid fractures occur rarely, treatment approach should be according to fracture pattern, time of fracture occurrence, presence of accompanying fracture, and severity of clinical symptoms. Considering fracture stability and the risk of post-traumatic ankylosis during treatment, it is important to continue rehabilitation with physical therapy exercises.

Etihics

Informed Consent: Informed consent was obtoined from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Consept: A.G., OK., R.C.T., Design: A.G., OK., R.C.T., Data Collection or Processing: A.G., OK., R.C.T., Analysis or Interpretation: A.G., OK., R.C.T., Literature Search: A.G., OK., R.C.T., Writing: A.G., OK., R.C.T.,

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

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Case Report

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Bezmialem Science 2019;7(4):342-4

A Rare Case: Granular Cell Tumor of the Tongue

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ABSTRACT

Granular cell tumors (GCT) are rare neoplasms and their diagnosis is mainly based on histopathologic examination of biopsies. Granular cell tumor is a neoplasm that has been reported in a variety of organs, including the extremities, head and neck skin, oral cavity and gastrointestinal tract. The cell of origin in GCT is controversial. In this article, we present a case of granular cell tumor of the tongue at the 49-year-old female patient with the knowledge of the literature.

Keywords: Granular cell tumor, tongue, immunohistochemistry

Introduction

Granular cell tumor (GCT) is a rarely seen lesion that mostly displays a benign course. It constitutes less than 1% of head and neck tumors (1). 1-2% of the cases show a malignant course (2-4). GCT is typically located in the extremities and head-neck skin, oral cavity and gastrointestinal system (5). Although it can be seen at any age, it is more common in the 4th and 5th decades. It is thought that peripheral nerves originate from "schwann" cells (2). Its benign forms can be treated by local excision (6).

Case Report

The lesion of a 49-year-old female patient with a complaint of cream-colored discomforting swelling (Figure 1), which had been growing on the left side of the tongue midline for 3 years, was excised. In the macroscopic examination, two pieces of light yellow - cream colored tissue measuring 0.8x0.5x0.4 cm and 0.5x0.5x0.3 cm were observed. Microscopic examination of hematoxylin-eosin staining revealed monomorphic, polygonal shaped cells with large, granular, eosinophilic cytoplasm and small nucleus with central or eccentric localization, which were unencapsulated in the form of nests between muscle fibers and showing infiltrative spread pattern (Figure 2, 3). Mitosis, pleomorphism and necrosis were not observed in these cells. In the immunohistochemical examinations, S100 (Figure 4), NSE (Figure 5), CD68 (Figure 6), inhibinα (Figure 7) and vimentin (Figure 8) were detected to be positive. Ki-67 proliferation index was found to be 2% (Figure 9). Based on these findings, the patient was diagnosed with granular cell tumor.

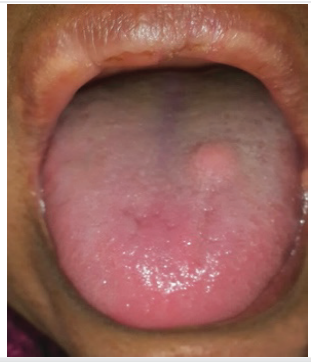

Figure 1. Gray-yellow colored swelling on the left side of the tongue midline

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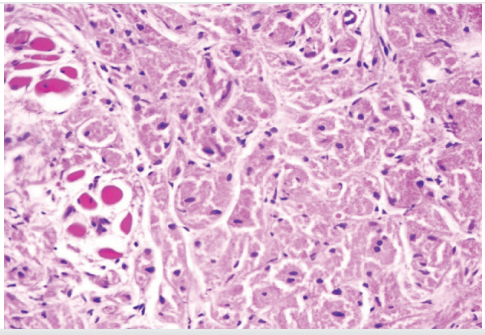

Figure 2. Monomorphic, polygonal shaped cells with large, granular, eosinophilic cytoplasm and small nucleus with central or eccentric localization, which were trabecular and in the form of nests between the muscle fibers (x200, H-E)

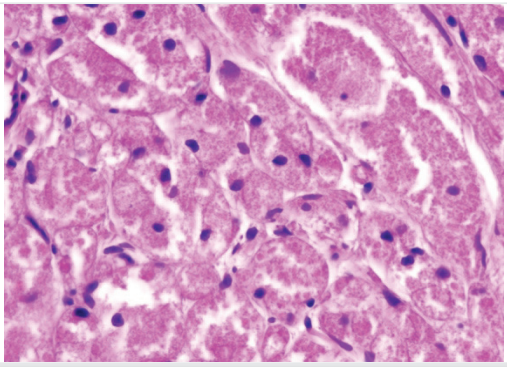

Figure 3. Monomorphic, polygonal shaped cells with fine granular, eosinophilic large cytoplasm and small nucleus with central or eccentric localization (x400, H-E)

