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EDITORIAL

Dear Colleagues;

We are proud and happy to meet you in the second issue of Bezmialem Science this year. First of all, I would like to thank our authors, referees and editorial board who contributed to our journal with their articles. Bezmialem Foundation University is a thematic university in the field of health. Among the foundation universities having faculty of medicine, we have the pride of being in the higher rankings in the URAP (University Ranking by Academic Performance) compared with many universities established before us. We continue our efforts to get a good place among the journals indexed abroad as in our country with your support.

In this issue; the article entitled " The Effects of Camellia sinensis Extract on Proliferation, Apoptosis and Oxidative Stress in Insulinoma INS-1 Cells" by KACAR et al., the article entitled "Aberrant Methylation Profile and Microsatellit Instability In Turkish Sporadic Colorectal Carcinoma" by EKMEKCI et al., the article entitled "The Role of PET/CT to Detect Bone Marrow Involvement in Hodgkin Lymphoma" by ESER et al., and the article entitled " Extra-Parenchymal Chest HRCT Findings of Patients With Systemic Sclerosis at The Time of Initial Diagnosis" by UFUK et al. are the articles that are prominent.

Welcome to our new editors who have joined our editorial board from different disciplines and our new scientists who have joined our scientific board and I wish them success in their duties.

Goodbye until we meet again in our next issue.

Prof. Dr. Adem AKCAKAYA

Editor-in-Chief

Original Article

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The Protective Effects of Long-term Probiotic Application on Experimental Sepsis-dependent Inflammation Process

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ABSTRACT

Objective: Probiotics are defined as live microorganisms that provide beneficial effects on the host when applied in appropriate amounts. The immun-modulatory effects of some probiotics are one of these. We aimed to investigate the effects of probiotics on some inflammatory cytokine levels in sepsis.

Methods: The mixture including 12 different live-probiotic bacteria was used in this study. Group 1 and 2 were evaluated as control and sepsis groups. Sepsis was developed in groups 2, 3, 4, 7 and 8 using cecal ligation and puncture. The probiotic mixture was given to rats in groups 3 and 4 for 21 days before sepsis, groups 7 and 8 a single dose after sepsis at 10^{10} and 10^{11} doses, respectively. Rats in groups 5 and 6, the same doses of probiotics were administered without sepsis. Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-10 and transforming growth factor (TGF)-β levels were measured by ELISA method.

Results: There was a serious increase on the level of TNF-α and IL-1β levels in sepsis group, and a serious decrease in 21 days of protective treatment groups. In accordance with IL-10 and TGF-β levels, although the cytokine levels of 21 days protective treatment groups were not reached in control group, a serious increase was observed compared to the sepsis group.

Conclusion: According to our findings, probiotics can be used for supportive purposes in addition to classical medical treatment of sepsis. However, we believe that more studies should be performed in order to standardize the effects of probiotic and determine its effects with different experimental groups of volunteers as well as experimental animal studies.

Keywords: Inflammation, sepsis, cytokine, probiotic

Introduction

The sepsis and septic shock are clinical pictures that may be very serious. They develop due to the spread of microorganisms or the toxic products of these microorganisms with blood circulation. The clinical features and findings of sepsis are results of "the fight" between the immune system of the host and the microorganism and its products. Sepsis stimulates the immunological, inflammatory and endocrine systems of the host, and the severity of these responses determines the clinical course (1).

Researches on the pathogenesis of sepsis have shown that; infection and traumatic injury in tissues activate the immunological mechanisms and cause inflammatory responses by releasing some mediators (especially some cytokines). In recent studies, the physiology of sepsis has been better understood. The metabolic and physiological changes continuously developed in the body have become clearer after the cytokines and other mediators and their mechanisms of action have been identified. As a result of these studies, it was understood that sepsis syndrome is a group of response sequences in which the released cytokines are largely responsible (1,2).

Under the name of "cytokine"; there are various groups of interleukins, monokines, lymphokines, growth factors, interferons

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and chemokines. Cytokines are produced by a wide variety of cell types (3,4). Cytokines are divided into two groups as pro-inflammatory and anti-inflammatory cytokines according to their mechanisms of action. While pro-inflammatory cytokines induce inflammation, anti-inflammatory cytokines prevent inflammation and promote healing (5,6). The pro-inflammatory cytokines necessary for the initiation and maintenance of the immune response are released at the onset of inflammation. The most important pro-inflammatory cytokines that play a key role in immune response are tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), IL-6 and IL-8. TNF-α and IL-1β are the first secreted cytokines and allow secretion of other cytokines that support migration and activation of the immune cells. Secondary or assisted cytokines are IL-6 and IL-8. Anti-inflammatory cytokines are released in later stages of inflammation, regulating the inflammatory response and down-regulation. The most important anti-inflammatory cytokines are IL-4, IL-10, IL-11, IL-13, and transforming growth beta factor (TGF)-β. Some cytokines may have both pro-inflammatory and anti-inflammatory effects (1,4).

Experimental studies on sepsis are continuing with a great acceleration. A variety of drugs and treatment protocols involving immune-modulatory substancesare being developed. One of the immune-modulators investigated and used for these purposes is probiotic bacteria (7).

The probiotics in the human gastrointestinal tract flora are located on the surface of the intestine and prevent pathogenic microorganisms from clinging, and the antimicrobial agents they produce control the growth of these bacteria. *Lactobacillus, Bifidobacterium, Streptococcus, Pediococcus, Leuconostoc* and *Enterococcus* species have been used as probiotics. There are significant differences between probiotic bacteria species and strains in terms of their effect, however the most effective probiotic bacteria are *Bifidobacterium* and *Lactobacillus* species according to the scientific studies (7,8).

Nowadays, probiotics can be used in many diseases and pathological conditions. They have beneficial effects such as strengthening the immunity of the host. Probiotics were shown to regulate immunological responses by stimulating host defense mechanisms both in *in vivo* and *in vitro* studies. It was shown in recent years that probiotics have anti-inflammatory, anti-oxidant, anti-bacterial, anti-parasitic, anti-fungal, apoptotic, anti-carcinogenic and many other immunological effects (7-9).

In our study, the aim was to evaluate the positive and/or negative effects of long-term probiotic use before sepsis and single/high dose probiotic administration after sepsis on TNF-α, IL-1β, IL-10 and TGF-β inflammatory parameters.

Methods

Experimental Procedure and Animals

This study was approved by the Local Ethics Committee of Animal Experiments with the date and approval numbers of 19.04.2016/48. Animal protocols were performed according to the local ethical rules and the rules of the Declaration of Helsinki.

Eighty female Wistar-albino rats, 12-week-old and weighing 220-300 g, were purchased. Rats were kept in polycarbonate boxes with a standard 12-hour light-dark cycle. The rats were randomly divided into 8 groups. Each group contained 10 rats (Table 1).

Probiotic Mixture and Preparation

A probiotic mixture (Enzibody®, Kenz BioTech, USA) that contained 12 different living probiotic bacteria strains was used in current study (Table 2). All bacteria were mixed in a 1:1 ratio. Twelve different probiotic bacteria (7 strains of *Lactobacillus* spp., 3 strains of *Bifidobacterium* spp., 1 strain of *Leuconostoc* spp. and 1 strain of *Lactococcus* spp.) were grown on de Man, Ragossa and Sharpe Broth overnight at 37 °C. Bacteria were separated from the culture supernatant by centrifugation (15 min at 3000 xg), washed three times with ice-cold phosphate saline buffer (PBS) (pH=7.2) and re-suspended in PBS. The final concentration of mixture contained $1x10^{13}$ colony-forming unit (cfu) lactic acid

Table 1. Number of animals in each group and group names

Group	Properties	Number
Group 1	Control	10
Group 2	Sepsis	10
Group 3	Septic group with 21-day 10^{10} cfu probiotic application	10
Group 4	Septic group with 21-day 10^{11} cfu probiotic application	10
Group 5	Healthy group with 21-day 10^{10} cfu probiotic application	10
Group 6	Healthy group with 21-day 10^{11} cfu probiotic application	10
Group 7	Septic group with single dose 10^{10} cfu probiotic application	10
Group 8	Septic group with single dose 10^{11} cfu probiotic application	10

cfu: Colony-forming unit

Table 2. Probiotic strains in the mixture

Probiotic bacteria	Strain
Bifidobacterium infantis	ATCC-15697
Bifidobacterium thermophilum	ATCC-25867
Bifidobacterium longum	ATCC-1570
Lactobacillus acidophilus	ATCC-43121
Lactobacillus casei	ATCC-393
Lactobacillus paracasei	ATCC-25302
Lactobacillus helveticus	ATCC-15009
Lactobacillus plantarum	ATCC-14917
Lactobacillus bifidus	ATCC-11863
Lactobacillus brevis	ATCC-14869
Leuconostoc mesenteroides	ATCC-8293
Lactococcus lactis	ATCC-19435

ATCC: American Type Culture Collection

bacteria. The final concentration of 10^{11} and 10^{10} doses was obtained by 1/100 and 1/1000 dilution series, respectively. The mixtures were stored at 4 ºC after the preparation till the day of experiment.

Experiments Details and Surgical Process

Group 1 was the "control group" and was consisted of ten rats. No surgical or treatment process was applied to group 1. Rats regularly consumed water and nutrients and blood samples were collected on the day of experiment.

Group 2 was the "septic group" and was consisted of ten rats. The rats were euthanized with 25 mg/kg dose of thiopental sodium anesthesia. After shaving the abdomen, the peritoneum was opened. The cecum was isolated and ligated with 3.0 silk ligature. The ligated part of the cecum was then punctured twice with a 16-gauge needle, and a small amount of cecal contents was expressed thought the punctures. After repositioning of cecum, the abdominal incision was closed with a 4.0 sterile absorbable suture. Blood was drawn from the apex of the cardiac ventricle, and collected into sterile blood collection tubes containing ethylenediaminetetraacetic acid after the rats were killed. Then, the blood samples were centrifuged at 4000 rpm for 10 minutes. After centrifugation, the serum supernatant to be used for further analysis was aliquoted into microcentrifuge tubes, and stored at -80 °C.

Group 3 and 4 were the "septic groups with 21-day probiotic application" (10^{10} cfu and 10^{11} cfu, respectively) and each was consisted of ten rats. 10^{10} cfu and 10^{11} cfu probiotic mixture were administered via oral gavage on a regular basis for 21 days to 100 mg/kg of each animal. Then, cecal-ligation and puncture (CLP) sepsis procedure was applied on 20 rats in these groups.

Group 5 and 6 were the "healthy groups with 21-day probiotic application" (10^{10} cfu and 10^{11} cfu, respectively) and each was consisted of ten rats. 10^{10} cfu and 10^{11} cfu probiotic mixture were administered via oral gavage on a regular basis for 21 days to 100 mg/kg of each animal. Nothing was applied on 20 rats in these groups.

Group 7 and 8 were the "septic groups with single dose probiotic application" (10^{10} cfu and 10^{11} cfu, respectively) and each was consisted of ten rats. Firstly, the CLP procedure was applied for these groups. Six hours later, single and high dose (500 mg/kg) of 10^{10} cfu and 10^{11} cfu probiotic mixtures were administered to evaluate the post-sepsis effectiveness of probiotic mixtures.

ELISA Procedure

To test the probiotic immunomodulation, the cytokine levels were detected by commercially available ELISA kits:

- Rat TNF-α ELISA kit (pg/mL) (cat no: CSB-E11987r, Cusabio, ABD).
- Rat IL-1β ELISA kit (pg/mL) (cat no: CSB-E08055r, Cusabio, ABD).
- Rat IL-10 ELISA kit (pg/mL) (cat no: CSB-E04595r, Cusabio, ABD).
- Rat TGF-β ELISA kit (pg/mL) (cat no: E-EL-R0084, Elabscience Biotechnology Ltd, ABD).

All of the chemicals and 96-well ELISA microplates were brought to room temperature before use. The first 7 wells were used for the standards; the 8th was used for the blank, and 100 µL of serum was added to each of the other wells. After incubation (at 37 °C, for 90 min), a 100 µL biotinylated detection antibody was added to each well, and then the plate was replaced in the incubator (at 37 °C, for 60 min). After a 3 step-washing, 100 µL of horseradish peroxidase conjugate was added to each well, placing the plate in the incubator again (at 37 °C, for 30 min). After the final 5 step-washing, 90 µL of substrate reagent was added, with 15 min incubation under darkened conditions. Finally, a 50 µL stop solution was added to the wells and the plate was read at a wavelength of 450 nm.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 statistical software (IBM, SPSS, Inc., Chicago, IL, USA). Continuous variables were analyzed using the one-way ANOVA and Kruskal-Wallis tests. The differences between the groups were defined as statistically significant when the p value was less than 0.05.

Results

Pro-inflammatory Cytokine Results

The level of IL-1β, a pro-inflammatory cytokine, showed statistically significant differences in some levels among experimental groups. IL-1β levels were significantly increased (146 pg/mL) in sepsis group and significantly decreased in the groups treated with probiotics for 21 days before the development of sepsis ($p<0.05$). In addition, the IL-1β level in the group treated with high-dose probiotic (10^{11} cfu) increased significantly ($p<0.05$) when compared with the control group (Figure 1). There were no statistically significant differences between sepsis and single-dose post-sepsis groups ($p>0.05$).

The level of TNF-α, which is another pro-inflammatory cytokine, in all experimental groups are presented in Figure 2. TNF-α levels were significantly increased (581 and 470 pg/mL, respectively) in sepsis groups compared with (621 pg/

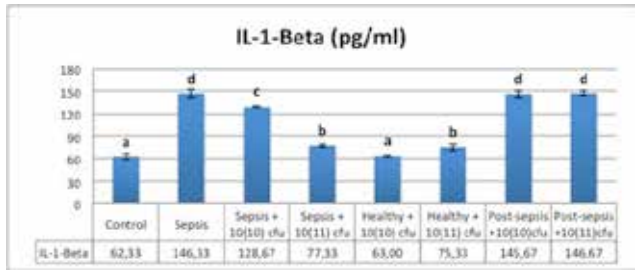

Figure 1. Interleukin-1-beta levels in each experimental group. The letters represent the difference between the groups ($p<0.05$)

IL-1: Interleukin-1, cfu: Colony-forming unit

mL) probiotic groups (p<0.05). TNF-α levels in healthy group significantly decreased when compared to the control group in a dose-dependent manner (p<0.05). A single dose probiotic application did not cause any significant decrease in IL-1β and TNF-α levels in post-sepsis groups (p>0.05).

After the evaluation of pro-inflammatory cytokine levels; it can be said that the present probiotic mixture suppresses excessive release of pro-inflammatory cytokines, which play an important role in the onset and permanency of the inflammatory process.

Anti-inflammatory Cytokine Results

The level of IL-10, an anti-inflammatory cytokine, showed statistically significant differences in some levels among experimental groups (Figure 3). In sepsis group, the level of IL-10 was the lowest among all groups (161 pg/mL), however; IL-10 levels were significantly increased in sepsis + probiotic treatment groups (p<0.05). In healthy groups, significant differences were observed depending on the dose manner of probiotic application. The level of IL-10 did not differ between the 10^{10} cfu healthy group and the control group (p>0.05), however; the level of IL-10 was significantly higher in the 10^{10} cfu healthy group than in the other groups except the control group (p<0.05).

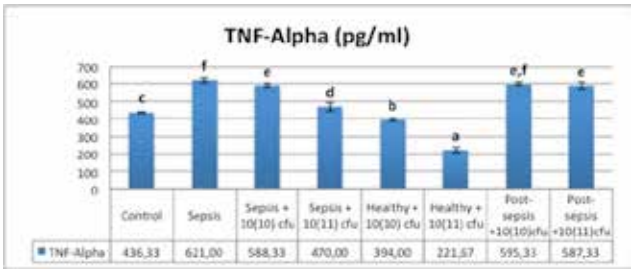

Figure 2. Tumor necrosis factor-alpha levels in each experimental group. The letters represent the difference between the groups (p<0.05)

TNF-alpha: Tumor necrosis factor-alpha, cfu: Colony-forming unit

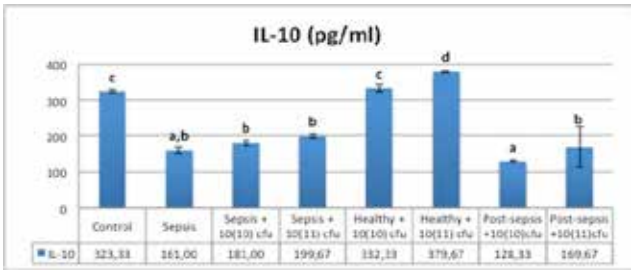

Figure 3. Interleukin-10 levels in each experimental group. The letters represent the difference between the groups (p<0.05)

IL-IA: İnterleukin-IA

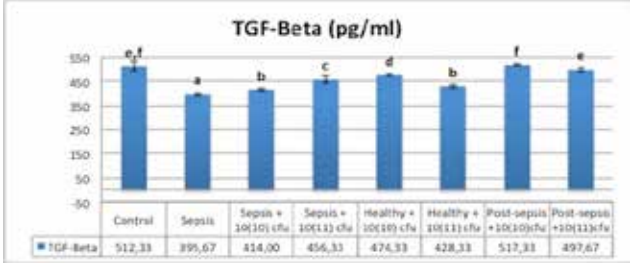

Figure 4. Transforming growth factor-beta levels in each experimental group. The letters represent the difference between the groups (p<0.05)

TGF-Beta: Transforming growth factor-beta, cfu: Colony-forming unit

TGF-β levels of all groups are shown in Figure 4. TGF-β level was 512 pg/mL in the control group and there was a significant decrease in TGF-β level (395 pg/mL) in sepsis group (p<0.05). This probiotic mixture, which we considered to be effective on anti-inflammatory cytokines in the process of inflammation caused by sepsis, resulted with an increase in TGF-β levels (414 and 456 pg/mL, respectively) in a dose-dependent manner (p<0.05).

When the test results were evaluated, the probiotic application used for therapeutic purposes (after sepsis) did not cause any changes, the probiotic application used for protective purposes showed differences in pro- and anti-inflammatory cytokine levels between the control group and the other groups.

Discussion

Sepsis is a serious infectious disease. Despite the developed intensive care conditions, high mortality rates have made it necessary to elaborate the mechanism of sepsis. Cytokines, which play a major role in the pathogenesis of sepsis, can be classified roughly as pro-inflammatory and anti-inflammatory mediators (5,6). The cytokines that are frequently studied in experimental studies on sepsis and inflammation are TNF-α, IL-1, IL-10 and TGF-β (10-13).