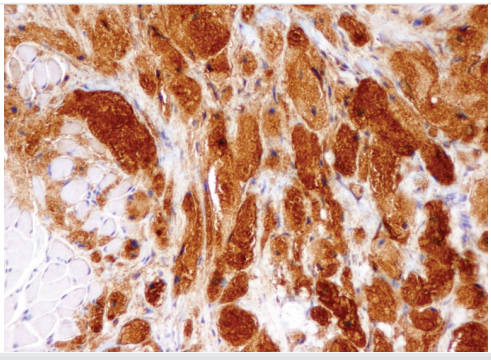

Figure 4. Cytoplasmic staining with S100 (x200, S100)

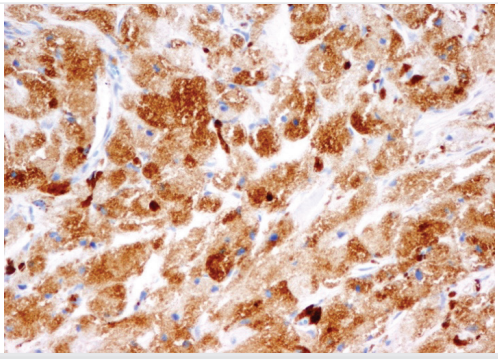

Figure 5. Cytoplasmic staining with NSE (x200, NSE)

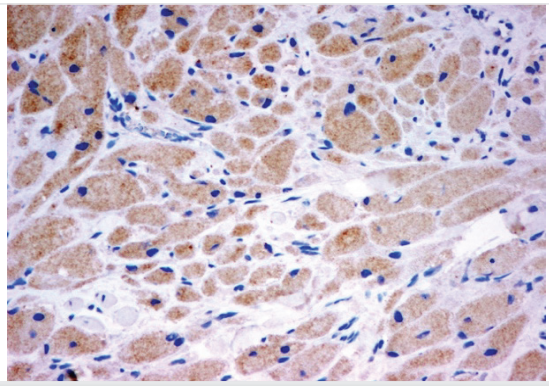

Figure 6. Cytoplasmic staining with CD68 (x200, CD68)

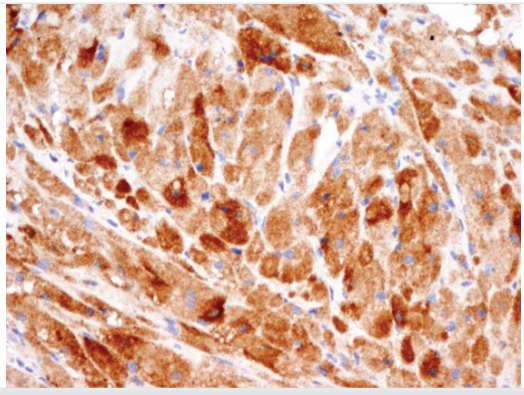

Figure 7. Cytoplasmic staining with Inhibinα (x200, Inhibin α)

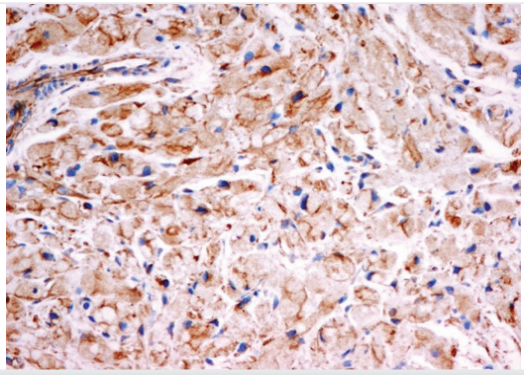

Figure 8. Cytoplasmic staining with vimentin (x200, Vimentin)

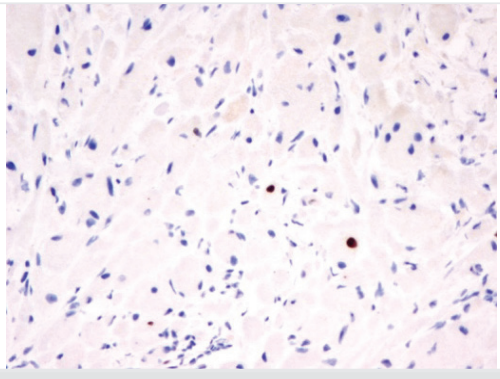

Figure 9. Nuclear staining with ki-67, proliferation index 2% (x200, ki-67)

Discussion

GCT accounts for less than 1% of all head and neck tumors. 50% of GHTs in the head and neck are located in the oral cavity. It is predominantly seen in women in the 4th and 5th decades (1,2,5,8,9). It was first reported by Weber in 1854 and then by Abrikossoff in 1926. It is called granular cell myoblastoma because it is thought to be of striated muscle origin (7).

Very different views have been made regarding the cells from which GCT originate due to its different cell histopathology. It is thought to develop from skeletal muscle, histiocytes, fibroblasts, nerve sheath cells, neuroendocrine cells and differentiated mesenchymal cells (2). Recent immunohistochemical and electronmicroscopic studies have shown that the origin of these tumors is "schwann "cells (1,2,9). While GCT is immunohistochemically stained with S100, p75, NSE and CD68, inhibinα, and vimentin, it is not stained with

smooth muscle actin (SMA), epithelial membrane antigen, HHF-35, synaptophysin, chromogranin, progesterone, androgen, estrogen, carcinoembryonic antigen (CEA), and PanCK (2,3,9). In our case, S100, NSE, CD68, vimentin, and inhibin alpha were positive. Ki-67 proliferation index was found to be 2%. PanCK, CEA, and SMA did not react immunohistochemically.

Although the majority of GCTs are benign, they have been reported to display a malignant behavior at a lower rate. GCT's being macroscopically well-demarcated, lack of necrosis and degeneration areas, and its slow growth suggests that it is benign (4). In general, 6 criteria were determined for malignancy. These include being spindle cells, large nucleolus-bearing vesicular nucleus, increased mitosis (>2 mitosis/10-200X-domain), increased nucleus/cytoplasm ratio, and the existence of pleomorphism and necrosis. The presence of 3 or more of these criteria supports the diagnosis of malignant GCT. If one or two of these criteria are present, a diagnosis of atypical granular cell tumor is made. In addition, p53 expression and high ki-67 index have been reported to be associated with aggressive progression in recent years. 1-2% of the cases have malignant course (4,9,10). In our case, none of the criteria for malignancy was found, and the ki-67 index was 2%.

The lesions with which GCT is most commonly confused clinically and macroscopically are other soft tissue tumors and traumatic fibromas (4,9).

Its treatment is surgical excision. It recurs approximately at the rate of 10% after it is completely excised. However, when excision cannot be performed completely, regular clinical radiological follow-up is required. In malignant patients, its effect is not known exactly and radiotherapy and chemotherapy are administered (7,9). If recurrence occurs, there are opinions suggesting the application of radiation after surgery (1,7,9).

In conclusion, GCT is a very rare tumor that can be confused with different lesions clinically and macroscopically, for which histopathological evaluation is very important.

Ethics

Informed Consent: Written informed consent was obtained from the patient who participated in this case.

Peer-review: Externally peer-reviewed.

Author Contributions

Concept: B.D.Y., Design: B.D.Y., Data Collection and/or Processing: B.D.Y, T.Y., A.B., B.G., Analysis and/or Interpretation: B.D.Y., A.B, Literature Review: B.D.Y., H.İ.Ö., Writing: B.D.Y.

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

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

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Case Report

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Bezmialem Science 2019;7(4):345-7

Extreme Hyperkalemia in a Hemodialysis Patient Presenting with Cardiac Arrhythmia

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ABSTRACT

Hyperkalemia is commonly seen in hemodialysis patients and is a potentially life-threatening condition. Serum potassium concentration greater than 10 mEq/L is defined as extreme hyperkalemia. Emergent treatment of hyperkalemia is made with intravenous calcium, insulin-dextrose solutions, diuretics, gastrointestinal cation-exchange resin and dialysis. We present here a 52-year-old chronic hemodialysis patient with extreme hyperkalemia and cardiac arrhythmia. She was treated with emergent hemodialysis. Her abnormal laboratory findings and electrocardiography recovered. Further examinations suggested insufficient hemodialysis treatment as the possible cause of hyperkalemia.

Keywords: Arrhythmia, extreme hyperkalemia, hemodialysis

Introduction

Hyperkalemia may cause paresis, life threatening cardiac rhythm disturbances and requires urgent management. Hyperkalemia is defined as a serum potassium concentration higher than 5.5 mEq/L. Severe hyperkalemia is defined as a serum potassium concentration exceeding 7 mEq/L. Extreme hyperkalemia (>10 mEq/L) is a very rare condition. It has a high death risk. Only a few patients are reported to survive it (1).

Case Report

A 52-year-old female patient was admitted to the emergency department with weakness. She was an anuric chronic hemodialysis patient. Her vascular access was provided by a left brachiocephalic fistula. Physical examination revealed generalized muscle weakness, collateral veins on her chest, scars of previous arteriovenous fistula operations on her left forearm and a blood pressure of 175/95 mmHg. In laboratory examinations, serum creatinine was 10.9 mg/dL (the normal range is 0.5 to 1.1 mg/dL) and urea was 287 mg/dL (the normal range is 21 to 43 mg/dL). Serum potassium concentration was reported to be higher than 10 mEq/L (the normal range is 3.5 to 5.1 mEq/L). A blood sample for the measurement of serum aspartat aminotransferase (AST) level to exclude hemolysis was taken at the same time and AST was found 12 U/L (the normal range is 5 to 34 U/L). Electrocardiography (ECG) showed widened QRS waves and disappearance of P waves (Figure 1A). Emergent medical treatment with intravenous calcium, insulin-dextrose solution and gastrointestinal cation-exchange resin was given and an emergent hemodialysis was performed. Blood test results after the hemodialysis session were as follows: Creatinine: 6.4 mg/dL, urea: 161 mg/dL, serum potassium: 7.5 mEq/L. After the second session of hemodialysis, laboratory results showed a serum creatinine of 4.65 mg/dL, urea of 79 mg/dL, and a serum potassium concentration of 5.2 mEq/L. The complaints of the patient and pathologic ECG findings disappeared completely and

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normal sinus rhythm was restored (Figure 1B). The patient was discharged after three days of hospital stay and three sessions of hemodialysis treatment.