There is excessive and irregular production of sepsis-mediated inflammatory mediators. Cytokine imbalance is seen in these inflammatory events as an increase in plasma pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines (reduction in the amount of IL-10 by an increase in the amount of IL-1 and TNF-α) (14). In the light of these data, we decided to evaluate the critical pro-inflammatory (TNF-α and IL-1β) and anti-inflammatory cytokines (IL-10 and TGF-β) in our study.

Today, increasingly prevalent and intensive studies on probiotics show the effects of direct or indirect stimulation of the endogenous flora and the immune system (9). Animal models and human clinical trials show that probiotics affect cytokine release and reduce inflammation (15,16). The application doses of probiotics are another important point in clinical trials. Probiotic activity requires high intakes of bacteria, such as 10^{9}-10^{11} cfu/mL per day, so that they can be measured significantly in humans. In our study, we benefited from available literature for probiotic doses (17).

During inflammation, macrophages and lymphocytes cause production of TNF-α and IL-1 which are pro-inflammatory

cytokines. Lots of scientific studies reported higher levels of TNF-α and IL-1 in patient groups when compared with the healthy donors (18-20). Our results are parallel with the current literature with regards to TNF-α and IL-1 levels. There are some important studies that have reported the possible positive effects of probiotic usage in patients with severe and multiple traumas (21). On the other hand, a study showed that IL-10 and IL-4 levels increased in patients with severe brain trauma by using probiotic mixture containing *Bifidobacterium longum, Lactobacillus bulgaricus, Streptococcus thermophilus* in $3x10^9$ cfu/mL dose (22).

Probiotics were proven to be effective in inflammation. In experimental studies, probiotic supplementation was found to have a potential to reduce TNF-α expression. *Lactobacillus* and *Bifidobacterium* species were especially used in these studies same as our studies (23-25). In another studies, Lammers et al. (26) and Menard et al. (27) showed that probiotic usage can reduce the IFN-γ, IL-1β, TNF-α and IL-8 levels. We also reported in the current study that the probiotic mixture reduced TNF-α and IL-1β levels in sepsis groups when compared with the control group.

On the other hand, the anti-inflammatory cytokine levels, IL-10 and TGF-β, are at least as important as pro-inflammatory cytokines in inflammation process. Same as pro-inflammatory cytokine results, probiotic application affects IL-10 levels in some severe conditions such as colitis. Some studies confirmed these probiotic effects and added that especially *Bifidobacterium* spp., *Lactobacillus* spp., *Leuconostoc* spp., and *Streptococcus thermophilus* were responsible of IL-10 reduction (28-30).

In our study, IL-10 level was detected at the lowest level (161 pg/mL) in the sepsis group among all experimental groups, however IL-10 levels significantly increased in sepsis groups treated with probiotic mixture. When the levels of anti-inflammatory cytokines, IL-10 and TGF-β, were examined in detail, a significant increase in the level of cytokines was observed in the sepsis-treated group. The present probiotic mixture increases the release of anti-inflammatory cytokines, which play an important role in the inflammatory process in accordance with the literature.

Study Limitations

More detailed experimental procedures should be considered. Extra methods should be used to get better results. Further studies should be carried out by performing more detailed methods. Additionally, a specific probiotic bacterium effects should be shown in further studies.

Conclusion

According to our findings, the use of probiotics decreased the levels of pro-inflammatory cytokines associated with the deterioration of sepsis pathology and increased the anti-inflammatory cytokine levels which limits the inflammatory process. We believe that probiotic support can be used in addition to classical medical treatments in the treatment of sepsis, which affects all organs and systems. However, in this regard, we believe that more studies should be performed in order to standardize the effects of probiotics and to determine their effects in different experimental groups of volunteers as well as experimental animal studies.

Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee of Animal Experiments with the date and approval numbers of 19.04.2016/48.

Informed Consent: As it was an animal experiment, only ethics committee approval was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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References

1. Cohen J. The immunopathogenesis of sepsis. Nature 2002;420:885-91.
2. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138-50.
3. Fijen JW, Muller Kobold AC de Boer P, Jones CR, van der Werf TS, Tervaert JW, et al. Leukocyte activation and cytokine production during experimental human endotoxemi. Eur J Intern Med 2000;11:89-95.
4. Al-Kharfy KM, Kellum JA, Matzke GR. Unintended immunomodulation: part I; Effect of common clinical conditions on cytokine biosynthesis. Shock 2000;13:333-45.
5. Horn DL, Morrison DC, Opal SM, Silverstein R, Visvanathan K, Zabriskie JB. What are the microbial components implicated in the pathogenesis of sepsis? report on a symposium. Clin Infect Dis 2000;31:851-8.
6. Chaudhry H, Zhou J, Zhong Y, Ali MM, McGuire F, Nagarkatti PS, et al. Role of cytokines as a double-edged sword in sepsis. In Vivo 2013;27:669-84.
7. Bergonzelli GE, Blum S, Brussow H, Corthesy-Theulaz I. Probiotics as a treatment strategy for gastrointestinal diseases? Digestion 2005;72:57-68.
8. Ashraf R, Shah NP. Immune system stimulation by probiotic microorganisms. Crit Rev Food Sci Nutr 2014;54:938-56.
9. Saarela M, Mogensen G, Fondén R, Mättö J, Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. J Biotechnol 2000;84:197-215.
10. Zhang A, Pan W, Gao J, Yue CL, Zeng L, Gu W, et al. Associations between interleukin-1 gene polymorphisms and sepsis risk: a meta-analysis. BMC Med Genet 2014;15:8.
11. Opal SM, Fisher CJ Jr, Dhainaut JF, Vincent JL, Brase R, Lowry SF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled,

multicenter trial. The interleukin-1 receptor antagonist sepsis investigator group. Crit Care Med 1997;25:1115-24.

12. Ji QW, Guo M, Zheng JS, Mao XB, Peng YD, Li SN, et al. Downregulation of T helper cell type 3 in patients with acute coronary syndrome. Arch Med Res 2009;40:285-93.
13. Kilic T, Ural D, Ural E, Yumuk Z, Agacdiken A, Sahin T, et al. Relation between proinflammatory to anti-inflammatory cytokine ratios and long-term prognosis in patients with non-ST elevation acute coronary syndrome. Heart 2006;92:1041-6.
14. Koca TT. Bağırsak mikroflorasının inflamatuvar hastalık patogenezindeki yeri. Arşiv Kaynak Tarama Dergisi 2015;24:78-91.
15. McFarland LV. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. World J Gastroenterol 2010;16:2202-22.
16. Ohland CL, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. Am J Physiol Gastrointest Liver Physiol 2010;298:807-19.
17. Karamese M, Aydin H, Sengul E, Gelen V, Sevim C, Ustek D, et al. The immunostimulatory effect of lactic acid bacteria in a rat model. Iran J Immunol 2016;13:220-8.
18. Mueller C. Tumour necrosis factor in mouse models of chronic intestinal inflammation. Immunology 2002;105:1-8.
19. Yan Y, Kolachala V, Dalmasso G, Nguyen H, Laroui H, Sitaraman SV, et al. Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. PLoS One 2009;4:6073.
20. Egger B, Bajaj-Elliott M, MacDonald TT, Inglin R, Eysselein VE, Büchler MW. Characterisation of acute murine dextran sodium sulphate colitis: cytokine profile and dose dependency. Digestion 2000;62:240-8.
21. Kotzampassi K, Giamarellos-Bourboulis EJ, Voudouris A Kazamias P, Eleftheriadis E. Benefits of a synbiotic formula (Synbiotic 2000Forte) in critically illtrauma patients: early results of a randomized controlled trial. World J Surg 2006;30:1848-55.
22. Tan M, Zhu JC, Du J, Zhang LM, Yin HH. Effects of probiotics on serum levels of Th1/Th2 cytokine and clinical outcomes in severe traumatic brain-injured patients: a prospective randomized pilot study. Critical Care 2011;15:290.
23. Peran L, Camuesco D, Comalada M, Nieto A, Concha A, Adrio JL, et al. Lactobacillus fermentum, a probiotic capable to release glutathione, prevents colonic inflammation in the TNBS model of rat colitis. Int J Colorectal Dis 2006;21:737-46.
24. Pagnini C, Saeed R, Bamias G, Arseneau KO, Pizarro TT, Cominelli F. Probiotics promote gut health through stimulation of epithelial innate immunity. Proc Natl Acad Sci U S A 2010;107:454-9.
25. Khailova L, Frank DN, Dominguez JA, Wischmeyer PE. Probiotic administration reduces mortality and improves intestinal epithelial homeostasis in experimental sepsis. Anesthesiology 2013;119:166-77.
26. Lammers KM, Vergopoulos A, Babel N, Gionchetti P, Gionchetti P, Rizzello F et al. Probiotic therapy in the prevention of pouchitis onset: decreased interleukin-1beta, interleukin-8, and interferon-gamma gene expression. Inflamm Bowel Dis 2005;11:447-54.
27. Ménard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. Gut 2004;53:821-8.
28. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75:263-74.
29. Wan YM, Zhu YQ, Xia B, Luo J. Treating TNBS-Induced colitis in rats with probiotics. Turk J Gastroenterol 2011;22:486-93.
30. Helwig U, Lammers KM, Rizzello F, Brigidi P, Rohleder V, Caramelli E, et al. Lactobacilli, bifidobacteria and E. coli nissle induce pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells. World J Gastroenterol 2006;12:5978-56.

Original Article

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Investigation of Carbapenemase Genes and Clonal Relationship in Carbapenem Resistant *Klebsiella pneumoniae* Strains

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ABSTRACT

Objective: Resistant Gram-negative bacteria isolated from health-related infections are a worldwide problem. Increasing frequency of infections particularly caused by *Enterobacteriaceae* producing expanded spectrum beta lactamase, leads to the use of more carbapenem group antibiotics which, in turn, leads to bacterial resistance. In this study, we aimed to evaluate carbapenem resistance in *Klebsiella pneumoniae* (*K. pneumoniae*) isolates, the mechanisms causing this resistance and the clonal relationship between these isolates.

Methods: Ninety-one *K. pneumoniae* strains isolated from clinical samples obtained in our laboratory were included to the study. The identification of the bacteria was performed with Matriks assisted laser desorption ionization time of flight mass spectrometry (bioMérieux, Marcy-l'Étoile, France) and antimicrobial susceptibility with VITEK-2 (bioMérieux), and the carbapenem resistance was confirmed by ertapenem E-test (bioMérieux). Reverse transcription polymerase chain reaction method was used for the investigation of genes causing carbapenemase production (bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, bla_{IMP}, bla_{VIM-1}). The clonal relationship between isolates was investigated by pulsed-field jel elektroforez.

Results: In carbapenem resistant isolates, bla_{OXA-48} positivity was found to be 55%, bla_{NDM-1} positivity 37.4%, bla_{KPC} and bla_{VIM-1} positivity 1.1%. A total of 10 isolates was identified with different resistance genes. In 73 of the isolates included in the study, the clonal relationship was examined, and 16 different groups were identified. Twenty isolates were not clonally associated with any other isolates. The most common resistance mechanism causing the carbapenem resistance was bla_{OXA-48} gene that is known to be endemic in Turkey.

Conclusion: As a result, the carbapenem resistance that we found as 3.13% in our study is similar to the rates obtained in other studies performed in our country which indicates that this resistance is not at a high level yet in our country. However, the ability of carbapenem resistance genes to spread between strains can be a major problem in the near future. Molecular methods are gold standard in carbapenemase detection, but because of having high cost they can not be used in laboratories routinely. Modified Hodge test or carbapenemase inactivation test are alternative tests with low costs that can be used in the determination of carbapenemase.

Keywords: Carbapenem, *Klebsiella pneumoniae*, resistance gene, pulsed-field gel electrophoresis

Introduction

Resistant Gram-negative bacteria isolated from health-related infections pose a worldwide problem (1). The incidence of infections is increasing in which bacteria, especially from the *Enterobacteriaceae* family, producing extended spectrum beta lactamase (ESBL), are isolated, leading to the further use of carbapenem group antibiotics (2).

Carbapenem resistance is mainly caused by two mechanisms: (i) overproduction of ESBL and/or AmpC along with the loss of porin, (ii) production of carbapenemase (3). Carbapenemases

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are classified in class A (bla_{KPC} vb.), B (bla_{VIM-1}, bla_{IMP}, bla_{NDM-1} vb.) and D (bla_{OXA-48} vb.) according to the Ambler classification (3,4). Phenotypic determination of carbapenem-producing *Enterobacteriaceae* members is difficult because carbapenems' minimum inhibitory concentrations may be low. Therefore, the genotypic determination of carbapenemase producing genes is gold standard (5).

In this study, we aimed to evaluate the carbapenem resistance in *Klebsiella pneumoniae* (*K. pneumoniae*)strains, the identification of resistance genes that cause this resistance, and the clonal relationship between resistant strains.

Methods

Bacterial Strains

Ninety one strains resistant to carbapenem among 2903 *K. pneumoniae* strains isolated in our hospital microbiology laboratory between January 2015 and December 2016 were included in this study. The identification of bacteria was made with Matriks assisted laser desorption ionization time of flight mass spectrometry (bioMérieux, Marcy-I'Etoile, France). İstanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee decision no: 2016/0034, 09.02.201.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test of isolated strains (colistin, ampicillin/sulbactam, piperacilin/tazobactam, ceftazidime, ceftriaxone, erapenem, meropenem, imipenem, ciprofloxacin, levofloxacin, gentamicin, amikacin and trimethoprim/sulfamethoxazole) was done with the automated system of VITEK-2 (bioMérieux, Marcy-I'Étoile, France). The authentication of strains with decreased sensitivity or resistance to any of the carbapenems was performed with erythromycin E-test (bioMérieux). Border values were evaluated in accordance with the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST) (6). *K. pneumoniae* American Type Culture Collection (ATCC) 700603 was used as the quality control strain.

Investigation of the Genes that Produce Carbapenemase

DNA extraction was carried out using the Qiagen (QIA)mp DNA mini kit (Qiagen GmbH, Hilden, Germany) in 18-24-hour culture of bacteria in accordance with the recommendations of the manufacturer. The genes causing carbapenemase production (bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, bla_{IMP}, bla_{VIM-1}) were evaluated with real-time polymerase chain reaction (PCR) in the Rotor-Gene Q device (Qiagen, Germany). The presence of the genes evaluated in the study was investigated using commercially produced Microbial DNA qPCR Assay (REF 330025) (Qiagen, USA) kits. Data obtained from PCR results were analyzed by setting the green channel threshold to 0.02 absolute value.

Pulsed-field Gel Electrophoresis (PFGE)

The epidemiological relation of carbapenem resistant *K. pneumoniae* (KDKp) strains was investigated in the the Molecular Microbiology Laboratory of Turkish Public Health Institution using pulsed-field gel electrophoresis (PFGE). Bionumerics program 7.1 version was used to evaluate the results. Clustering analysis was performed using "unwighted pair group method with mathematical averaging". The relationship between the strains was determined according to "dice" similarity coefficient. Tolerance was taken as 1.5% and optimization as 1% in the calculation of similarity coefficient. PFGE types were determined according to the DNA tailing patterns, in accordance with the criteria defined by Tenover et al. (7) and the strains with a similarity of ≥85% were defined as "related strains". Normalization was done by using *K. pneumoniae* ATCC 700603 standard strain.

Results

Ninety-one (3.13%) of a total of 2903 *K. pneumoniae* strains isolated from various clinical samples in our laboratory were found to be resistant to at least one antimicrobial agent from carbapenem group.

Of carbapenem resistant strains, 49.5% were isolated from intensive care unit, 12.1% from internal medicine, 11% from general surgery, 8.8% from brain surgery and 8.8% from child health and disease services and 9.8% from other services (emergency service, infectious diseases, neurology and urology). Of resistant strains, 58.2% were isolated from blood, 19.8% from tracheal aspirate, 11% from deep tissue aspirate, 8.8% from urine and 2.2% from phlegm culture. All strains were resistant to ertapenem, 93.3% to meropenem, 91.1% to imipenem. In addition, all KDKp strains were resistant to piperacillin/tazobactam and ampicillin/sulbactam, 95.6% to ceftazidime and ceftriaxon, 86.7% to ciprofloxacin and levofloxacin, 82.2% to trimethoprim/sulfamethoxazole. Resistance rates to colistin, amikacin and gentamicin were shown as 9%, 42.2% and 62.2%, respectively and they were relatively sensitive antibiotics.

All strains were tested for bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, bla_{IMP} and bla_{VIM-1} resistance genes, and bla_{OXA-48} (55%) and bla_{NDM-1} (37.4%) were the most frequently found resistance genes (Figure 1). Resistance genes were not detected in a total of 15 strains and two different resistance genes were found in 10 strains.

Seventy three of 91 KDKp strains included in the study were investigated in terms of clonal relations with PFGE due to the technical problems experienced in the reanimation of bacteria. In

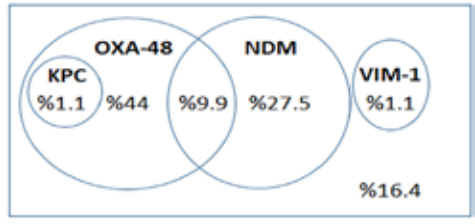

Figure 1. Distribution of resistance genes in carbapenem resistant 91 strains

KPC: *Klebsiella pneumoniae* carbapenemase, OXA: Oxacillin sodium, NDM: New Delhi metallo-beta-laktamaz-1, VIM: Vimentin

20 of these strains, a clonal relationship was not detected, while others formed 16 clonal groups (Figure 2). The presence of *K. pneumoniae* strains carrying the same resistance genes in different groups suggested that mobile genetic elements carrying these resistance genes might be transferred horizontally.

Discussion

Carbapenem resistance has been spreading rapidly recently and is a major problem threatening public health. The solution of this problem requires studies to explain the mechanisms of resistance and the development of methods to determine the resistance to carbapenem quickly and easily (3,8).

EUCAST indicates that ertapenem has high sensitivity and low specificity and meropenem has good sensitivity-specificity balance in detecting carbapenem resistance in *Enterobacteriaceae* (9). Therefore, in our study, ertapenem E-test was used to confirm carbapenem resistance. All strains were resistant to ertapenem and some strains were resistant to both meropenem and imipenem. Our findings supported that the use of ertapenem in detection of carbapenem resistance was more appropriate.