The patient's consent to publication was obtained.

Discussion

Hyperkalemia is frequently seen in patients with end stage renal disease. A total of 26.4%, 13.8%, and 4.9% of hemodialysis patients were found to be hyperkalemic with pre-dialysis serum potassium levels of more than 5.1 mEq/L, 5.5 mEq/L and 6 mEq/L, respectively (2). Hyperkalemia in hemodialysis patients is associated with mortality, hospitalization and emergency department admissions (3).

Most of the hemodialysis patients are anuric or have a very small volume of residual urine. In this group of patients, colonic potassium excretion increases. By help of the cellular adaptation mechanisms, hyperkalemia is better tolerated with less cardiac complications and pathologic electrocardiographic changes compared to the healthy population (4). However, a serum potassium level of >6 mEq/L requires prompt treatment. The major causes of hyperkalemia are increased dietary intake, inadequate potassium removal during hemodialysis, excessive potassium release from the cells into the extracellular space and pseudohyperkalemia. Increased dietary intake is mostly related to dietary incompliance. Dialytic removal of serum potassium depends on the frequency and duration of the hemodialysis session, surface area of the dialysis membrane, blood and dialysis solution flow rates, potassium concentration of the dialysis solution and the adequacy of the vascular access (5).

Our patient was evaluated for the possible causes of hyperkalemia. She was compliant with the diet and she was not skipping or shortening her hemodialysis sessions. Rhabdomyolysis or hemolysis were excluded with blood tests. She did not use medications which may have increased serum potassium levels like spironolactone, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or beta blockers. Extensive collateral veins on her chest, scars of previous unsuccessful arteriovenous fistula operations on her arm and the finding that the arterial and venous cannulation sites on the arteriovenous fistula were only 2 cm apart, suggested that hemodialysis insufficiency was the presumptive reason for the extreme hyperkalemia. Insufficient hemodialysis was further verified by a kt/v value of 1 and an urea reduction ratio of 55% in her last hemodialysis visit.

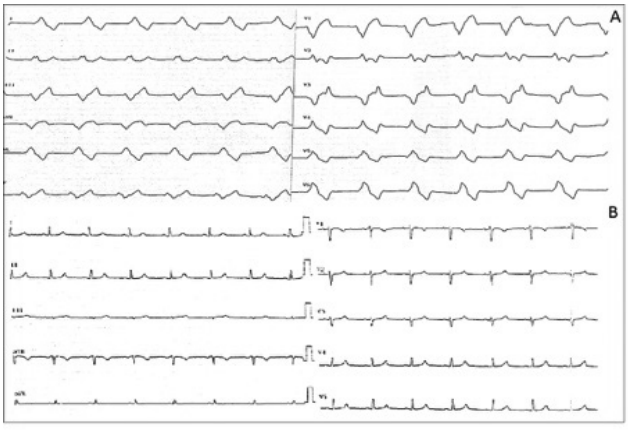

Figure 1. ECG recordings of the patient pre- (A) and postdialysis (B) with a potassium concentration of >10 mEq/L and 5.2 mEq/L respectively

ECG: Electrocordiography

Emergent treatments of hyperkalemia are intravenous calcium to antagonize the membrane actions of hyperkalemia, intravenous insulin-dextrose solutions to drive extracellular potassium into cells, loop diuretics, gastrointestinal cation-exchangeresins and dialysis to rapidly remove excess potassium from the body (5).

The most effective treatment of hyperkalemia in hemodialysis patients is hemodialysis itself. Potassium binders and insulin-dextrose solutions provide a transient but possibly life-saving treatment when hemodialysis treatment cannot be done immediately. Our patient's complaints, physical, and laboratory findings recovered after three efficient hemodialysis sessions. Her ECG recovered completely.

Extreme hyperkalemia is fatal unless treated without delay. To the best of our knowledge, our patient had one of the highest serum potassium levels reported until now (1). Serum potassium concentration in her first drawn blood sample was found to be higher than 10 mEq/L. A precise value of serum potassium could not be obtained because the laboratory kits in our hospital are not suited for readings of serum potassium concentrations above 10 mEq/L.