In the multi-center SENTRY study conducted in the United States (USA) between 2007-2009, carbapenem resistance in *K. pneumoniae* strains was found to be 6.1% (10). This resistance was 1-5% in the European

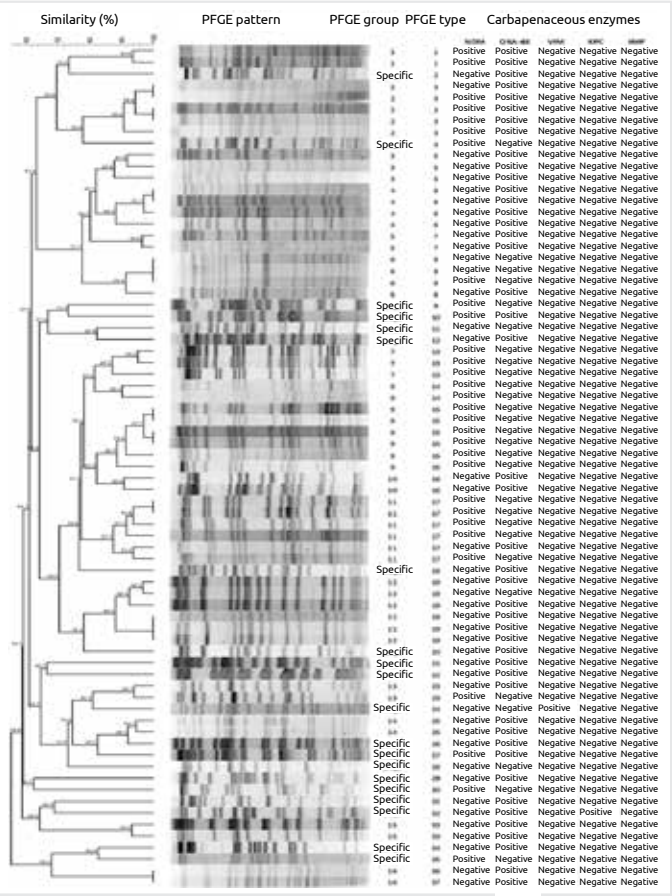

Figure 2. Clon analysis results of 73 strains by using pulsed-field gel electrophoresis

PFGE: Pulsed-field gel electrophoresis

Antimicrobial Resistance Surveillance System-2008 study in Europe and 3.2% in the high ıntensity tactical training-2 study in Turkey (11,12). Karbapenem resistance was found 3.13% in our study, consistent with the European and Turkish Data.

KDKp strains were most frequently isolated from intensive care units, compatible with another study in our country (3). Mechanical ventilation, prolonged intubation time, the use of paralytic agents and advanced age were found to increase the risk of infection. Even staying in the intensive care unit was stated as a risk factor alone (13).

All of the strains undergoing antimicrobial susceptibility test performed with KDKp strains were reported to be resistant to ertapenem. The most sensitive antimicrobial was reported as colistin (3,14). The data we obtained in our study showed the same situation.

Carbapenemase resistance was first identified in *Enterobacter clocae* strains more than 20 years ago. During this time, a large number of carbapenem resistance has been reported in different bacteria with different resistance mechanisms from many countries of the world (15).

In the present study, we found that bla_{OXA-48} and bla_{NDM-1} were the most frequent carbapenem resistance genes (55% and 37.4%, respectively). *K. pneumoniae* strains carrying bla_{OXA-48} are considered endemic in Turkey (3,16,17). In a multi-center study conducted in Europe, *K. pneumoniae* strains with decreased sensitivity to carbapenem with bla_{OXA-48} positivity was found to be 79% for Turkey (18). Tekintaş et al. (19) showed bla_{OXA-48} positivity in 52 of 54 (96.3%) KDKp strains in a study published in 2017.

An endemic region for bla_{NDM-1} positive *K. pneumoniae* has not been identified It was seen in Sweden in 2015 and it was isolated from a patient in India in 2009 (17,20). Bla_{NDM-1} positivity in carbapenem resistant *K. pneumoniae* strains was detected in 2011 for the first time in Turkey in a patient with leukemia who underwent a bone marrow transplantation (3). In a multicenter study published in 2017, bla_{NDM-1} positivity was reported as 7.3% in KDKp strains in Turkey (18). Other studies in our country reported bla_{NDM-1} positivity in 38.9% and 29.5% of strains (19,21). These ratios coincide with our findings.

In our study, bla_{KPC} and bla_{VIM-1} genes were detected in one patient and bla_{IMP} gene was not detected. The presence of $bla_{IMP,}$ bla_{VIM-1} and bla_{KPC} in *K. pneumoniae* strains in Turkey was first detected in 2003, 2005 and 2014, respectively (22-24). In a study conducted in our country in 2016, only one of 279 KDKp strains was found to have bla_{IMP} gene (25). These genes are endemic in some countries such as Italy and Greece (15,17).

In our study, resistance genes other than the five most common carbapenemase enzymes in Turkey were not searched, which constituted the limitation of our study. PFGE patterns of a total of 73 KDKp strains showed differences. Although isolating

times of strains in group 1 were close to each other, the services they were isolated were different. Being isolated of the strains in groups 2, 7, 9, 11 and 15 at different times from the same service suggested that a health worker or material might be a reservoir. In other groups, it was observed that the strains were isolated in services at different times from each other. *K. pneumoniae* strains carrying the same resistance gene could be found in different groups or even they did not belong to any group, suggesting that mobile genetic elements carrying these resistance genes might be transferred horizontally between bacteria.

Conclusion

As a result, carbapenem resistance in our country is not yet at the highest level. This was found to be 3.13% in our study. However, the horizontal spread of the genes that cause carbapenem resistance can be a major problem in the near future. Because of the more deadly surveillance of infections with KDKp strains and the lack of treatment options; rapid and reliable tests are needed to determine resistance status (26-28). Although the molecular methods in detection of carbapenemase are gold standard, they can not be done routinely in each laboratory because of the high cost. Alternatively, practical and less costly methods such as carbapenemase inactivation test or modified hodge test can be used in the determination carbapenemase.

Ethics

Ethics Committee Approval: İstanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee decision no: 2016/0034, 09.02.201.

Informed Consent: Our study was an in vitro study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: M.S., İ.D., M.E.K., Design: İ.D., M.E.K., Data Collection or Processing: İ.D., M.E.K., Analysis or Interpretation: İ.D., M.E.K., H.V., Literature Search: H.Ç., İ.D., M.E.K., Writing: İ.D., M.E.K.,

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References

1. Kollef MH, Fraser VJ. Antibiotic resistance in the intensive care unit. Ann Intern Med 2001;134:298-314.
2. Gülmez D, Woodford N, Palepou MF, Mushtaq S, Metan G, Yakupogullari Y et al. Carbapenem-resistant Escherichia coli and Klebsiella pneumoniae isolates from Turkey with OXA-48-like carbapenemases and outer membrane protein loss. Int J Antimicrob Agents 2008;31:523-6.
3. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceaein a tertiary-level reference hospital in Turkey. Ann Clin Microbiol Antimicrob 2016;15:20.
4. Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JD. Surveillance and Molecular Epidemiology ofKlebsiella pneumoniae Isolates That Produce Carbapenemases: First Report of OXA-48-Like Enzymes in North America. Antimicrob Agents Chemother 2013;57:130-6.
5. Cohen Stuart J, Leverstein-Van Hall MA. Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae. Int J Antimicrob Agents 2010;36:205-10.
6. http://www.eucast.org/clinical_breakpoints/ , EUCAST Breakpoint Tables v 7.1 , Aralık 2017.
7. Tenover FC, Arbeit RD, Goering RV. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. Infect Control Hosp Epidemiol 1997;18:426-39.
8. Kuskucu MA, Karakullukcu A, Ailiken M, Otlu B, Mete B, Aygun G. Investigation of carbapenem resistance and the first identification of Klebsiella pneumoniae carbapenemase (KPC) enzyme among Escherichia coli isolates in Turkey: A prospective study. Travel Med Infect Dis 2016;14:572-6.
9. http://www.eucast.org/resistance_mechanisms/ Temmuz 2017.
10. Kaiser RM, Castanheira M, Jones RN, Tenover F, Lynfield R. Trends in Klebsiella pneumoniae carbapenemase-positive K.pneumoniae in US hospitals: report from the 2007-2009 SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis 2013;76:356-60.
11. 11.https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/1011_SUR_annual_EARS_Net_2008.pdf
12. Gur D, Hascelik G, Aydin N, Telli M, Gültekin M, Ogunc D et al. Antimicrobial resistance in gram-negative hospital isolates: results of the Turkish HITIT-2 Surveillance Study of 2007. J Chemother 2009;21:383-9.
13. Rodríguez-Acelas AL, de Abreu Almeida M, Engelman B, Cañon-Montañez W. Risk factors for health care–associated infection in hospitalized adults: Systematic review and meta-analysis. Am J Infect Control 2017;45:e149-e156.
14. Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JD. Surveillance and molecular epidemiology of Klebsiella pneumoniae isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. Antimicrob Agents Chemother 2013;57:130-6.
15. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers in Enterobacteriaceae worldwide. Clin Microbiol Infect 2014;20:821-30.
16. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of Oxacillinase-Mediated Resistance to Imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother 2004;48:15-22.
17. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL ve group., European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill 2015;20.
18. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT et al. Occurrence of carbapenemase-producing Klebsiella pneumoniae and. Escherichia coli in the European survey

of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis 2017;17:153-63.

19. Tekintaş Y, Çilli F, Eraç B, Yaşar M, Aydemir SŞ, Hoşgör Limoncu M. Comparison of phenotypic methods and polymerase chain reaction for the detection of carbapenemase production in clinical Klebsiella pneumoniae isolates. Mikrobiyol Bul 2017;51:269-76.
20. Rolain JM, Parola P, Cornaglia G. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect 2010;16:1699-701.
21. Haciseyitoglu D, Dokutan A, Abulaila A, Erdem F, Cag Y, Ozer S et al. The First Enterobacter cloacae Co-Producing NDM and OXA-48 Carbapenemases and Interhospital Spread of OXA-48 and NDM-Producing Klebsiella pneumoniae in Turkey. Clin Lab 2017;1:1213-22.
22. Labarca J, Poirel L, Ozdamar M, Turkoglü S, Hakko E, Nordmann P. KPC-producing Klebsiella pneumoniae, finally targeting Turkey. New Microbes New Infect 2014;2:50-1.
23. Yildirim I, Ceyhan M, Gur D, Mugnaioli C, Rossolini GM. First detection of VIM-1 type metallo-beta-lactamase in a multidrug-resistant Klebsiella pneumoniae clinical isolate from Turkey also producing the CTX-M-15 extended-spectrum beta-lactamase. J Chemother 2007;19:467-8.
24. Aktaş Z, Poirel L, Salcioğlu M, Ozcan PE, Midilli K, Bal C et al. First IMP-1-producing Klebsiella pneumoniae isolate in Turkey. Clin Microbiol Infect 2006;12:695-6.
25. Zarakolu P, Eser OK, Aladag E, Al-Zahrani IA, Day KM, Atmaca O et al. Epidemiology of carbapenem-resistant Klebsiella pneumoniae colonization: a surveillance study at a Turkish university hospital from 2009 to 2013. Diagn Microbiol Infect Dis 2016;85:466-70.
26. Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. Epidemiology of Carbapenemase-Producing Enterobacteriaceae and Acinetobacter baumannii in Mediterranean Countries. Biomed Res Int 2014;2014:305784.
27. Sahin K, Tekin A, Ozdas S, Akın D, Yapislar H, Dilek AR et al. Evaluation of carbapenem resistance using phenotypic and genotypic techniques in Enterobacteriaceae isolates. Ann Clin Microbiol Antimicrob 2015;14:44.
28. Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. J Clin Microbiol 2014;52:4003-9.

Original Article

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Prolonged Mechanical Ventilation Predictors in Patients Undergoing Liver Transplantation

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ABSTRACT

Objective: Liver transplantation is the current treatment modality for end-stage liver disease. While there is increasing drive towards early extubation in patients undergoing liver transplantation, prolonged meachanical ventilation (PMV) related to various factors can be observed. We tried to determine the risk factors associated with PMV.

Methods: One hundred twenty five patients (age>18 years) of with the American Society of Anesthesiology (ASA) III-IV group undergoing liver transplantation were enrolled in this retrospective study. Patient charts, intraoperative and intensive care unit follow-up forms, and electronic medical recording system were used for data collection. Patients were categorized as having received (with) or not received (without) prolonged mechanical ventilation (>24 hours), and perioperative risk factors were attempted to determine.

Results: No significant intergroup differences were found in demographic variables but ASA and model for end-stage liver disease (MELD) scores were significantly higher in the with- than in the without-PMV group. Total amount of suspension of erythrocytess suspension, fresh frosen plasm, and cell-saver blood used intraoperatively was higher in the with-PMV group but difference did not reach statistical significance. Patients with-PMV had significantly higher cryoprecipitate transfusion rates than did those without-PMV (p=0.007). While no significante intergroup differences were found in mortality and lenght of hospital stay; lenght of intensive care unit (ICU) stay was higher in the with- PMV group (p=0.001). Extravascular lung water index, global ecosystem dynamics ınvestigation, and pulse volume index values obtained from pulse contour cardiac output (PiCCO) monitorization were significantly lowver in the with- than in the without-PMV group.

Conclusion: We found that preoperative high ASA and MELD scores, high blood product transfusion rates, and hypovolemia supported by the PiCCO measurements are were closely related to PMV and long lenght of ICU stay.

Keywords: Liver transplatation, weaning, prolonged mechanical ventilation

Introduction

Liver transplantation is a safe and effective way of treating end-stage liver disease (ESLD) due to better understanding of the pathology of liver diseases, the development of organ protection techniques, the use of safer and effective immunosuppressive drugs, advances in surgical techniques, anesthesia and monitorization. With these improvements, both perioperative complication and mortality rates have decreased significantly, and 1-year life expectancy has exceeded 90%.

Liver transplant candidates are often characterized by a hyperdynamic circulatory state of cirrhotic cardiomyopathy (1) and a complex process in which all systems are affected. Need for massive transfusion due to severe surgical blood loss and intravascular volume shift, surgical large vein maneuvers and the effects of current coagulopathy, metabolic dysfunction, hemodynamic instability and reperfusion on systems require intraoperative detailed monitorization (2). In addition to standard monitorization, cardiac output monitorization is required for the

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management of intraoperative hemodynamic changes. For this reason, nowadays, pulse contour cardiac output (PiCCO) ve hemodynamic monitor methods which combine thermodilution and arterial waveform analysis methods, have less complication rates than pulmonary artery catheter and are less invasive, have gained importance. PiCCO, also used in our clinic on a routine basis, is an invasive hemodynamic monitoring method which uses the combination of these two techniques and shows important parameters such as cardiac index (CI), stroke volume index (SVI), global end-diastolic index (GEDI), stroke volume variation (SVV), global ejection fraction, systemic vascular resistance index (SVRI), extravascular lung water index (ELWI) and pulmonary vascular permeability index (PVPI).

Although early extubation trend increases in most of the centers where liver transplantation is performed due to improvements in perioperative care (3,4), prolonged mechanical ventilation is required due to various factors such as massive blood transfusion, pulmonary edema, lung damage, acid, capillary leakage, primary pulmonary and heart disease (5). In this study, we aimed to determine the risk factors associated with prolonged mechanical ventilation requirement in patients undergoing liver transplantation.

Methods

Anesthesia

After obtaining ethics committee approval, American Society of Anesthesiologists (ASA) III-IV 125 patients aged over 18 years who underwent liver transplantation between 2015 and 2017 were included in this retrospective study. Patients whose preoperative anesthetic evaluations were completed one day before, were taken to the operation room without premedication and heart rate, noninvasive blood pressure, SpO_2, body temperature and $EtCO_2$ monitorizations were performed. After induction of anesthesia, invasive arterial pressure, central venous pressure (with right internal jugular catheterization accompanied by ultrasound) and PiCCO monitorization (with right femoral artery catheterization using the existing central venous catheter and PiCCO catheter with special thermistor) were performed. Propofol and fentanyl was used for induction of anesthesia and cis-atracurium was used for muscle relaxation. After endotracheal intubation, desfluran (6-8%) was given in 40-50% oxygen-air mixture and intravenous infusion of remifentanyl [0.05-0,25 μg kg^{-1} minimum $(min)^{-1}$] was used for maintenance of anesthesia. Muscle relaxation was maintained with cis-atracurium infusion. Mechanical ventilation parameters were adjusted to keep $PaCO_2$ values between 30-35 mmHg, tidal volume 6-8 mL kg^{-1}, respiration rate 10-14/min and peep 4-6 mmHg. Restrictive fluid management was performed in patients. The replacement of albumin was determined according to the level of albumin of the patients. Erythrocyte suspension (ES) replacement was performed to keep hematocrit values at 26-28%. In our study, thromboelastography was not used and fresh frozen plasma (FFP), cryopresipitat or platelet replacement (complete blood count and prothrombin time, activated partial thromboplastin time, international normalized ratio and fibrinogen levels measured in standard dissection phase and anhepatic and neohepatic phases). Inotrop/vasopressor agents were initiated due to the data of invasive hemodynamic monitorization (blood pressure, heart rate, cardiac flow, SVRI) and the presence of hemodynamic instability. The patients were heated with lower heating blankets and blown heating systems. In addition, hypotermia was prevented by using fluid heating systems for all intravenous fluids and blood products. In order to prevent rejection, all patients were administered 10 mg kg^{-1} methyl prednisolone intravenously prior to perfusion during anhepatic period. Blood glucose levels of patients were kept between 80-160 mg/dL. Acıbadem Mehmet Ali Aydınlar University ATADEK-2017/16.

Surgical Technique

The patients were operated with bilateral subcostal incision or inverted L incision. Greft implantation was started with the main hepatic vein (left/right hepatic vein) and vena cava anastomoses were performed under partial clamp using piggy-back method. Then, if available, other vein anastomoses (segment 5-8 or inferior vein) were made by parachute technique on openings on vena cava. Portal vein anastomosis was performed end-to-end, if possible and anhepatic phase was completed and graft perfusion stage was initiated. Following perfusion, the hepatic artery anastomosis was made preferably anatomical and in case of a problem in recipient artery, extra-anatomic arterial anastomosis was made. Gallbladder anastomosis was performed by end-to-end/hepatico-jejunostomy method. In none of the patients portacaval shunt was used. After anastomoses were ultrasonographically shown as open and flow was shown as good by the radiologist, bleeding control was done and surgery was completed.

Intensive Care and Extubation

The patients were sent to intensive care unit as intubated at the end of the operation and they were followed up with electrocardiography, heart rate, invasive blood pressure, central venous pressure, $EtCO_2$ and temperature monitorizations. Analgesia and sedation management were performed for each patient in accordance with the patient's own intensive care process. When intensive care patients met the standard criteria (Table 1), they were extubated with T-tube method, one of spontaneous breathing methods (Table 2) by the intensive care team (6). Mechanical ventilation was accepted as prolonged mechanical ventilation when the duration of the ventilation exceeded 24 hours.