Hyperkalemia is a common finding in hemodialysis patients. The most effective treatment for hyperkalemia is hemodialysis. After the emergency treatment, a careful investigation aimed to enlighten the cause of hyperkalemia should be made. Dialysis adequacy, patient's treatment compliance and vascular access should be evaluated at first.

Ethics

Informed Consent: A consent form was completed by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.K., Design: A.S.A., Data Collection or Processing: A.S.A., Y.D.B., Analysis or Interpretation: M.G., Ö.C.E., Literature Search: A.S.A., Writing: A.S.A., M.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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Case Report

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Bezmialem Science 2019;7(4):348-51

New Diagnosis Diabetes and Deep Venous Thrombosis with Glucagonoma Cases; Case Report

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ABSTRACT

Glucagonomas; is a rare neuroendocrine tumor originating from alpha cells of the pancreas. Glucagonoma syndrome is a paraneoplastic entity known as diarrhea, weight loss, stomatitis, thrombosis, diabetes, and necrotizing migratory erythema. It is difficult to come to mind in the differential diagnosis with the very rarely seen and little known reason. We also wanted to present a patient with bilateral deep vein thrombosis and a new diagnosis of diabetes that we were difficult to diagnose.

Keywords: Deep ven thrombosis, new diagnosis diabetes, glukagonoma, diarrhea

Introduction

Neuroendocrine tumors (NETs) are the tumors originating from the neuroendocrine system in any part of the body. NETs are rare tumors that are mostly benign but may also be aggressive. Today, NETs are divided into two groups as pancreatic NETs and other NETs (1). 60-90% of NETs occur in the gastrointestinal tract and pancreatic system. They are rarely seen in other organs (lung, adrenal gland, thymus, bladder, ovary, testis) (2). Pancreatic NETs, 90-95% of which are insulinoma and gastrinoma, constitute 7% of all NETs. Glucagonoma constitutes only 4% of pNETs (3). 80% of glucagonomas are malignant. It is often sporadic and is associated with genetic factors in 20% of cases (MEN1). Glucagonomas may present with migratory necrotic skin rash (dermatitis), diabetes, depression, diarrhea (4D syndrome), glossitis, stomatitis, angular cheilitis and severe weight loss (4). We present the case because it is a rare entity.

Case Report

Our case was a 72-year-old male patient. The patient was brought to the emergency unit with fatigue, loss of appetite, diarrhea, swelling and redness on the legs. He was admitted to our hospital because his serum glucose level was 553 mg/dL. Lower extremity Doppler ultrasonography (USG) showed bilateral acute deep vein thrombosis (DVT). It was thought that it might be associated with malignancy, especially the pancreas, due to DVT and diabetes newly diagnozed at advanced age. Ca19-9 (655 U/mL) was found to be high in the examinations. Other test results are given in Table 1. Abdominal USG revealed a 81*64 mm mass between the superior of the left kidney and the spleen. Gastroscopy and colonoscopy were unremarkable. In the abdominal computed tomography, there were homogeneously contrasting soft tissue appearances in the spleen lodge and anterior pararenal area, the largest of which was measured as 90x67 mm. A nodular soft tissue density of 13x10 mm was observed in the neighborhood of the superior of the pancreatic corpus. Abdominal magnetic resonance imaging revealed solid mass lesions measuring 80x65 mm in the spleen hilus and 46x40 mm in the medial neighborhood and showing different intensities with the splenic parenchyma (Figure 1, 2). The patient was aspirated with two 22 G from the paraaortic area with the help of endoscopic US, and smear and cell block were prepared. The liver was aspirated from a 50

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Table 1. Patient results

	13.03.2018	31.03.2018	02.04.2018	23.04.2018	Referans değer	
Glucose	183	500	183	159	70-105	mg/dL
BUN	22	29	84	24.3	8.4-25.7	mg/dL
Creatinine	0.96	1.46	3	1.07	0.72-1.25	mg/dL
Uric acid	3.3		11.2	3.3	3.5-7.2	mg/dL
T. Protein	5.3		4.9	5.5	6.2-8.1	gr/dL
Albumin	3.0		2.2	2.7	3.4-4.8	g/dL
AST	29	48	39	11	5-34	U/L
ALT	61	49	50	11	0-55	U/L
ALP	110	157	129		40-150	U/L
GGT	36	34	49		12-64	U/L
LDH	219	498	382	192	125-220	U/L
CK	34		189	15	30-200	U/L
T. BİL	0.50	0.79	0.38	0.42	0.3-1.2	mg/dL
D. BİL	0.17	0.16	0.28	0.20	0-0.5	mg/dL
Amylase	90		30		20-160	U/L
Lipase	9		14		8-78	U/L
Calcium	8.2		7.8	6.8	8.4-10.2	mg/dL
Magnesium	2.27			1.37	1.6-2.6	mg/dL
Phosphorus. inorg	2.0			2.1	2.3-4.7	mg/dL
Na	136	134	142	138	135-145	mmol/L
K	4.48	5.17	4.43	3.01	3.5-5.1	mmol/L
Cl	107		112	107	98-107	mmol/L
Folic acid	5.4		5.0		3.1-20.5	ng/mL
B12	503				157-883	pg/mL
Ferritin	620		3397		21.81-274.66	ng/mL
CK-MB			4.3		0-7.2	ng/mL
Troponın I			30		0.40	pg/mL
D-Dimer			4449		0-300	ng/mL
PTH	63		247		15-68.3	pg/mL
CRP	7.2	21	25	5.37	<0.5	mg/dL
Procalcitonin	0.6		335	2.94	<0.5	ng/mL
ESR	15		71		<20	mm/h
Hb	9.8	9.65		8.55	14.1-17.5	g/dL
Hct	10	30.3		25.7	40-52	%
MCV	87	87		85	80-97	fL
WBC	7500	14900		7540	4.6-10.2	10*3/mL
PLT	158.000	222.000		158.000	142-424	10*3/mL
Glukagon				>500	<209	pg/
TSH	0.26				0.35-4.94	mIU/mL
CEA	3.52				0-5	ng/mL
Ca19-9	655.54				0-37	U/mL
C-peptide	2.82				0.78-5.19	ng/mL

AST: Aspartate transaminase, ALP: Alkali phosphatase, ALT: Alanine transaminase, GGT: Gamma-glutamyl transpeptidase, LDH: Lactate dehydrogenase, CK: Creatine kinase, T. BİL: Total bilirubin, D. BİL: Direkt bilirubin, Na: Sodium, K: Potassium, Cl: Chlorine, CK-MB, Creatine kinase myocardial isoenzyme, PTH: Parathyroid hormone, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean erythrocyte volume, WBC: White blood cell, PLT: Platelets, TSH: Thyroid-stimulating hormone, CEA: Carcinoembryonic antigen, BUN: Blood urea nitrogen

mm cyst and carcinoembryonic antigen (CEA), amylase and cytology were sent for evaluation. In the cyst fluid, amylase value was found as 15 and CEA as 0.9 ng/mL. After the treatment of the patient was arranged and glycemic regulation was achieved, he was discharged for outpatient follow-up of biopsy results. However, the patient was admitted to the emergency service again and hospitalized in the clinic after twelve days because of deterioration in his general condition and swelling of his feet. The patient's previous biopsy result was evaluated as 'fine-chromotine neuroendocrine cell groups'. At this time, diarrhea and rashes on the lower extremities developed (Figure 3) and the patient was thought to have glucagonoma. Serum glucagon was >500 pg/mL (N<209 pg/mL). Surgery was recommended to the patient, but the patient and his relatives did not accept this recommendation. By the department of oncology, lanreotide was initiated to be administered every 28 days. Within days, the need for insulin decreased. The patient is still being followed up in the outpatient clinics of general internal diseases and oncology with lanreotide, glargine insulin and coumadin treatment.