Data Collection

Anesthesia preoperative evaluation forms, intraoperative monitoring forms, intensive care monitoring forms, consultation notes, and epicrisis were examined and the following data were recorded.

Preoperative Data: Demographic data, comorbid diseases, smoking habit, etiology of end-stage liver disease, model for end-stage liver disease (MELD) scores, presence and degree of preoperative encephalopathy, presence of hepatopulmonary and hepatorenal syndrome.

Intraoperative Data: Intraoperative fluid amounts, blood and blood product transfusion amounts, hemodynamic data (heart rate, invasive arterial pressures, central venous pressures) and PiCCO data (CI, SVI, SVV, GEDI, SVRI ELWI, PVPI), inotrop-vasopressor needs, anhepatic phase and surgery durations.

Postoperative Data: Duration on mechanical ventilator in intensive care unit and stay in intensive care unit-hospital, need for retransplantation, mortality and morbidity.

Patients were divided into two groups based on the time periods for mechanical ventilation determined in the previous studies (7,8): Patients needing prolonged mechanical ventilation (>24 hours) and patients not needing prolonged mechanical ventilation (<24 hours) and the risk factors for prolonged duration of mechanical ventilation were determined.

Table 1. Standard extubation criteria

Objective criteria	• Adequate oxygenation (PO_2 >60 mmHg when FiO_2 <0.4 and PEEP: 5-10 cm H_2O, PO_2/FiO_2 >150-300 • Stable hemodynamic status (heart rate <140 beats/min, stable blood pressure without vasopressor or with minimal vasopressor support) • Fever <38 °C • The absence of respiratory acidosis • Adequate hemoglobin level (8-10 g/dL) • Stable mental status (GCS >13, no sedative infusion) • Stable metabolic status (electrolyte, blood glucose…) • Amelioration of acute phase of disease
Subjective clinical evaluation	• Clinician considering suitability for extubation • Adequate cough reflex

GCS: Glasgow coma scale, PEEP: Positive end-expiratory pressure

Statistical Analysis

Statistical analysis was performed using the SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, NY). Two-tailed tests were used in all statistical analyses. In comparisons between the two groups, independent samples t test was used in evaluation of parametric data and Pearson's chi-square and Fisher's exact test, if needed, were used in evaluation of non-parametric data. The relationship between etiological factors and prolonged mechanical ventilation was examined by Bivariate correlation test. Categorical data were expressed as mean ± standard deviation, non-categorical data were expressed as number (n) and percent (%). A p value less than 0.05 was considered statistically significant.

Results

The data and files of 125 patients over 18 years of age who underwent liver transplantation from living donor were retrospectively analyzed. All data of 10 patients could be reached and 115 patients were included in the study. In 10 (8.69%) of 115 patients, prolonged mechanical ventilation need was observed. Demographic data and ASA and MELD scores of the patients were summarized in Table 3. ASA and MELD scores were significantly higher in the prolonged mechanical ventilation group than in the other group. There was no difference between the groups in terms of the presence of comorbid disease, hepatopulmonary syndrome, hepatorenal syndrome, portal hypertension and hepatic encephalopathy and etiological factors.

When intraoperative variables were examined, there was no significant difference between the groups in terms of the amount of crystalloid and albumin given with restrictive liquid approach. Although ES, FFP, cell saver blood and platelet amounts were higher in the prolonged mechanical ventilation group, statistical significance was not found (Figure 1). The amount of cryoprecipitate used (1.67±3.39 U) was significantly higher in the prolonged mechanical ventilation group than in the other

Table 2. Weaning methods

Method	Advantage	Disadvantage
T-tube method	- Testing patient's spontaneous breathing ability - Allow working/resting periods - Faster weaning than SIMV	- Sudden loading of respiratory work to patient - Endotracheal tube resistance - Disabled alarm systems - Careful observation requirement
Pressure support ventilation (PSV)	- Synchronization - Increased patient comfort - Slow loading of respiratory work on the patient - Faster weaning than SIMV - Prevention of muscle fatigue	- Observation of major changes in minute ventilation
Synchronized intermittent mandatory ventilation (SIMV)	- Guaranteed minimum minute ventilation - Use of alarm systems - Applicability with PSV/CPAP	- Desynchronization - Long weaning process - Increased muscle fatigue
CPAP	- Allowing tidal volume monitoring	- Sudden loading of respiratory work to patient

SIMV: Synchronized intermittent mandatory ventilation, PSV: Pressure support ventilation, CPAP: Continious positive airway pressure

group (0.21±1.27 U) (p=0.007). The duration of the surgery was 495±76 min, the duration of the anhepatic phase was 95±41 min in the prolonged mechanical ventilation group and the duration of the surgery was 529±125 min, the duration of the anhepatic phase was 90±34 min in the other group. Differences in terms of duration of the surgery and the anhepatic phase between groups were not statistically significant.

The results of PiCCO measurements in dissection phase, anhepatic phase and neohepatic phase were summarized in Table 4.

In our study, 10 of 115 patients died and one had a need for retransplantation. There was no difference between the groups in terms of mortality and hospital stay, whereas the duration of stay in intensive care unit was 8.10±9.0 days in the prolonged mechanical ventilation group and 3.13±3.51 days in the other group (p=0.001).

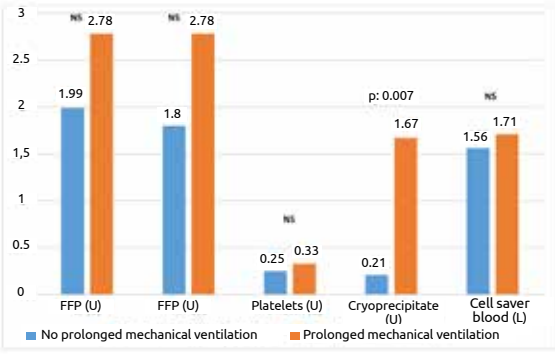

Figure 1. Blood product utilization rates in patients undergoing liver transplantation

ES: Erythrocyte suspension, FFP: Fresh frozen plasma, NS: Not significant

Table 3. Demographic data and risk scores

	Prolonged mechanical ventilation No (n= 105)	Yes (n= 10)	p value
Age (year)	49.61±12.64	47.10±16.03	0.558
Weight (kg)	73.99±15.75	66.35±23.20	0.164
Height (cm)	168.07±12.48	165.30±13.87	0.507
BMI (kg/m²)	25.75±4.77	23.56±6.36	0.179
ASA (I-V)	3.18±0.386	3.60±0.516	0.002
MELD	18.71±4.24	22.55±5.89	0.013

ASA: American Society of Anesthesiologists, MELD: Model for end-stage liver disease, BMI: Body mass index

Table 4. Intraoperative hemodynamic monitorization data in patients undergoing liver transplantation

	Prolonged MV	Dissection phase	p	Anhepatic phase	p	Neohepatic phase	p
CI	Yes	4.03±0.8	0.443	3.31±0.95	0.144	3.99±0.85	**0.018**
	No	4.28±1.02		3.82±1.04		4.75±0.96	
SVI	Yes	46.00±11.12	**0.027**	32.70±11.05	**0.008**	41.60±11.80	**0.000**
	No	53.89±10.56		43.76±12.37		55.01±11.10	
GEDI	Yes	592.70±134.64	**0.028**	483.40±143.89	**0.006**	510.50±139.15	**0.010**
	No	681.94±119.98		588.05±108.94		621.66±127.40	
SVV	Yes	7.10±4.41	0.556	12.80±6.65	0.073	11.10±5.53	**0.004**
	No	6.34±3.83		9.27±5.82		6.66±4.45	
GEF	Yes	29.63±10.34	0.057	29.70±5.21	0.389	32.40±6.15	**0.022**
	No	33.47±5.50		31.63±6.87		37.08±6.07	
SVRI	Yes	1506±468	0.631	1925±498	0.732	1337±328	0.357
	No	1433±452		1848±691		1230±350	
ELWI	Yes	7.50±1.65	**0.003**	8.25±1.72	**0.016**	7.40±1.43	**0.008**
	No	9.25±1.76		12.00±1.51		8.88±1.68	
PVPI	Yes	1.86±0.38	0.424	2.01±0.41	0.802	2.09±0.50	0.723
	No	1.96±0.37		2.05±0.49		2.04±0.42	
CVP	Yes	12.50±4.28	0.633	10.20±4.59	0.480	11.6±5.76	0.399
	No	13.10±3.71		9.30±3.76		12.65±3.53	

CI: Cardiac index, ELWI: Extravascular lung water index, GEDI: Global end-diastolic index, GEF: Global ejection fraction, PVPI: Pulmonary vascular permeability index, SVI: Stroke volume index, SVRI: Systemic vascular resistance index, SVV: Stroke volume variation, CVP: Entral venous pressure MV: Mechanical ventilation

Discussion

In patients undergoing major surgery, it may be necessary to maintain mechanical ventilation support in intensive care unit. The duration of prolonged mechanical ventilation in these patients is accepted as 48 hours in some clinical studies (9,10) and as 24 hours in some studies (7,8). In our study, we accepted mechanical ventilation for more than 24 hours as "prolonged mechanical ventilation need". Prolonged mechanical ventilation after major surgery is found to be associated with increased morbidity and mortality (11,12). The need for prolonged mechanical ventilation in patients undergoing liver transplantation, although traditionally considered as a postoperative pulmonary complication, may occur as a result of graft dysfunction, impairment of consciousness, preoperative sedentary condition, hemodynamic instability, bleeding diathesis, residual anesthetic effect, heart failure and prolonged surgical process. For this reason, general practice in patients undergoing liver transplantation during the postoperative period is to monitor the patient in the mechanical ventilator for a while.

Weaning is the gradual reduction of mechanical ventilator support until the patient has spontaneous and sufficient breathing. In this process, the physician should gradually increase the patient's effort, reduce mechanical ventilator support, and initiate the weaning process at the appropriate time. Weaning types are summarized in Table 2. Studies show that early and inappropriate weaning tryings are associated with increased cardiac ischemia risk and prolonged mechanical ventilation durations. When appropriate clinical and laboratory findings were obtained and when the weaning indexes were suitable for extubation in our intensive care unit, patients were extubated frequently using T-tube method.

In our study, we found that postoperative mechanical ventilation support was prolonged (>24 hours) in 8.69% of adult patients undergoing liver transplantation. The preoperative ASA and MELD scores and ES, FFP, cell saver blood, platelet and cryoprespit amounts used in the operation were higher in the prolonged mechanical ventilation group than the other group.

In patients undergoing liver transplantation, it may be necessary to give blood and blood products in serious amounts to the patients for various reasons during the intraoperative and postoperative periods. Therefore, high bleeding rate and high amount of transfusion of blood products during perioperative period are among the most important factors responsible for prolongation of mechanical ventilation support and increased mortality and morbidity after liver transplantation (13). In a recent study by Prasad et al. (14) the volume of blood products used in patients undergoing orthotopic liver transplantation was found to be a significant predictor of postoperative poor outcome. In our study, although the amount of transfusion of erythrocyte suspension and FFP was very low compared with the other studies (15,16), it was found to be higher in the prolonged mechanical ventilation group than the other group without reaching statistical significance. On the other hand, the use of cryopresipitat was found to be statistically significantly higher in the prolonged mechanical ventilation group. Therefore, we believe that high blood transfusion rates should be a stimulant for prolonged mechanical ventilation, so that blood conservation techniques and intraoperative autologous transfusions will affect patient outcomes.

Pulmonary edema, another risk factor for prolonged mechanical ventilation after liver transplantation, may be hydrostatic or non-hydrostatic. Alveolocapillary membrane integrity is impaired and this impairment may be aggravated by ischemia-reperfusion damage during transplantation in patients with ESLD (17,18). The clinical outcome is the increase of protein-rich extravascular fluid in the lung. Measurement of parameters showing the integrity of this liquid and alveolocapillary membrane may provide important information to clinician. In the literature, there are studies showing that ELWI and PVPI values obtained by PiCCO monitoring had prognostic value in terms of results in critical patients, and that PVPI values could be used effectively to differentiate hydrostatic pulmonary edema from non-hydrostatic pulmonary edema (19-21). In those study, high ELWI levels were associated with decreased survival, and PVPI was defined as a good indicator for results in patients in intensive care units. However, studies on the use of these parameters during the perioperative period are limited. Garutti and colleagues showed that ELWI values ≥12 mL/kg and PVPI values ≥2.3 obtained by PiCCO measurements made at the end of surgery in patients with liver transplantation, were associated with longer mechanical ventilation and longer duration of hospital stay (22). In our study, we found that ELWI values were lower in all three phases of transplantation in the prolonged mechanical ventilation group than the other group and that there was no significant difference between the two groups in terms of PVPI values. In support of these results, GEDI ve SVI values obtained in all three phases of surgery were found to be lower in the prolonged mechanical ventilation group. Contrary to the results of the studies in the literature which showed that high ELWI, PVPI and GEDI levels were risk factors for prolonged mechanical ventilation; low values found in our study seems to be a stimulating factor. Prolonged mechanical ventilation may be due to having lower intravascular volumes of these patients or it may be due to having greater fluid and blood transfusion need during surgery in these patients, as well as it may be due to organ damage caused by hypoperfusion due to inadequate fluid resuscitation.

Another risk factor for prolonged mechanical ventilation is preoperative poor condition. There are many studies in the literature that show that high ASA and MELD scores are associated with poor outcomes (14,15,23). In our study, ASA and MELD scores were significantly higher in the prolonged mechanical ventilation group, but the effect of age, gender and comorbid disease on mechanical ventilation duration was not shown.

There were a number of limitations in our study. One of these was the retrospective design of our study. In addition to this, a low number of patients who needed prolonged mechanical ventilation might be a parameter that seemed to affect the statistical value of the results. In addition, the results of arterial wave analysis and

PiCCO measurements might have been unexpectedly affected by vascular impedance and compliance, which have been impaired in ESLD. It is well known that the current-dependent invasive hemodynamic monitorization methods have no proven effects on the results, mortality and morbidity.

Study Limitations

One limitation of our study was the retrospective design of the study. In addition, the dose and duration of the analgesic and sedative agents, which directly affect the duration of ventilation support, were not known. However, patients undergoing liver transplantation in our center were monitored by the same team using standard protocols in intensive care unit.

Conclusion

We observed that preoperatively high ASA and MELD scores in patients undergoing liver transplantation, and the use of blood and blood products in intraoperative period and hypovolemia observed in PiCCO measurements extended the duration of stay in mechanical ventilation and in intensive care unit during the postoperative period. Since prolonged duration of mechanical ventilation and stay in the intensive care unit are known to be directly correlated with morbidity and mortality; the determination of factors that constitute a risk to the need for prolonged mechanical ventilation and the determination of appropriate treatment strategies by taking the necessary measures will reduce the duration of hospitalization in the intensive care unit and mortality and morbidity rates. We believe that there is a need to work more for it.

Ethics

Ethics Committee Approval: Acıbadem Mehmet Ali Aydınlar University ATADEK-2017/16.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.G.C., Design: M.G.C., A.Ö., Data Collection or Processing: M.G.C., A.Ö., Analysis or Interpretation: M.G.C., A.Ö., Literature Search: M.G.C., Writing: M.G.C., A.Ö.

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

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References

1. Siniscalchi A, Aurini L, Spedicato S, Bernardi E, Zanoni A, Dante A, et al. Hyperdynamic circulation in cirrhosis: predictive factors and outcome following liver transplantation. Minerva Anestesiol 2013;79:15-23.
2. De Wolf AM. Pulmonary artery catheter: rest in peace? Not just quite yet. Liver Transpl 2008;14:917-8.
3. Zeyneloglu P, Pirat A, Guner M, Torgay A, Karakayali H, Arslan G. Predictors of immediate tracheal extubation in the operating room after liver transplantation. Transplant Proc 2007;39:1187-9.
4. Mandell MS, Stoner TJ, Barnett R, Shaked A, Bellamy M, Biancofiore G, et al. A multicenter evaluation of safety of early extubation in liver transplant recipients. Liver Transpl 2007;13:1557-63.
5. Lee JM, Chang HW, Park CS, Park HJ, Kim JE, Choi JH. Evaluation of mechanical ventilation and its influencing factors between the living related liver transplantation and cadaveric whole liver transplantation. Korean J Anesthesiol 2005;49:816-21.
6. MacIntyre NR, Cook DJ, Ely EW Jr, Epstein SK, Fink JB, Heffner JE, et al. Evidence-based guidelines for weaning and discontinuing ventilatory support: a collective task force facilitated by the American College of Chest Physicians; the American Association for Respiratory Care; and the American College of Critical Care Medicine. Chest 2001;120:375-95.
7. Glanemann M, Langrehr J, Kaisers U, Schenk R, Müller A, Stange B, et al. Postoperative tracheal extubation after orthotopic liver transplantation. Acta Anaesthesiol Scand 2001;45:333-9.
8. Choi JH, Kim TH, Lee JM. Evaluation of usefulness of perioperative risk factors which affect early or delayed extubation after liver transplantation. Korean J Anesthesiol 2003;44:847-52.
9. Mandell MS, Campsen J, Zimmerman M, Biancofiore G, Tsou MY. The clinical value of early extubation. Curr Opin Organ Transplant 2009;14:297-302.
10. Neelakanta G, Sopher M, Chan S, Pregler J, Steadman R, Braunfeld M, et al. Early tracheal extubation after liver transplantation. J Cardiothorac Vasc Anesth 1997;11:165-7.
11. O'Meara ME, Whiteley SM, Sellors JM, Luntley JM, Davison S, McClean P, et al. Immediate extubation of children following liver transplantation is safe and may be beneficial. Transplantation 2005;80:959-63.
12. Rajakaruna C, Rogers CA, Angelini GD, Ascione R. Risk factors for and economic implications of prolonged ventilation after cardiac surgery. J Thorac Cardiovasc Surg 2005;130:1270-7.
13. Schrem H, Klußmann A, Focken M, Emmanouilidis N, Oldhafer F, Klempnauer J, et al. Post-operative hemorrhage after liver transplantation: risk factors and long-term outcome. Ann Transplant 2016;21:46-55.
14. Prasad V, Guerrisi M, Dauri M, Coniglione F, Tisone G, De Carolis E, et al. Prediction of postoperative outcomes using intraoperative hemodynamic monitoring data. Sci Rep 2017;7:16376.
15. Lee S, Jung HS, Choi JH, Lee J, Hong SH, Lee SH, et al. Perioperative risk factors for prolonged mechanical ventilation after liver transplantation due to acute liver failure. Korean J Anesthesiol 2013;65:228-36.
16. Yıldız İ, Sabuncuoğlu MZ, Koca YS, Solmaz Alkaya F, Şenol A. Yeni kurulan organ nakli merkezimizde yapılan karaciğer nakli sonuçlarımız. Sdü Sağlık Bilimleri Enstitüsü Dergisi 2017;8:18-20.

17. Schraufnagel DE, Malik R, Goel V, Ohara N, Chang SW. Lung capillary changes in hepatic cirrhosis in rats. Am J Physiol 1997;272:139-47.

18. Chang SW, Ohara N. Increased pulmonary vascular permeability in rats with biliary cirrhosis: role of thromboxane A2. Am J Physiol 1993;264:245-52.

19. Brown LM, Liu KD, Matthay MA. Measurement of extravascular lung water using the single indicator method in patients: research and potential clinical value. Am J Physiol Lung Cell Mol Physiol 2009;297:547-58.

20. Kushimoto S, Taira Y, Kitazawa Y, Okuchi K, Sakamoto T, Ishikura H, et al. The clinical usefulness of extravascular lung water and pulmonary vascular permeability index to diagnose and characterize pulmonary edema: a prospective multicenter study on the quantitative differential diagnostic definition for acute lung injury/acute respiratory distress syndrome. Crit Care 2012;16:232.

21. Jozwiak M, Silva S, Persichini R, Anguel N, Osman D, Richard C, et al. Extravascular lung water is an independent prognostic factor in patients with acute respiratory distress syndrome. Crit Care Med 2013;41:472-80.

22. Garutti I, Sanz J, Olmedilla L, Tranche I, Vilchez A, Fernandez-Quero L, et al. Extravascular lung water and pulmonary vascular permeability index measured at the end of surgery are independent predictors of prolonged mechanical ventilation in patients undergoing liver transplantation. Anesth Analg 2015;121:736-45.

23. Nafiu OO, Carello K, Lal A, Magee J, Picton P. Factors associated with postoperative prolonged mechanical ventilation in pediatric liver transplant recipients. Anesthesiol Res Pract 2017;2017:3728289.

Original Article

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The Inhibitory Effect of Ileal Mucosal Media Originated from FVB/N mice strain on *Escherichia coli* LF82 Invasion

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ABSTRACT

Objective: The aim of this study was to investigate the effect of healthy mucosa on adhesive and invasive properties of AIEC reference strain *Escherichia coli (E. coli)* LF82. For this purpose, we had designed special medias that contained cell culture medium and mucosal content obtained from different regions (colon and ileum) of the digestive tract.

Methods: We tested the infecting ability of AIEC reference strain *E. coli LF82* on I-407 cells in the presence of mucosal media (Muc-M) under *in vitro* conditions. Muc-M composed of certain rates of cell culture medium or M63 minimal medium and mucosal contents obtained from different part of intestine were designed for cell-infection experiments and biofilm-formation assays.

Results: The result showed that the mucosal media decreased the infection percentage of *E. coli* LF82 strain when compared with control group. It was seen that the mucosal media originating from ileum almost completely inhibited the invasion of LF82 strain. On the other hand, it was observed that the mucosal media prepared from colon) reduced the bacterial invasion only in half the rate when compared with control.

Conclusion: The findings showed that these medias obtained from different regions of the intestinal tract affected LF82 invasion at different rates. Therefore, this study provided crucial information that could contribute to the future studies on the localization of bacteria

Keywords: *Escherichia coli* LF82, Crohn's disease, biofilm, pathobiont, adhesion, invasion

Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease that can affect various parts of the intestinal tract and complex genetic and environmental factors are involved in its development. On the other hand, the intestinal flora plays an important role in the development of this disease because there are some strong findings about the role of the intestinal flora in the development of Crohn's disease. One of these is the detection of the significant inflammatory process in response to the contact of luminal content with terminal ileum, shown after surgical operations in Crohn's patients. However, when the fecal flow is directed to another side, the signs of healing are observed in these regions (1). And the others are the positive results of antibiotic treatment in some Crohn's patients, the reduction of ulceration symptoms following antibiotic treatment in multiple animal experiments and no findings about colitis in germ-free animals (2).

On the other hand, scientific studies performed about the relationship between intestinal flora and CD have shown the presence of a significant increase in the amount of *Escherichia coli (E. coli)* in individuals with the ileal type of CD when compared to normal individuals (3-5). After some detailed studies, a new *E. coli* member, adherent-invasive (AIEC), was isolated from the inflamed tissue of an individual with ileal type of CD (6). *In vitro* studies have shown that this new strain can adhere to and invade intestinal epithelial cells. Similarly, it has been determined by in vitro methods that this strain can live and multiply in

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macrophages and cause a high amount of tumour necrosis factor-alpha (secretion. These bacteria was also shown to cause high-level expression of carcino-embryonic antigen-related cell adhesion molecule-6 molecules, a kind of adhesion receptor in patients with CD (7).

Despite all the research done with AIEC, it is still unclear how these bacteria play a role in the development of Crohn's disease. Furthermore, it is not possible to determine the location of these bacteria in the intestinal flora because these bacterial specific molecules have not yet been identified. The intestinal mucus covering the intestinal epithelium along the digestive tract serves as a barrier to pathogenic microorganisms reaching the epithelial cells as well as hosting many commensal bacteria (8).

The basic structure of the mucus is composed of mucins which are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) produced by epithelial tissues in most animals. Typically, these mucins are glycoproteins containing 80% carbohydrate and consisting of proline, serine and threonine amino acid repeats (referred to as peroxisomal targeting signal sequence) (8,9). Although there are several types of mucin glycoproteins that form the mucous membrane throughout the digestive tract, goblet cells secrete MUC-2 mucin in the colon and small intestine of the intestinal tract. However, the same mucin exhibits different behaviors in the small intestine and the colon (10-12). Studies suggest that this difference originates from the differences in glycan epitopes in MUC-2. It is emphasized that these oligosaccharide units present in the mucus may also play an important role in the distribution of the intestinal flora in the digestive tract (13). Therefore, the mucin glycoprotein in different compartments of the digestive tract may have a key role in studies on the localization of AIEC bacteria, or on the detection of its specific molecules.

The aim of current study was to investigate the effect of healthy mucosa on adhesive and invasive properties of AIEC. For this purpose, we designed special medias that contained cell culture medium and mucosal content obtained from different regions (colon and ileum) of the digestive tract.

Methods

Bacterial Strain

The AIEC reference strain, *E. coli* LF82 isolated from an ileal lesion of a patient with CD was kindly provided by Dr. Nicolas Barnich and Dr. Elisabeth Billard (Universite´ d'Auvergne, Clermont-Ferrand, France). Bacteria were grown routinely in the Luria-Bertani (LB) broth overnight at 37 °C and without shaking.

Cell Line and Cell Culture Procedure

The intestine-407 (I-407) cell line (The Global Bioresource Center, chemokine (C-C motif) ligand 6, Manassas, VA, USA) was also kindly provided by Dr. Nicolas Barnich and Dr. Elisabeth Billard (Universite ´ d'Auvergne). The cells were maintained in an atmosphere containing 5% CO_2 at 37 °C in Eagle minimum essential medium (MEM) (MEM; Sigma-Aldrich, MO, USA) supplemented with 10% (v/v) heat- inactivated fetal bovine serum [Fetal bovine serum (FBS); Life Technologies, CA, USA], 1% nonessential amino acids (Life Technologies), 1% L-glutamine (Life Technologies), 100,000 units/L penicillin, 100 mg/L streptomycin, 25 mg/L amphotericin B and 1% MEM vitamins solution X-100 (Life Technologies).

Mice Strain

FVB/N female mice were housed under specific pathogen-free conditions in the animal care facility of Universite ´ d'Auvergne (Clermont-Ferrand, France). Animal protocols were performed according to the local ethical rules during ERASMUS+ Traineeship Program between 23th and June 19th 2015 in Universite ´ d'Auvergne (Clermont-Ferrand, France).

Isolation of Ileum & Colon and Preparation of Mucosal Media (Muc-M)

Mucosal media (Muc-M) was designed as an artificial medium, which was isolated from the 8- to 10-week-old female healthy FVB/N mice intestine. Muc-MIR is originated from ileum regions and Muc-MCR is originated from colon regions of healthy mice intestines. The 8- to 10-week-old female FVB/N mice were euthanized by cervical dislocation. The mouse was opened by ventral midline incision under 70% ethanol anesthesia. Colon was isolated as the distance (~5 cm) from rectum to cecum. Then, the ileum was isolated from cecum to (~8 cm) superior region of cecum. The colon and ileum regions of intestine were separately collected in sterile Petri dishes containing physiological water. The colon was brought into the open using scissors and the inner surface of the colon scrapped by a glass slide. This procedure was repeated also for ileum. Scrapped contents obtained from three mice were collected in a sterile 2 mL eppendorf tube for each region.

Cell infection procedure and biofilm assays were performed in different times. For cell-infection experiments, the tubes were labelled then, 1 mL sterile MEM added into tubes. Samples were treated for 15 min at +4 °C at a maximum speed of the disruptor. After disruption, samples were centrifuged for 10 min at +4 °C in 10,600x g. Supernatant was collected and transferred into falcon tubes separately for colonic regions and ileal regions.

Sterilization was performed by filtration method in the laminar air-flow cabinet. Samples were passed through filter with 0.45μm to new sterile falcons, then the filtrate was passed through filter with 0.20-μm pore diameter to another new sterile falcons and finally, it was diluted with sterile MEM as half of their total volume. The mentioned procedures were similarly performed for biofilm-formation assays. The only difference between two methods is that M63 minimal medium supplemented with 8 g/L (0.8%) glucose was used instead of MEM for biofilm-formation assay. To test the sterility of these media, Muc-M were inoculated onto LB and Mac Conkey (Oxoid, Basingstoke, UK) agar plates, and afterward, it was seen no colony forming on both plates after overnight incubation at 37 °C.

By the way, the effects of Muc-M alone on I-407 cell monolayers were also tested for cell infection experiments with a similar experiment performed by Aygun et al (14). Briefly, I-407 cell monolayers in the cell culture medium including 20% of Muc-M was incubated for 3-4 h at 37 °C with 5% CO_2. After incubation period, it was seen that the cell culture medium including 20% of Muc-M had no negative or deleterious effects on cell monolayers. Finally, Muc-M was ready for cell infection and biofilm-formation assays.

Cell Infectivity and Adhesion&Invasion Experiments

The cell infectivity, adhesion and invasion experiments were performed according to Darfeuille-Michaud method (15). I-407 cells were seeded in 24-well tissue culture plates (Sigma-Aldrich) at a density of $4x10^5$ cells per well and incubated for 20 h. The cell monolayers were washed twice with phosphate-buffered saline (PBS) (pH 7.2). 200 µL of Muc-M was prepared with MEM, was added to monolayers and brought to 1 mL with 10% FBS-supplemented MEM for each regions. 10% FBS-supplemented MEM was only designed and used as control. Each monolayer was infected with a multiplicity of infection (MOI) of 10 bacteria per epithelial cell (MOI: 10). In 3 h incubation period, it was performed at 37 °C with 5% CO_2.

For adhesion assay, after 3 h incubation period at 37 °C with 5% CO_2, the monolayers were washed three-times with PBS and the epithelial cells were then lysed with 1% Triton X-100 (Sigma-Aldrich) in deionized water. The samples were diluted and plated onto LB agar plates to determine the number of colony forming unit (CFU), and the mean number of bacteria per cell was determined. Adhesion assays were performed in triplicate.

For invasion assay, after 3 h incubation period at 37 °C with 5% CO_2, the monolayers were washed three-times with PBS. One hundred µL/m fresh culture medium containing gentamicin (Sigma-Aldrich) was added to cell media to kill the extracellular bacteria and 1h incubation was performed. After incubation for an additional hour, monolayers were washed with PBS and 1% Triton X-100 in deionized water placed in the wells to lysis the eukaryotic cells for 5 min. The samples were diluted and plated onto LB agar plates to determine the number of CFU. Invasive ability of LF82 with I-407 cell lines were expressed as the percentage of intracellular bacteria compared with the initial inoculum, taken as 100%. Invasion assays were performed in triplicate.

Statistical Analysis

One-way ANOVA followed by Dunnet T3 test was used for the assessment of the numerical data. P values ≤ 0.05 were considered statistically significant. All assays were performed at least three-times in separate experiments. Microsoft Excel and SPSS version 24.0 (IBM, NY, USA) programs were used for statistical analysis.

Results

We evaluated the effects of sterile mucosal media, derived from different parts of healthy intestines of FBV/N mice strain, on LF82 adhesion and invasion to human intestinal epithelial cell line (I-407) by adding each Muc-M (20; Muc-MCR or Muc-MIR) to the cell culture medium before infection and during 3 h infection period for adhesion/invasion assays.

After incubation period, it was seen that the cell culture medium including 20% of Muc-M had no negative or deleterious effects on cell monolayers (Figure 1, Figure 1a, Figure 1b, Figure 1c and Figure 1d).

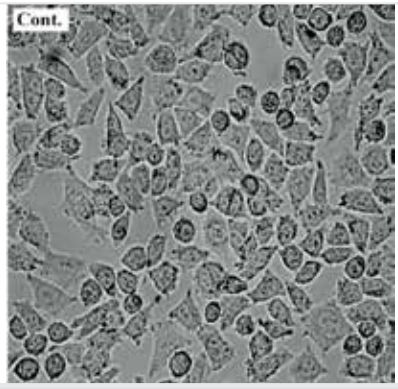

Figure 1. The apperance of cells in normal cell culture medium.

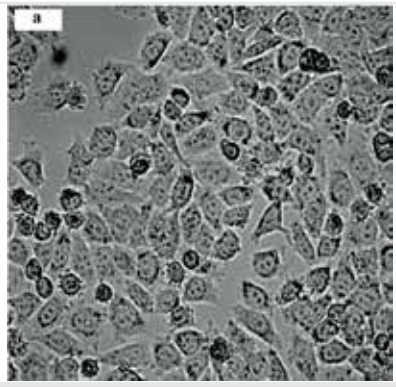

Figure 1a: Micrographs of crystal violet stained biofilms in the different mucosal medias originated from colon or ileum. The appearance of cells in mucosal media (Muc-MCR) after 3 hour incubation

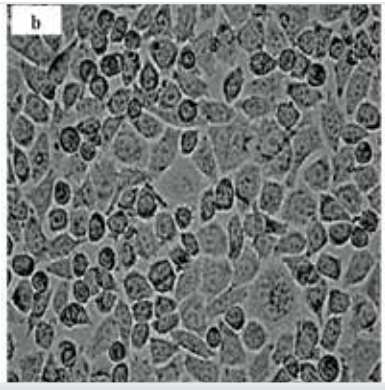

Figure 1b. The apperance of cells following 3- hour incubation, after replacement mucosal media (Muc-MCR) with fresh cell culture medium

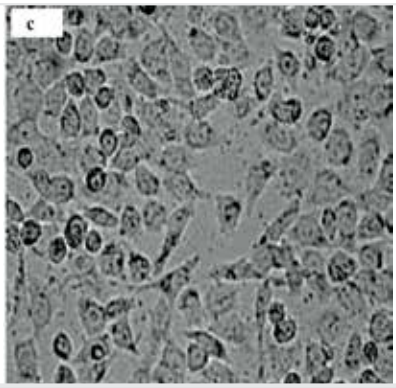

Figure 1c: Micrographs of crystal violet stained biofilms in the different mucosal medias originated from colon or ileum. Cell culture in mucosal media (Muc-MIR) after 3 hour incubation

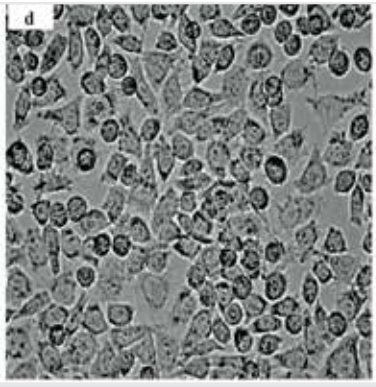

Figure 1d. The apperance of cells following 3- hour incubation, after replacement mucosal media (Muc-MIR) with fresh cell culture medium

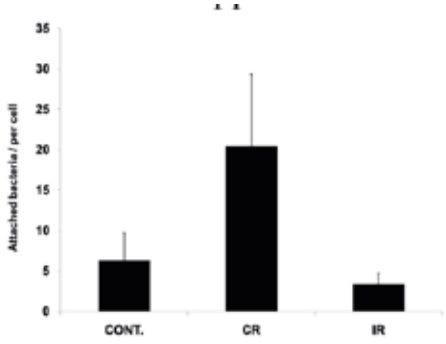

Figure 2. Adhesion abilities of adherent-invasive *Escherichia coli* LF82 in different medias including mucosal media originated from colon or ileum regions. Cell-associated bacteria were quantified after a 3-h infection period. Results are expressed as cell-associated (adherent+intracellular) bacteria per cell. ANOVA was used for multiple comparisons.
CONT.: Control, CR: Colon region, IR: Ileum region

Adhesion results showed that the Muc-MIR medium almost completely inhibited LF82 invasion ($p<0.001$), while the Muc-MCR medium reduced the LF82 invasion by 50% compared to the control, however this decline was not statistically significant ($p>0.001$) as seen in Figure 2. LF82 exhibited a higher adherent

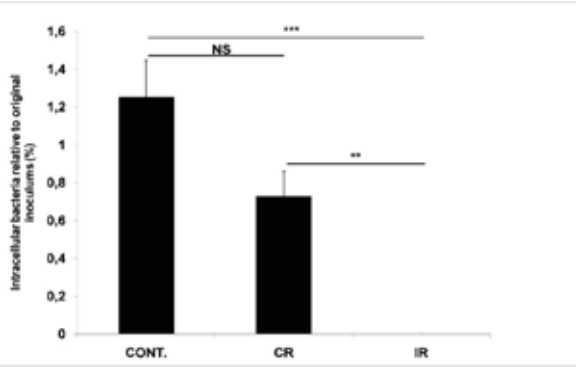

Figure 3. Invasion abilities of adherent-invasive *Escherichia coli* LF82 in different medias including mucosal media originated from colon or ileum regions. Cell-associated bacteria were quantified after a 3-h infection period. Results are expressed as cell-associated (adherent+intracellular) bacteria per cell. ANOVA was used for multiple comparisons.
CONT.: Control, CR: Colon region, IR: Ileum region

ability to I-407 cell monolayers in cell culture medium including Muc-MCR than those including Muc-MIR ($p<0.01$; as attached bacteria per cell, control: 7±3; Muc-MCR: 21±7; Muc-MIR: 4±1; Figure 2). On the other hand, after gentamicin treatment following extra 1 h incubation period, LF82 invasion significantly decreased ($p<0.05$) in the cell culture media including 20% of Muc-MCR or Muc-MIR.