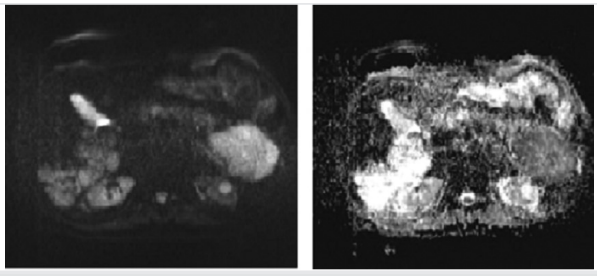

Figure 1. Abdominol MR image 1
MR: Magnetic resonance

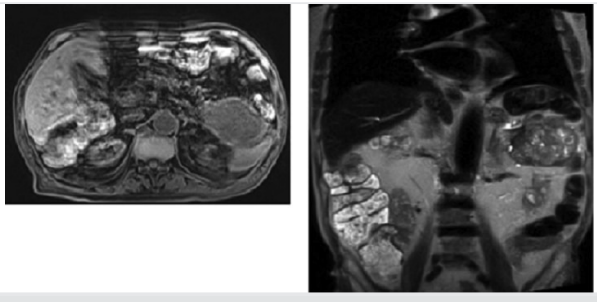

Figure 2. Abdominol MR image 2
MR: Magnetic resonance

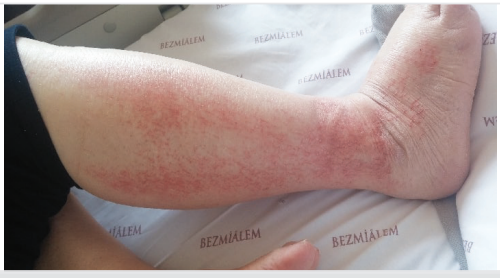

Figure 3. Lower extremity skin rash

Discussion

NET incidence is two per million and constitutes 0.5% of all cancers. It should be noted that NETs may coexist with other solid organ cancers. The gastrointestinal tract is the largest neuroendocrine system in the body, and NETs are often localized here. Gastroenteropancreatic NET (GEP-NET) cells are caused by diffuse endocrine system cells, which are phenotypically similar. These tumors are referred to as Neuroendocrine because they express proteins such as synaptophysin, neuron-specific enolase (NSE) and chromogranin A associated with neural cells. 65% of NETs occur in the gastrointestinal tract, 25% in the lungs, and the rest in other endocrine tissues. Clinical findings vary depending on the location of the NETs and the hormone that they secrete. Diagnosis is made by elevated blood glucagon level. The diagnosis is supported by the measurement of chromogranin-A (CgA) levels, 5-HIAA, CA19-9 and CEA levels. CgA has a sensitivity of 80% and specificity of 90% and has a better diagnostic value than 5-HIAA, NSE and pancreatic polypeptide. Plasma NSE identifies poorly differentiated NETs with 85% specificity and 70% sensitivity (5). Mitosis rate and ki-67 index are well below 2% in well-differentiated NETs and necrosis is not seen, whereas in poorly differentiated group, these rates increase above 10% and necrosis can be seen (6). Pancreatic NETs are also classified according to the hormones they secrete and they are called insulinoma, gastrinoma, glucagonoma, VIPoma. It can also be classified as local, regional and distant spread according to the extent of the disease. NETs may occur with metastases below 2 cm. Similarly, the rate of metastasis was 50% at the time of diagnosis (7). The metastasis rate is determined by the organ from which the tumor originated. Glucagonomas are located in the distal pancreas at a rate of 85%. In our case, the patient was examined considering GIS malignancy initially because there were weight loss, anemia, and DVT, and Ca19-9 was high (8). Abdominal imaging revealed a mass between the spleen and kidney; therefore, biopsy was performed under endoultrasonography. Meanwhile, basal-bolus insulin therapy for glycemic regulation and coumadin therapy for DVT were performed. After stabilization of the patient, he was discharged for outpatient follow-up. Then, the patient was brought back to the emergency unit due to deterioration of his general condition. Meanwhile, diarrhea and non-necrotic erythematous skin rash on the right leg were added to the complaints. In the immunohistochemical study of the previous biopsy, chromogranin (+), synaptophysin (+), pancytokeratin (+), LCA (-), ki 67 index was less than 1%. Results were evaluated to be consistent with neuroendocrine tumor (Grade I). Glucagon level sent to support the diagnosis was found to be significantly higher (9). Surgical intervention was planned but was not accepted. As a result of the consultation with oncology, lanreotide treatment was planned (10). After the treatment, his general condition recovered and glycemic regulation was achieved. The patient was discharged for follow-up in the outpatient clinics of oncology and general internal medicine.

We wanted to emphasize that glucagonoma should be considered in cases with newly diagnosed diabetes and DVT, erythematous

skin lesions, diarrhea and a mass in the abdomen and examinations for it should be performed.

Ethics

Informed Consent: Informed consent was obtained from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Consept: T.K., C.K., Design: T.K., C.K., Data Collection or Processing: T.K., C.K., Analysis or Interpretation: T.K., C.K., Literature Search: T.K., C.K., Writing: T.K., C.K.

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

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Case Report

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Physiotherapy and Rehabilitation in Congenital Facial Paralysis

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ABSTRACT

Congenital facial paralysis (CFP) is a paralysis of facial nerve that occurs in birth or shortly after birth due to congenital or traumatic causes, has a prevalence of 1.2-2.4/1000. Our aim is to determine the effectiveness of physiotherapy and rehabilitation in CFP. We assessed a 9-year-old girl, who had no congenital injuries, with muscle test, House Brackmann Facial Grading Systems (HBFGS) and Sunnybrook Facial Grading Systems (SFGS). She got level 4 in HBFGS scale; and 20 points for resting symmetry, 72 points for voluntary movement symmetry and 4 points for synkinesis in SFGS scale. The patient received our treatment including face massage, electrotherapy, mimic muscle and functional exercises lasting one hour per session, 3 days a week for 24 weeks. Biopsychosocial positive feedback was obtained after the ongoing treatment program, although there were no numerical changes in assessment scales. Although reported cases in the literature are irreversible, studies on rehabilitation are needed.

Keywords: Congenital facial paralysis, physiotherapy, rehabilitation

Introduction

Facial paralysis is the paralysis of the VII. Cranial nerve (Nervus facialis), which innervates the facial muscles and mimic muscles. The facial nerve has motor, sensory and parasympathetic fibers. The motor fibers innervate the facial muscles, the sensory fibers innervate the 2/3 front part of the tongue, and the autonomous fibers innervate the lacrimal gland. Congenital Facial Paralysis (CFP) can manifest before, during or after birth, with a frequency of 1.2-2.4/1000 (1). It causes facial asymmetry and affects eating and talking due to involvement of mimic muscles (2). Facial paralysis is a rare condition in the neonatal period, and it can be developmental or caused by by trauma. Although the most common cause is trauma during delivery, it may be observed in association with genetic syndromes and inner-ear structural abnormalities.