The results of invasion assay showed that both of these medias considerably reduced bacterial invasion (Figure 3). It was quite remarkable that the Muc-MIR medium originating from the ileum inhibited more LF82 invasion than the Muc-MCR medium prepared from the colon region of intestine ($p<0.01$; intracellular bacteria relative to original inoculums (%), control: 1.3±0.2; Muc-MCR: 0.7±0.2; Muc-MIR: 0; Figure 3).

Discussion

Many studies examined the relationship between etiopathogenesis of CD and AIEC strains which are found in high amounts in the inflamed ileal tissues of patients with Crohn's disease. However, the current literature does not have enough evidence about the role of AIEC on Crohn's disease. The same studies showed that although the *E. coli* strains are relatively found in higher amounts in ileal tissues both in healthy and CD patients, the localization of AIEC in digestive tract has not yet been identified (16,17). Therefore, studies to find these bacterial specific molecules will help illuminate the role of AIEC strains in the progression of CD. In the current study, we designed special medias that contained mucosal content obtained from different regions (colon and ileum) of FBV/N healthy female mice digestive tract. Then, we tested the infecting ability of AIEC reference strain *E. coli* LF82 to I-407 cells in the presence of Muc-M originating from the colon (Muc-MCR) and ileum (Muc-MIR) regions, under *in vitro* conditions.

After adding Muc-MCR or Muc-MIR media to the cell culture medium at 20% percentage, we infected the cells with LF82 bacteria. There was no statistically significant difference when we

examined the results of the adhesions after the 3 hour infective period. However, the results of invasion assay showed that both of these media considerably reduced bacterial invasion. According to our results; the Muc-MIR medium almost completely inhibited LF82 invasion ($p<0.001$), while the Muc-MCR medium reduced the LF82 invasion by 50% compared to the control, however this decline was not statistically significant ($p>0.001$). The results we obtained from the current study strongly supported our previous study that performed with Balb/c mouse strain (14). The strong inhibition of bacterial invasion, in particular, may be due to glycosylated oligosaccharide units. Likewise, in a study supporting this result, the role of mannose oligosaccharide in the adhesion and invasion of LF82 bacteria was investigated by cell infection experiments using the I-407 cell line. The results showed that mannose strongly inhibited LF82 invasion and that inhibition largely occurred via type-1 pili/mannosyl interaction (18). In another study, commercially purchased mucin was added to the cell culture medium at a certain rate before it was infected with *E. coli* C25 strain. The results obtained after the infection period showed that mucin inhibited bacterial translocation strongly in both cell lines and the bacterial pili retained by the mucin-bound oligosaccharide units without reaching the cell (19). On the other hand, it was quite remarkable that the Muc-MIR medium originating from the ileum inhibited more LF82 invasion than the Muc-MCR medium prepared from the colon region of intestine. Similarly, goblet cells in both the ileum and the colon secrete the same mucin called MUC-2. Thus, these results support the view that the mucin in the colon and the small intestine may exhibit different properties although they have the same structure (10-12). In addition, it is suggested that specific receptor-ligand interactions with LF82 strain may occur, because medias originated from colon and ileum regions of intestine affect LF82 invasion in different ratios. Also, oligosaccharide units associated with mucin may play a decisive role in the distribution of gut flora in the gut system (13). If these interactions can be identified in further studies, specific molecules can be identified that facilitate the detection of LF82.

Conclusion

In conclusion, this study showed that AIEC LF82 strain has different patterns in terms of the adhesion and invasion capabilities in mucosal medium originated from ileum region of healthy FBV/N female mice strain. Therefore, our data have a potential importance to give new ideas about determining the localization of AIEC bacteria within the healthy intestinal mucosa as in our previous work (14).

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Ethics

Ethics Committee Approval: Animal protocols were performed according to the local ethical rules during ERASMUS+ Traineeship Program between 23th and June 19th 2015 in Universite ´ d'Auvergne (Clermont-Ferrand, France).

Informed Consent: *In vitro* study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: H.A., F.U., Design: H.A., M.K., Data Collection or Processing: H.A., M.K., Analysis or Interpretation: H.A., F.U., M.K., Literature Search: H.A., F.U., M.K., Writing: M.K.

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References

1. Rutgeerts P, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, et al. Effect offaecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 1991;338:771-4.
2. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577-94.
3. Schultsz C, Moussa M, van Ketel R, Tytgat GN, Dankert J. Frequency of pathogenic and enteroadherent *Escherichiacoli* in patients with inflammatory bowel disease and controls. J Clin Pathol 1997;50:573-9.
4. Baumgart M, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, et al. Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of *Clostridiales* in Crohn's disease involving the ileum. ISME J 2007;1:403-18.
5. Kotlowski R, Bernstein CN, Sepehri S, Krause DO. High prevalence of *Escherichia coli* belonging to the B2+D phylogenetic group in inflammatory bowel disease. Gut 2007;56:669-75.
6. Boudeau J, Glasser AL, Masseret E, Joly B, Darfeuille-Mıchaud A. Invasive Ability of an *Escherichia coli* Strain Isolated from the Ileal Mucosa of a Patient with Crohn's Disease. Infect Immun 1999;67:4499-509.
7. Carvalho FA, Barnich N, Sauvanet P, Darcha C, Gelot A, Darfeuille-Michaud A. Crohn's disease-associated *Escherichia coli* LF82 aggravates colitis in injured mouse colon via signaling by flagellin. Inflamm Bowel Dis 2008;14:1051-60.
8. Johansson ME, Ambort D, Pelaseyed T, Schütte A, Gustafsson JK, Ermund A, et al. Composition and functional role of the mucus layers in the intestine. Cell Mol Life Sci 2011;68:3635-41.
9. Lang T, Hansson GC, Samuelsson T. Gel-forming mucins appeared early in metazoan evolution. Proc Natl Acad Sci U S A 2007;104:16209-14.
10. Audie JP, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP. Expression of human mucin genes in respiratory, digestive, and reproductive tracts as certained by in situ hybridization. J Histochem Cytochem 1993;41:1479-85.
11. Weiss AA, Babyatsky MW, Ogata S, Chen A, Itzkowitz SH. Expression of MUC2 and MUC3 mRNA in human normal, malignant, and inflammatory intestinal tissues. J Histochem Cytochem 1996;44:1161-6.

12. Hansson GC, Johansson ME. The inner of the two Muc2 mucin dependent mucus layers in colon is devoid of bacteria. Gut Microbes 2010;1:51-4.

13. Larsson JM, Karlsson H, Sjovall H, Hansson GC. A complex, but uniform O-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MSn. Glycobiology 2009;19:756-66.

14. Aygun H, Karamese M, Ozic C, Uyar F. The effects of mucosal media on some pathogenic traits of Crohn's disaese-associated *Escherichia coli* LF82. Future Microbiol 2018;13:141-9.

15. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, et al. High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology 2004;127:412-21.

16. Seksik P, Rigottier-Gois L, Gramet G, Sutren M, Pochart P, Marteau P, et al. Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. Gut 2003;52:237-42.

17. Vasquez N, Mangin I, Lepage P, Seksik P, Duong JP, Blum S, et al. Patchy distribution of mucosal lesions in ileal Crohn's disease is not linked to differences in the dominant mucosa-associated bacteria: a study using fluorescence in situ hybridization and temporal temperature gradient gel electrophoresis. Inflamm Bowel Dis 2007;13:684-92.

18. Boudeau J, Barnich N, Darfeuılle-Mıchaud A. Type-1 pili-mediated adherence of *Escherichia coli* strain LF82 isolated from Crohn's disease is involved in bacterial invasion of intestinal epithelial cells. Mol Microbiol 2001;39:1272-84.

19. Gork AS, Usui N, Ceriati E, Drongowski RA, Epstein MD, Coran AG, et al. The effect of mucin on bacterial translocation in I-407 fetal and Caco-2 adult enterocyte cultured cell lines. Pediatr Surg Int 1999;15:155-9.

Original Article

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Clinical and Radiological Outcomes in Arthroscopic Repair of Shoulder Rotator Interval Lesions

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ABSTRACT

Objective: Rotator interval lesion (RIL) is a distinct rotator cuff (RC) injury patern consisting of subscapularis and supraspinatus tear with biceps problem. This pathology is an underdiagnosed RC entitiy and not studied in-depth. Aim of this study is to report the functional and radiological results of RIL surgeries performed.

Methods: Surgeries performed in a single center, in a 7-year-period were retrospectively reviewed. Sixteen cases (n=16) who underwent arthroscopic RC repair including subscapularis with biceps tenodesis or tenotomy were called for an up-to-date assessment. Fourteen shoulders of 13 patients (3 females, 10 males) were included. Constant, disabilities of the arm shoulder and hand (DASH), standardized shoulder assessment form (ASES) scores, and RC thickness measurements with magnetic resonance imaging (MRI) were recorded. Preoperative and postoperative results were compared.

Results: Average age of the patients were 60.6 (47-74), and follow-up period was 3.2±1.9 years. Average preoperative Constant, DASH and ASES scores were 44.43±15.4, 22.11±17.21 and 51.37±27.6, respectively. Postoperative values improved to 90.45±6.44, 6±13.68 and 95.82±7.82, respectively ($p<0.05$). Average subscapularis and supraspinatus thicknesses measured with MRI were 3.85±0.87 and 4.60±0.65 mm respectively. MRI revealed subscapularis tendinitis in 1 patient. Re-tear was not observed in any patients.

Conclusion: Arthroscopic subscapularis and supraspinatus repair with biceps tenodesis or tenotomy is an effective treatment method in RIL. No retear was observed with MRI. Clinical results are similar with other RC pathologies.

Keywords: Rotator cuff, rotator interval, subscapularis, magnetic resonance imaging

Introduction

Rotator interval (RI) lesions (anterior-superior tears) represent a special subset of rotator cuff (RC) tears. In these lesions, pathology is localised to anterior structures, and they always present with supraspinatus tear, biceps tendon pathology and subcapularis tear (1). They were classified as type A tears by Collins et al. (2). RI lesions are thought to account for 4% of all RC tears.

A small number of articles have been published in the literature, due to the rare occurrence of them compared with posterior-superior tears. It is suggested that natural course and prognosis are worse than conventional supraspinatus and infraspinatus tears or isolated subcapularis tears (1). Good results are reported in arthroscopic treatment of subcapular tears (3), for this reason, it is thought that RI lesions with similar patho-anatomy will have good results with arthroscopic treatment (3,4). However, due to

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the small number of studies on the subject, the results are still considered to be unclear.

The hypothesis of this study is that the arthroscopic treatment of RI lesions is comparable to the arthroscopic treatment of other RC lesions reported in the literature and can be accepted as successful. For this purpose, functional clinical and magnetic resonance imaging (MRI) results in our patients who were diagnosed as having RI lesions and underwent arthroscopic repair were reported in this study.

Methods

Following the approval of the ethics committee, all RC tears operated by a single surgeon in a single center in 7-year period (2007-2014) were retrospectively screened. The inclusion criteria were: (1) Superior 1/3 complete tendon tear of subcapularis tendon and repair, (2) Tenodesis or tenotomy due to biceps tendon pathology, (3) Supraspinatus tear and repair, (4) One year and over follow-up period and (5) Arthroscopic surgery used as surgical technique. Owners of sixteen shoulders which met the inclusion criteria among 337 RC surgeries during the identified period were invited for functional evaluation and radiological examinations. Fourteen shoulder joints of 13 patients (10 males, 3 females) who agreed to participate in the study and completed the appropriate clinical and radiological examinations were included in the study.

All patients underwent the same surgical procedure. Following arthroscopic joint examination and biceps tenotomy with radiofrequency, subacromial decompression and acromioplasty were performed. Tendon footprints in large and small tubercles were prepared and tendon debridement was performed until healthy edge was obtained. For subscapularis repair, first suture, no: 2 high strength braided suture, is put from superior edge as a cement, and second suture is put from the torn body of the tendon in matrix form (Fiberwire, Arthrex, Florida, USA), and then, both of them are fixed on tendon footprints with the help of a nodeless anchor. Single medial anchor was used in supraspinatus and infraspinatus tears below 1.5 cm, double medial anchors were used in tears above 1.5 cm and double lateral anchors were used in total tears. As a result, all of them were repaired with double raw method which is equivalent of nodeless transosseus method. In all patients, biocomposite absorbable anchor was used (Biocomposite Swivelock, Arthrex, Florida, USA). After the operation, the patients were followed up with a padded arm hanger for 1 month and activities on table were allowed immediately. Active assistive exercises were initiated at the end of the first month and resistive exercises were initiated at the end of the second month.

The patients were examined by a single physical therapist with over 15 years of shoulder and elbow surgery experience. Examination of subscapularis was performed using lift-off, bear-hug and belly-press tests (5-7) and clinical evaluation was performed using Constant, disabilities of the arm shoulder and hand (DASH) and standardized shoulder assessment form (ASES) scores (8-10). Preoperative Constant, ASES and DASH scores were obtained from the files of the patients.

MRI was performed in supine position using standard shoulder protocols (3T System; Skyra; Siemens Medical Solutions, Erlangen, Germany) and with a dedicated shoulder coil. T1-weighted spin-echo images (TR/TE 420/11) were obtained in the sagittal oblique plan (parallel to glenohumeral joint) and proton density-T2 weighted images (TR/TE 2300-2800/45-46) were obtained in the axial, sagittal and coronal oblique plan (straight to glenohumeral joint). The cross-sectional range was set to 3 mm, inter-sectional range was set to 0.4 mm and the matrix dimensions were determined as 384x288. The field of study was between 14-18 cm. The images were interpreted by a single radiologist with over 20 years of musculoskeletal radiology experience. Tendon thicknesses of supraspinatus, infraspinatus and subscapularis muscles were recorded as means of mm. S.B. Koç University Faculty of Medicine Clinical Research Ethics Committee decision no: 2014.110.IRB1.001.

Statistical Analysis

Statistical analysis was performed using MedCalc 12.5 Statistical Software (Ostend, Belgium). In addition to descriptive statistical methods (mean and standard deviation), a dependent student's t-test was used to compare the clinical scores before and after surgery. P value below 0.05 was accepted as statistically significant.

Results

The mean age of 13 patients (10 males, 3 females) was 60.6 (47-74) years and the mean follow-up period was 3.2±1.9 years. Tenodesis was performed in 9 shoulders and tenotomy in 5 shoulders for the pathology of biceps tendon.

Preoperative constant, DASH and ASES scores were found 44.43±15.4, 22.11±17.21 and 51.37±27.6, respectively. These values were measured as 90.45±6.44, 6±13.68 and 95.82±7.82 in the last control after the operation. The increase in all scores was statistically significant ($p<0.05$). Subcapularis tests (lift-off, belly-press, and bear-hug) were positive in only one shoulder, while they were negative in the other shoulders (n=1/14).

Constant, DASH and ASES scores of the pathological shoulders of the patients are summarized in Table 1.

Tendon thicknesses in MRI performed in the last control are summarized in Table 2. No re-tear was detected in any patient. Tendinitis of supraspinatus and subcapularis tendons were observed in a patient.

Table 1. Clinical scores of the patients before and after operation

	Constant	DASH	ASES
Preop	44.43±15.39 25-62	22.11±17.21 0-50	51.37±27.60 10-86.6
Postop	90.45±6.44 79.46-98	5.99±13.68 0-49.13	95.82±7.82 73.3-100
p	<0.001*	0.001*	0.006*

DASH: Disabilities of the arm shoulder and hand, ASES: Standardized shoulder assessment form

Table 2. Tendon thickness measurements obtained by magnetic resonance imaging

	Subscapularis (mm)	Supraspinatus (mm)	Infraspinatus (mm)
Magnetic resonance			
Mean	3.85±0.87	4.60±0.65	3.90±1.14
Min-max	2.80-5.3	3.60-5.9	1.40-5.7

Min: Minimum , Max: Maximum

Discussion

RI refers to the region between subscapularis and supraspinatus muscles, and includes biceps tendon, coracohumeral ligament and superior glenohumeral ligament. This region also includes the suspension system where the biceps tendon leaves the glenohumeral joint (11). Because of involving multiple structures, pathologies of this region are with involvement of both subscapularis and supraspinastus tendons, with biceps tendon pathologies, and affects the stability of the glenohumeral joint in superior-inferior direction. For this reason, it is considered as a separate entity and it has clinical characteristics.

Subcapularis tears was previously classified according to the tear width by Lafosse et al. (12,13) And this classification is frequently used in clinical practice. Clinical results of subcapularis lesions with a spectrum ranging from partial superior 1/3 tears (Lafosse I) to complete layer tears (Lafosse V) in which centralization of the head deteriorates, also vary according to the type of tear. There is still no consensus on how much the definition of "RI tear" covers subscapularis muscle. In order to obtain a homogenous group, only tears of Lafose type II (superior 1/3 complete layer) were included in this study. Another publication that classifies the pathology of structures within RI belongs to Bennett (14). This classification is based on the relationship between subscapularis tear and coracohumeral and glenohumeral ligaments, the displacement of the biseps pulley and whether the subscapularis tear is intraarticular or complete layer. This classification was not used in our study, because patients were operated before the study and there was a lack of of adequate reliable records on coracohumeral and glenohumeral ligaments.

In this study, Constant-Murley, DASH and ASES, Turkish version of which were shown as valid and reliable before, were used as clinical evaluation methods (8-10, 15-19). Significant improvement was observed in all scores. Kim et al. (20) reported that ASES score was 90.8±6.5 in patients with RI lesions in the postoperative 2nd year. Lanz et al. (21) reported that postoperative Constant score was 81.2±14.6 in patients who were repaired for anterior superior tears. The results of our study are similar to those studies (ASES: 95.82±7.82, constant: 90.45±6.44) (ASES: 95.82±7.82, constant: 90.45±6.44). DASH score was 5.99±13.68 in our study which was also similar to those studies.

In this study, MRI was preferred as a radiological evaluation method. MRI is an accepted method for providing higher sensitivity compared to ultrasound, as well as not dependent on the individual and providing information about tendon quality beyond the tear. MRI is considered to be a reliable diagnostic examination in subscapularis tears (22). In our study, MRI in last control was used to see whether supraspinatus, infraspinatus and subscapularis tendons were intact. Radiological tendinopathy findings and tendon thicknesses were also recorded by using MRI. No patients with re-tear of supraspinatus or subchapularis tendons suggested that the surgical technique was effective. Only one patient had radiological tendinitis findings.

Small number of patients and no comparison made with other RC pathologies were the limitations of this study in which the clinical and radiological results of RI lesions were reported. RI lesions constitute a small portion of all RC tears which makes it difficult to report large series of cases. The inclusion of multiple clinical scores and the reported radiological results constituted the strengths of our study.

Conclusion

RI lesions constitute the least known group in all RC tears. Arthroscopic repair results show similar properties with other reported RC pathologies. Radiologically, the fact that there was no re-tearing suggests parallelism with clinical results. Comparative studies and studies on the natural course of RI lesions followed conservatively will enable a better understanding of this particular antithesis.

Ethics

Ethics Committee Approval: Koç University Faculty of Medicine Clinical Research Ethics Committee decision no: 2014.110.IRB1.001.

Informed Consent: It was not taken due to *in vitro* study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.D., S.A., İ.E., Design: M.D., İ.E., H.Ö., Data Collection or Processing: İ.E., H.Ö., Ş.M.E., A.U., Analysis or Interpretation: İ.E., N.C., M.D., Literature Search: İ.E., H.Ö., Writing: İ.E., H.Ö., M.D.

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References

1. Warner JJ, Higgins L, Parsons IM 4th, Dowdy P. Diagnosis and treatment of anterosuperior rotator cuff tears. J Shoulder Elbow Surg 2001;10:37-46.
2. Collin P, Matsumura N, Ladermann A, Denard PJ, Walch G. Relationship between massive chronic rotator cuff tear pattern and loss of active shoulder range of motion. J Shoulder Elbow Surg 2014;23:1195-202.
3. Nho SJ, Frank RM, Reiff SN, Verma NN, Romeo AA. Arthroscopic repair of anterosuperior rotator cuff tears combined with open biceps tenodesis. Arthroscopy 2010;26:1667-74.

4. Ide J, Tokiyoshi A, Hirose J, Mizuta H. Arthroscopic repair of traumatic combined rotator cuff tears involving the subscapularis tendon. J Bone Joint Surg Am 2007;89:2378-88.
5. Barth JR, Burkhart SS, De Beer JF. The bear-hug test: a new and sensitive test for diagnosing a subscapularis tear. Arthroscopy 2006;22:1076-84.
6. Gerber C, Hersche O, Farron A. Isolated rupture of the subscapularis tendon. J Bone Joint Surg Am 1996;78:1015-23.
7. Gerber C, Krushell RJ. Isolated rupture of the tendon of the subscapularis muscle. Clinical features in 16 cases. J Bone Joint Surg Br 1991;73:389-94.
8. Celik D. Turkish version of the modified Constant-Murley score and standardized test protocol: reliability and validity. Acta Orthop Traumatol Turc 2016;50:69-75.
9. Celik D, Atalar AC, Demirhan M, Dirican A. Translation, cultural adaptation, validity and reliability of the Turkish ASES questionnaire. Knee Surg Sports Traumatol Arthrosc 2013;21:2184-9.
10. Düger T, Yakut E, Öksüz Ç, Yörükan S, Bilgütay BS, Ayhan Ç. Reliability and validity of the Turkish version of the Disabilities of the Arm, Shoulder and Hand (DASH) Questionnaire. Turk J Physiother Rehabil 2006;17:99-107.
11. Jost B, Koch PP, Gerber C. Anatomy and functional aspects of the rotator interval. J Shoulder Elbow Surg 2000;9:336-41.
12. Lafosse L, Jost B, Reiland Y, Audebert S, Toussaint B, Gobezie R. Structural integrity and clinical outcomes after arthroscopic repair of isolated subscapularis tears. J Bone Joint Surg Am 2007;89:1184-93.
13. Lafosse L, Lanz U, Saintmard B, Campens C. Arthroscopic repair of subscapularis tear: Surgical technique and results. Orthop Traumatol Surg Res 2010;96(8 Suppl):99-108.
14. Bennett WF. Subscapularis, medial, and lateral head coracohumeral ligament insertion anatomy: arthroscopic appearance and incidence of "hidden" rotator interval lesions. Arthroscopy 2001;17:173-80.
15. Constant CR, Gerber C, Emery RJ, Sojbjerg JO, Gohlke F, Boileau P. A review of the Constant score: modifications and guidelines for its use. J Shoulder Elbow Surg 2008;17:355-61.
16. Constant CR, Murley AH. A clinical method of functional assessment of the shoulder. Clin Orthop Relat Res 1987;214:160-4.
17. Beaton DE, Wright JG, Katz JN; Upper Extremity Collaborative Group. Development of the QuickDASH: comparison of three item-reduction approaches. J Bone Joint Surg Am 2005;87:1038-46.
18. Hudak PL, Amadio PC, Bombardier C. Development of an upper extremity outcome measure: the DASH (disabilities of the arm, shoulder and hand) [corrected]. The Upper Extremity Collaborative Group (UECG). Am J Ind Med 1996;29:602-8.
19. Richards RR, An KN, Bigliani LU, Friedman RJ, Gartsman GM, Gristina AG, et al. A standardized method for the assessment of shoulder function. J Shoulder Elbow Surg 1994;3:347-52.
20. Kim SJ, Jung M, Lee JH, Park JH, Chun YM. Arthroscopic repair of a significant (>50%) partial-thickness subscapularis tear concomitant with a full-thickness supraspinatus tear: technical considerations for subscapularis repair (transtendon technique versus tear completion). J Shoulder Elbow Surg 2015;24:875-81.
21. Lanz U, Fullick R, Bongiorno V, Saintmard B, Campens C, Lafosse L. Arthroscopic repair of large subscapularis tendon tears: 2- to 4-year clinical and radiographic outcomes. Arthroscopy 2013;29:1471-8.
22. Adams CR, Brady PC, Koo SS, Narbona P, Arrigoni P, Karnes GJ, et al. A systematic approach for diagnosing subscapularis tendon tears with preoperative magnetic resonance imaging scans. Arthroscopy 2012;28:1592-600.

Original Article

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Preparation and Evaluation of Inflammation Targeted Nano-micellar Formulation of Celecoxib

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ABSTRACT

Objective: Celecoxib (CLX), brand named Celebrex, which belongs to non-steroidal anti-inflammatory drugs family, selectively inhibits the cytokine related cyclooxygenase-2 isoenzyme and thus, possesses less gastrointestinal side effects, have shown to cause stroke, myocard infarction and even death in some cases. In this study we aimed to target inflammation site using CLX uploaded nano-micelles (nano-CLX) made of poly (lactic-co-glycolic) acid (PLGA) to protect other tissues from its side effects.

Methods: CLX was physically entrapped in PLGA micelles using w/o/w emulsion method, resulted in obtaining mono-dispersed particles with 112 nm size. 50 mg PLGA was able to carry 50 mg CLX in 20 mL (2.5 mg/mL) with encapsulation efficiency of 85%. Rheumatoid arthritis model was achieved by injection of complete Freund's Adjuvant to the hint paw of Wistar rats. Infected groups received oral Celebrex, intravenous (i.v.) Celebrex and nano-CLX. Each group was compared with a healthy control group receiving the drug via the same routes. The obtained serums and the hint paw sizes were studied for 6 hours in 3 time periods.

Results: Prostaglandin E2 and tumor necrosis factor-α levels were found to be decreased for longer time period by application of nano-CLX compared to oral and i.v. Celebrex. Interleukin-1 (IL-1) and IL-6 levels showed a dramatic decrease at orally administered Celebrex groups, showing the accumulation of these pro-inflammatory factors at inflammation area.

Conclusion: Based on the hypothesis that the ratio of blood parameters is inversely proportional to accumulation at inflammation site, thus, our nano-formulation is targeted to the tissues in the systemic blood flow and have a better selective inhibition.

Keywords: Targeted inflammation therapy, Celecoxib, nano drug delivery system, PLGA, COX2

Introduction

Rheumatoid arthritis (RA) is a multisystem chronic inflammatory disease which especially affects the peripheral joints in a symmetric manner and often causes bone erosions, cartilage destruction and joint deformities. RA has a worldwide distribution, with a prevalence of approximately 1%. Major complaint is pain and it is aggravated with movement (1). Preferably, non-steroidal anti-inflammatory drugs (NSAIDs) are used, which tend to control the symptoms by blocking cyclooxygenase-2 (COX-

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2) and the production of the thromboxanes, prostacyclins and prostaglandins. Gastrointestinal (GI) intolerance and the cardiovascular complications are the common side-effects of the NSAIDs and COX-2 inhibitors (1).

Celecoxib (Celebrex®) (CLX), a selective COX-2 inhibitor, was approved by the FDA for the treatment of chronical poly-arthritis and osteoarthritis in 1998 (2). Certainly, selective COX-2 inhibitors decrease the risk of the GI side effects. Nevertheless, COX-2 is not only a pro-inflammatory inducible enzyme, it also has various physiological functions which means it is constitutively expressed to a high extent in the human body (3). The clarified paradigm of constitutive COX-1 and inducible COX-2 has several exceptions: COX-1 may be modulated during development, whereas COX-2 is constrictively expressed in the kidney, brain and reproductive tissues. Additionally, selective inhibitors of COX-2 decrease prostacyclin, an atheroprotective agent, however it does not decrease COX-1 derived thromboxane A_2, a vasoconstrictor and proaggregatory mediator, which can predispose patients to stroke and heart attack. Therefore, the use of CLX requires further improvement (4).

Nano-technology is a science area dedicated to the construction, design and utilization of functional structures on the nanometer scale (often 100 nm or even smaller). Biodegradable nanoparticles are regularly used to improve the therapeutic value of different water soluble/insoluble medicinal drugs and bioactive molecules by improving their retention time, solubility, bioavailability or by bringing the targeting property to the small molecules such as CLX. These nanoparticle-drug formulation lowers the expenses and risks of toxicity in patients (5). Enhanced Permeability and Retention (EPR) are the most known phenomena in targeting disease site using nano-drug delivery systems (NDDS). This elegant strategy uses the enhanced gap between the endothelial cells of veins at site of inflammation or cancer compared to that of healthy tissues (6). This enhanced gap reaches to around 200 nm, while it has been measured as 10-15 nm in healthy tissue. Entrapping the small molecules within NDDS with size less than 200 nm provides accumulation of drug molecules only at the inflammation site.

In order to transport the drug molecules to the cells and enzymes, a lot of biodegradable polymers are synthesized (7,8). Polyesters and co-polyesters of the alpha, beta and gamma-hydroxy acids are the most common synthetic biodegradable polymers in the last 20 years. Poly-lactic acid, poly-glicolic acid and Poly (lactic-co-glycolic) acid (PLGA), which are copolymer molecules, are the best defined polymers based on to their activities and the designs (9,10). PLGA is accepted as gold standard and is known for self-assembling in aqueous media entrapping the hydrophobic drug molecules at the core part of the formed spherical micelles. PLGA is converted into the lactic acid and the glycolic acid by hydrolysis in the body, thus is bio-compatible and bio-degradable (11).

The aim of this study was prepare and characterize a novel PLGA- CLX nano micellar formulation in order to target the inflammation site to provide high efficacy and low toxicity.

Methods

Materials

Complete Freund's Adjuvant (#F5881), Poly-(D,L-lactide-co-glycolide) (#P2192) and CLX (#PZ0008) was purchased from Sigma Aldrich, Rat tumor necrosis factor (TNF)-α Kit (#YHB20170406928), Rat PG-E2 Kit (#YHB20170406931), Rat interleukin (IL)-1β Kit (#YHB20170406929) and Rat IL-6 Kit (#YHB20170406930) was purchased from Yehua, all other chemicals were analytical grade, unless mentioned.

Preparation of the Nano-celecoxib Formulation

Fifty mg PLGA and increasing amounts of CLX (10-60 mg) were dissolved in 5 mL of acetone. Acetone solutions were then combined and added wisely by dripping on 20 mL of d.d. water containing 0.005% tween 80 in a very slow manner while stirring on magnetic stirrer (12). Stirring was continued overnight allowing all acetone to be evaporated and the day after the mixture's volume was adjusted to 20 mL and particle size was measured as below. This process is summarized in Figure 1.

Particle Size Measurements

The sizes of PLGA-CLX and free PLGA particles were measured directly on Zetasizer Nano ZS (model ZEN 3600; Malvern Instrument, Inc., London, UK) at 25 °C. Default setting on the Zetasizer Nano ZS was used, i.e. refractive index, absorption. The dispersant used was water and measurement angle was 173. Measurements were repeated 5 times, 3 minutes each and data were analyzed by number distributions (13). All samples were measured in triplicate. Presence of aggregates in micron size indicated the precipitated drug molecules. The max drug concentration with mono-modelar particle distribution was chosen as the optimized formulation. Further studies were carried out using the optimized formulation. According to our experiences, in case of presence of traces of amorphous aggregates in the system, measurements based on the volume and intensity distributions reveal these aggregates better than the ones based on number distribution. Therefore, all above mentioned measurements were conducted to make sure the obtained toxicity results were caused by the micelles, not aggregates (12).

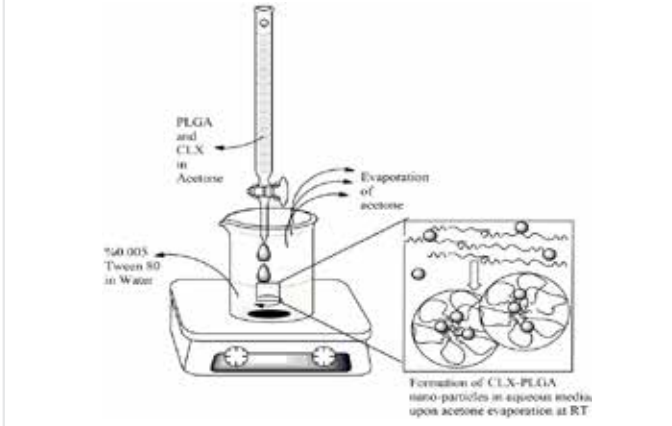

Figure 1. Preparation of CLX-PLGA nano-particles
CLX: Celecoxib, PLGA: Poly (lactic-co-glycolic acid)

***In vivo* Experiment Process**

The approval for animal experiments was obtained from Bezmialem Vakıf University Experimental Animal Ethics Committee, numbered 2016-241. Since this study was aimed to compare the toxicity of commercially available CLX formulation with the novel nano PLGA-CLX, the toxic dose of CLX was chosen to use in the animal study. It has been demonstrated that 5 and 7 mg/kg CLX is tolerable, thus 10 mg/kg of CLX was chosen to be evaluated at all samples.

Thirty two 5-week-old female Wistar rats (Bezmialem Vakıf University Experimental, Animal Research Center, Istanbul-Turkey) were separated randomly within 8 weight-matched groups consisted of 4 animals at each group and were given free access to a standard chow and water. All process followed were in complete agreement with The ARRIVE guidelines (14). 0.1 mL Complete Freund's Adjuvant (F5881) was injected to the right hint paw of the infected groups to induce RA model. At 0 point (before Freud's adjuvant injection), 30 min, 3rd and 6th hours after Freud's adjuvant injection, 0.5 mL blood was taken from all animals and their right hint paw size to which the adjuvant was injected was measured. First group was "Healthy Control Group" (HC) which received neither adjuvant nor medication. Second group, receivedthe adjuvant without medication [RA Control Group rheumatoit artritis control group (RAC)]. The third group received commercially available celebrex formulation orally, dissolved in water without getting infected by the adjuvant (HO) and fourth group received oral celebrex while treated with adjuvant rheumatoit artritis model (RAO). In the same manner, the fifth and sixth groups received CLX intravenously, to compare the efficacy of commercial product while receiving intravenously, and the sixth group was treated with the adjuvant (group 5: healthy rats treated with intravenous and group 6: rheumatoit artritis model treated with intravenous). The last two groups received nano PLGA-CLX formulation intravenously (group 7: home non-invasive ventilation and group 8: RANIV). Oral and intravenöz formulations were applied as max. 0.5 and 1 mL, respectively (the dose was equal to 10mg/kg of each animal).

Determination of Cytokines and Prostaglandins in Blood Circulation

All the blood samples were transferred to empty tubes and were centrifuged in 4000 rpm for 10 minutes to obtain the serum samples. Obtained serum samples were kept at -80 °C till the test day. Cytokine and prostaglandin levels in blood were measured by sandwich enzyme-linked immunosorbent assay (ELISA), using IL-1β, IL-6, TNF-α, prostaglandin E2 (PGE2) ELISA kits. Colorimetric absorbance was measured at 450 nm on a Bio-Tek ELISA Synergy-H1 microplate reader.

Release Study

Three mL CLX-PLGA with optimum drug and polymer ratio was transferred into dialysis membrane tubing SPECTRA/POR 3 Dialysis Membrane (Molecular weight cut-off 3,5 Lot No: 9200373) which then placed into 300 mL of dialysis medium (Phosphate-buffered saline buffer, pH 7.4) at 37 °C, while the media was stirred in a slow manner (15). One mL samples were withdrawn from the dialysis media at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 20, 22 and 24 hours of the experiment and CLX concentration was measured using high performance liquid chromatography (HPLC).

Measuring CLX concentration using HPLC

An analytical method for measuring CLX at HPLC was developed in order to measure the amount of drug incorporated with PLGA micelle, and to follow the release results. HPLC trials were conducted on a Shimadzu LC 20 liquid chromatograph equipped with a LC-20AT pump, SIL AT-HT auto-sampler part, and ultraviolet detector, which was set at 260 nm and CLX was passed through the colon with 1 mL/min speed with acetone-water (70:30) solution system from the C18 type colon with 4.6 cm inner diameter, 5µm particle size (Shimadzu; Kyoto, Japan), where the colon heat was stabilized to 25 °C. The CLX retention time was 5 min under these conditions (16,17).

Drug Loading Capacity

The concentration of CLX used in the preparation of the nano-formulation process was increased step wisely. Prepared CLX-PLGA formulation was incubated at room temperature for two hours after which samples with certain volumes were taken from the formulation and diluted with methanol 1/5 (V:V) to obtain free drug. Triple samples from supernatant were injected to the HPLC. Encapsulation Efficiency was measured using Equation (1:1) (18).

$$EE\% = \frac{\text{Total drug incorporated with PLGA}}{\text{Initial quantity of drug}} \times 100$$

Stability Studies

Obtained optimized PLGA-CLX formulation was frozen at -80 °C overnight following by freeze drying at -80 °C and 0.03 mbar (lyophilizer, Labconco, Freezone 2.5 plus). The obtained fluffy material was incubated at either room temperature or at -20°C for 1 week, 2 and 6 months, after which all were re-hydrated with the amount of water equal to that of pre-drying. Re-hydrated formulations were studied for particle size and CLX content as a factor of stability, using above mentioned methods (15).