Congenital asymmetric crying face caused by unilateral hypoplasia or aplasia of depressor angularis oris muscle should also be considered in differential diagnosis. It is estimated that 78-91% of the CFP is due to birth trauma and 89-94% of cases are fully cured within a few weeks (3). In these patients, a multidisciplinary approach involving genetic, pediatric neurology and physiotherapy is required. Here, we present a 9-year-old girl who had CFP not related to congenital trauma and to whom we performed physiotherapy and rehabilitation program. With this case, it is aimed to review the literature about physiotherapy and rehabilitation treatment approaches for CFP.

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Case Report

It was learnt that the patient weighing 3650 g was delivered via caesarean section at the 40th gestational week. It was the third pregnancy of the mother who had a medical therapeutic abortion history. There was no drug use or infectious history . In the family story, it was learnt that there was no consanguineous marriage. Post-natal physical examination revealed that the left side of the face was in a neutral state and also during crying there was no wrinkling, frowning, blinking, nostril winging, and mouth movements in the left side of the face. Left facial paralysis was diagnosed in the patient. The baby's position in the mother's womb was thought to cause damage to the left facial nerve. The patient's needle electromyography results showed that left frontal muscle input activity was not detected and the motor unit could not be ignited by voluntary contraction. Normally configured motor unit potentials were observed during voluntary contraction of the left orbicularis oris muscle. These electrophysiological findings suggested a congenital anomaly in the left facial nucleus or nerve.

The patient who did not receive physiotherapy and rehabilitation before was referred to our clinic when she was 9 years old. The informed consent was taken from the patient and her family. The manual muscle test [Medial Research Council (MRC)] was used to assess the strength and contraction of the mimic muscles during mimic movements such as smile; while the patient was in neutral posture and was closing her eyes, wiggling her forehead, twisting her nose, showing her teeth and whistling. The MRC scores are between 0 and 5. The score of 5 (Normal) means the patient completes normal joint motion with maximum resistance to muscle gravity. The score of 0 means no contractions and full paralysis. According to this, the left side of the patient's forehead and the muscles involved in the front part of the head are MRC0, muscles around the eye are MRC1, muscles around the mouth were MRC2. House Brackmann Facial Grading Systems (HBFGS) and Sunnybrook Facial Grading Systems (SFGS) scales were used to assess facial function (4,5. The patient received fourth level in HBFGS scale; 20 points for resting symmetry, 72 points for voluntary movement symmetry, and 4 points for synkinesis in SFGS scale.

The patient was included in the physiotherapy and rehabilitation program for 24 weeks, 3 days/week, 1 hour/day. Positive feedbacks for both motor developments and biopsychosocial improvements were obtained after the dynamically continuing treatment program. While smiling, appearance of the muscles surrounding mouth was found almost same with the right side of the face.

Discussion

Our case was a patient with left facial paralysis without any additional diseases. In post-treatment assessments, it was observed that physiotherapy and rehabilitation had positive effects in the treatment of CFP. Improvements on the prognosis of the disease were seen with the physiotherapy and rehabilitation program. In the study by Wang et al. (6); steroid treatment was given, but no improvement was observed (6). For this reason, physiotherapy and rehabilitation is a treatment approach that should be considered in these patients. Electrotherapy and proprioceptive neuromuscular facilitation techniques should be applied to patients who have CFP. In the literature, it has been reported that children benefit from rehabilitation approaches in the treatment of CFP (7). Studies involving rehabilitation approaches such as physiotherapy, electrotherapy, biofeedback, massage, botulinum toxin and exercises (relaxation, coordination, face lift) have low levels of evidence. The purpose of rehabilitation is to reduce the muscle rigidity, and to make facial/mimic movements. It has been reported that the effects of botulinum toxin is temporary and may be more effective after nerve grafts (8). Studies on acupuncture and electrical nerve stimulation are not found in the literature. When we look at the literature, there are few studies about these kind of cases. More research on physiotherapy and rehabilitation is needed in congenital facial paralysis.

Conclusion

In conclusion, this case was presented in terms of the rare occurrence of CFP and its association with the genetic diseases. We can conclude that these patients may benefit from the physiotherapy and rehabilitation, and that these patients need guidance.

Ethics

Informed Consent: A consent form was completed by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.T., Design: B.A., A.C.Ü., M.T., B.Y., M.Ç., Data Collection or Processing: B.A., A.C.Ü, B.Y., M.Ç., Analysis or Interpretation: M.T., B.A., A.C.Ü, B.Y., M.Ç., Literature Search: M.T., B.A., A.C.Ü., B.Y., M.Ç., Writing: M.T., B.A., A.C.Ü., B.Y., M.Ç.

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