Statistical Analysis

Regarding hint paw size and cytokine and prostaglandin measurements, each group at same time periods was compared separately and analyzed using Kruskal Wallis variance analysis. Different groups were determined using post hoc Dunn test. The p value <0.05 was considered as statistically significant. All statistical analyzes were done by using SPSS package program (ver.23) (19).

Results

Particle Size of the CLX Loaded PLGA Micelles

Particle sizing results of CLX-PLGA are listed in Table 1. As it is seen in Number, volume and intensity results, particle size

is monomodelar up to 30 mg/mL CLX. Excess amounts of CLX uploading causes obtaining bimodelar particles. In case of application of nano-CLX-PLGA for studying efficacy of this drug, this ratio could be used as the optimized formulation. However, in this study, we tried to use the toxic dose which corresponded to 10 mg/kg animal (each rat is around 250 g). Thus, we used 50 mg CLX in 50 mg PLGA/20 mL which the ratio of aggregate was less than 10% in terms of volume and intensity distribution and the particle size distribution was exactly 100% in terms of number distribution. Figure 2 shows the particle characteristics of the optimized formulation in which the phenylene diisocyanide is 0.415 and the majority of particles are less than 200 nm. It is noteworthy that all particles were smaller than 200 nm, which was necessary for a particle to be used in EPR effect (6).

Hint Paw Size Measurement Results

Figure 3 shows the increase in the hint paw size of the healthy and infected animals with adjuvant. The differences between the paw size of the healthy groups (encoded with H as HC, HO etc.) and the RA (encoded by RA) groups increased significantly by time. However, this increase in difference was less significant in the RA group treated with nano-CLX-PLGA ($p<0.01$).

Evaluation of Cytokines and Prostaglandins *In-vivo*

Figure 4 shows the variations in ratios of some biological indicators of inflammation before and after administration of oral and intravenous Celebrex and intravenous application of nano-CLX-PLGA to both healthy animals and RA models.

As it could be seen above, average results of TNF-α at the 6th hour showed meaningful decrease in RANIV group compared to that of RAO ($p<0.05$ or $p<0.10$), however, orally administered Celebrex was significantly more successful in decreasing the level of PGE2 within 3 hours (not till 6 hours). Nevertheless, intravenously administered celebrex was able to decrease the level of this prostaglandin in the long-term (6 hours). Similarly, RANIV group showed significantly decreased levels of PGE2 in the long-term (6 hours). No significant difference observed

Table 1: Particle size distribution of CLX-PLGA nano micelles with increasing amount of CLX

CLX Conc.(mg/mL)	Distribution by Number-nm (%)	Distribution by Volume-nm (%)	Distribution by Intensity-nm (%)	PDI
10	112 (100)	153 (100)	165 (100)	0.186
20	122 (100)	158 (100)	151 (100)	0.475
30*	125 (100)	156 (100)	150 (100)	0.457
40	118 (100)	158 (86) 5305 (14)	168 (95) 5146 (5)	0.295
45	143 (100)	185 (81) 5403 (19)	189 (94) 5290 (6)	0.402
50**	119 (100)	157 (91) 5537 (9)	169 (97) 5481 (3)	0.415
55	123 (100)	159 (92) 5527 (8)	167 (98) 5467 (2)	0.359
60	116 (100)	166 (67) 5322 (33)	177 (87) 5172 (13)	0.565

*: Optimized formulation for the effective dose (6 mg/kg), **: Optimized formulation for the toxic dose (10mg/kg) used in this study

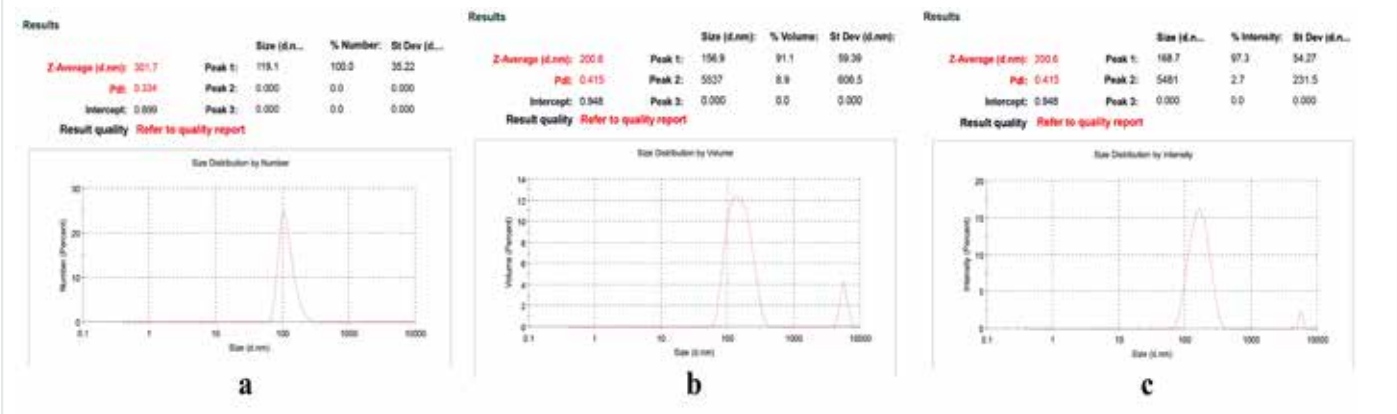

Figure 2. Particle size distribution of the optimized formulation; 50mg CLX in 50mgPLGA/20mL

CLX: Celecoxib, PLGA: Poly (lactic-co-glycolic acid)

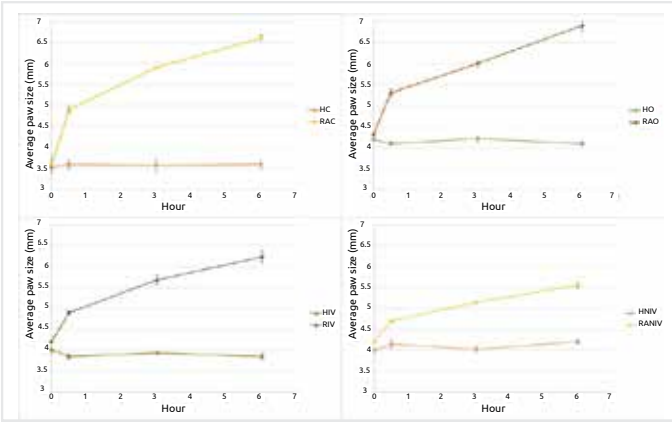

Figure 3. The size of the infected hint paw during the observations for 6 hours. The graphics are classified according the drug application groups: Up-left; Control, up-right: Orally administered Celebrex, bottom-left: intravenous CLX and bottom-right: intravenous nano-CLX-PLGA (H: Healthy and RA: Rheumatoid Arthritis)

Figure 4. The variations in the blood ratios of PGE2, IL 1, IL6 and TNF-α. HC: healthy control Group, RAC: rheumatoit artritis control group, HO: healthy rats treated with oral celebrex, RAO: rheumatoit artritis model, treated with oral celebrex, HIV: healthy rats treated with intravenous celebrex, RAIV: rheumatoit artritis model treated with intravenous celebrex, HNIV: healthy rats treated with intravenous Nano-CLX-PLGA, RAIV: rheumatoit artritis model treated with Nano-CLX-PLGA. A: significantly lower than RANIV, b: significantly lower than RAO.3 and lower tan RIV.6 ($p<0.10$), c: Significant decrease compared to HNIV.6, d: significant decrease compared to HO. 6, e: significant decrease compared to HO.6, d: significant decrease compared to HO.6, e: significant decrease compared to HNIV.6 and RAIV 6. (in each group $p<0.05$ or $p<0.10$)

between the group which orally received CLX and the control group in terms of the level of biological indicators. According to other results, differences between HC and RAC groups were similar to differences between HO and RAO groups. Except that, RAO group showed significant decrease compared to HO ($p>0.05$), in terms of IL-6 and PGE2.

Results of Release Study of CLX-PLGA

The release profile of CLX from nano-PLGA micelles is shown in Figure 5. Almost 70% of the drug was released within 6 hours with an accelerated behavior which was expected from an anti-inflammatory agent. After 6 hours, the release profile showed a very fast increase, probably due to disruption in the structure of the nano-micelle in the dialysis membrane.

Drug Loading Capacity

As it could be seen in particle sizing results, the optimized formulation was determined as the 50 mg CLX uploaded to 50 g PLGA. Thus, this formulation was injected to HPLC to detect the amount of drug inside polymeric micelles and at the aqueous media. EE% was calculated as:

Stability Studies

Figure 6 shows the results of stability studies made on CLX-PLGA at two different incubation conditions. While incubation at room temperature caused a short period of stability, incubation at -20 °C resulted in obtaining perfect stability, revealed with keeping almost all drug inside of the NDDS.

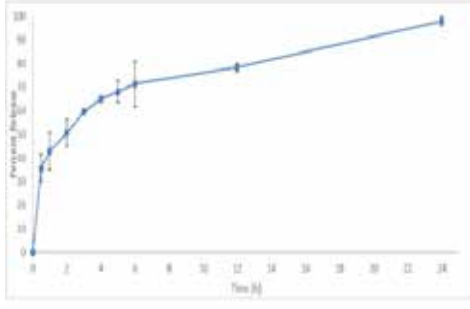

Figure 5. Celecoxib Loaded Nano-PLGA release Results

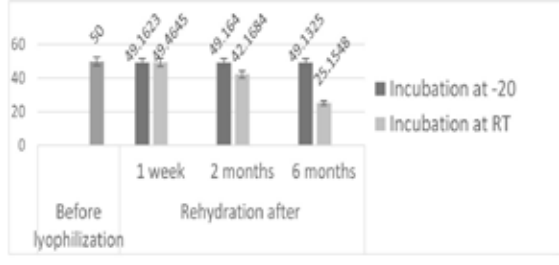

Figure 6. Stability study results of optimized CLX-PLGA formulation containing 50 mg CLX, rehydrated after lyophilization and incubation at RT or -20°C (RT: room temperature)

Discussion

Nonsteroidal antiinflammatory drugs (NSAIDs) were announced in the 1960s and became the most widespread prescribed class of drugs in the world, with over 100 million prescriptions issued per year in the United States alone (20). They were used especially for the treatment of the inflammation, mostly arthritis, and pain. NSAIDs inhibit the COX which decreases the inflammation and the pain through the prostaglandin inhibition. But, the COX enzyme is also existing in gastric mucosa where it stimulates the gastro-protective prostaglandins. The identification of two isoforms, COX-1 and COX-2, and recognition of direct relationship between COX-1 inhibition and gastrointestinal system side effects, resulted in development of selective COX-2 inhibitors that offered potential to maintain efficacy while decreasing the GI adverse effects (21). Consequently, administration of the NSAIDs unavoidably leads to a lack of prostaglandins which are essential for the physiological functions. Therapeutic and side effects of this class of anti-inflammatory drugs are strongly related to their biochemical mechanism of action (8). As a result, long term NSAID users suffer from a high incidence of GI irritation. The risk of death or hospitalization from a GI adverse event varies from 1.3% to 1.6% per year in patients with RA (22). These rare, however potentially serious GI side effects of NSAIDs have become a major healthcare problem (23).

There are several nanotechnological administrations. Among them, the treatment, diagnosis, monitoring and control of the biological systems have lately been referred as "nano-medicine" by the National Institute of Health (Bethesda, MD, USA). We can easily say that the "targeting" property of nanotechnology-managed particles is the most attractive subject in this area.

The size of the 50 mg CLX incorporated with 50 mg PLGA/20 mL nano-formulation is under 200 nm and is very suitable for targeting site of inflammation and also for studying the toxic dose of CLX due to the high drug loading efficacy. The release properties of this nano-formulation is long enough to carry the drug molecule in NDDS and is accelerated enough to show the fast effect in urgent cases however according to the *in vivo* results, the commercially available oral formulation of Celebrex shows much faster results.

Previously, Harirforoosh et al. (24) have prepared nano-PLGA-CLX to assess its pharmacodynamics and pharmacokinetics in rats. However, no efficacy studies were done in this research. Their results were in great accordance with our results. Since the surfactant used in their formulation was different, the particle sizes were not comparable however, the increasing ratio of CLX in serum showed similarity to our release results. Interestingly, in the study of Harirforoosh et al. (24) there was no inflammation site and the NDDS releasedthe drug within the blood flow similar to that of conventional drug and in this condition no difference between PGE2 ratios were observed. This could be an evidence of the success obtained by our group in targeting the inflammation site and inhibiting PGE2 more significantly by nano-CLX-PLGA. Similarly, Amrite et. al (14) showed 40% reduction in PGE2 by local administration of CLX-PLGA microparticles (diameter= 1140±15 nm), containing 14.93%±0.21% of CLX. In another study, CLX-PLGA was prepared to study its antitumor activity against brain tumor cells. In this study, the drug/ polymer ratio was completely in accordance with the results obtained by our group, where acetone was used as solvent, however the particle size was different due to the applied preparation method. Since this study was an *in vitro* study, the targeting property of CLX-PLGA was not revealed however, the release study which was similar to ours, showed very similar profile (25).

The biochemical experiments of the nano-CLX effect prove that CLX has elongated and sustained efficacy on inflammation while incorporated with micelles comparing to the conventional CLX; CLX. Comparing the paw size of the healthy and inflammated animal groups showed that CLX which was given by gavage and also administered intravenously showed almost the same efficacy. It was showed that PGE2 which is directly related to COX-2 enzyme inhibition was decreased longer with intravenous nano-CLX formulation than Celebrex.

In an overview, our results are cohesive and correlated with the hypothesis that the counted parameters are being decreased in blood and therefore increased in the tissues.

In general, differences occurred in the blood levels of IL-1 and IL-6 were not significant, probably due to their "pro" inflammation role. However, comparing IL-1 levels in the RAO and RANIV revealed that RAO group had a dramatic decrease within 3 hours. The results obtained from the IL-6 and IL-1 were correlated to each other.

Conclusion

As a conclusion, according to the results obtained from this study, it is proved that the suggested nano-CLX-PLGA formulation is able to target the inflammated tissues in the systemic blood flow and have a better selective COX-2 inhibition activity. It is obvious that the results obtained from RAO, RAIV and RANIV groups are obtained from a single dosage administration and to achieve complete response, multiple doses are necessary.

Ethics

Ethics Committee Approval: The Bezmialem Vakıf Universty Experimental Animals Ethics Committee received confirmation from the experimental animals used. Numbered 2016-241.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.B., Design: F.B., A.K., Data Collection or Processing: Ş.E.K.T., Z.E., A.Ş.B., A.S.K., Analysis or Interpretation: F.B., A.K., H.A., H.N.K., Literature Search: F.B., Z.E., A.Ş.B., A.S.K., Writing: F.B.

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

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References

1. Peakman M,Vergani D. Basic and Clinical Immunology. Elsevier Health Sciences;2009.
2. Simon LS, Lanza FL, Lipsky PE, Hubbard RC, Talwalker S, Schwartz BD et al. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor: efficacy and safety in two placebo-controlled trials in osteoarthritis and rheumatoid arthritis, and studies of gastrointestinal and platelet effects. Arthritis Rheum 1998;41:1591-602.
3. Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. Inflamm Res 2000;49:367-92
4. de Gaetano G, Donati MB,Cerletti C. Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors. Trends Pharmacol Sci 2003;24:245-52.
5. Qiao W, Wang B, Wang Y, Yang L, Zhang Y, Shao P. Cancer therapy based on nanomaterials and nanocarrier systems. J Nanomater 2010;2010:7.
6. Maeda H. Macromolecular therapeutics in cancer treatment: the EPR effect and beyond. J Control Release 2012;164:138-44.
7. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci 2007;32:762-98.
8. Tian H, Tang Z, Zhuang X, Chen X, Jing X. Biodegradable synthetic polymers: preparation, functionalization and biomedical application. Prog Polym Sci 2012;37:237-80.
9. Gupta AP, Kumar V. New emerging trends in synthetic biodegradable polymers–Polylactide: A critique. Eur Polym J 2007;43:4053-74.
10. Mohamed F, van der Walle CF. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. J Pharm Sci 2008;97:71-87.
11. Muthu MS. Nanoparticles based on PLGA and its co-polymer: An overview. AJP 2019;3:266-73.
12. Bahadori F, Kocyigit A, Onyuksel H, Dag A, Topcu G. Cytotoxic, Apoptotic and Genotoxic Effects of Lipid-Based and Polymeric Nano Micelles, an In Vitro Evaluation. Toxics 2017;6:7.
13. Banerjee A,Onyuksel H. Human pancreatic polypeptide in a phospholipid-based micellar formulation. Pharm Res 2012;29:1698-711.
14. Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. Invest Ophthalmol Vis Sci 2006;47:1149-60.
15. Bahadori F, Topçu G, Eroğlu MS, Önyüksel H. A new lipid-based nano formulation of vinorelbine. AAPS PharmSciTech 2014;15:1138-48.
16. Emami J, Fallah R, Ajami A. A rapid and sensitive HPLC method for the analysis of celecoxib in human plasma: application to pharmacokinetic studies. DARU 2008;16:211-17.
17. Baboota S, Faiyaz S, Ahuja A, Ali J, Shafiq S, Ahmad S. Development and validation of a stability-indicating HPLC method for analysis of celecoxib (CXB) in bulk drug and microemulsion formulations. Acta Chromatogr 2007;18:116.
18. Wong HL, Bendayan R, Rauth AM, Wu XY. Development of solid lipid nanoparticles containing ionically complexed chemotherapeutic drugs and chemosensitizers. J Pharm Sci 2004;93:1993-2008.
19. Cakal B, Akoz AG, Ustundag Y, Yalinkilic M, Ulker A, Ankarali H. Red cell distribution width for assessment of activity of inflammatory bowel disease. Dig Dis Sci 2009;54:842-47.
20. Bello AE, Holt RJ. Cardiovascular risk with non-steroidal anti-inflammatory drugs: clinical implications. Drug Saf 2014;37:897-902.
21. Whittle B. COX-1 and COX-2 products in the gut: therapeutic impact of COX-2 inhibitors. Gut 2000;47:320-25.
22. Fries JF. NSAID gastropathy: the second most deadly rheumatic disease? Epidemiology and risk appraisal. J Rheumatology Suppl 1991;28:6-10.
23. Silverstein FE, Graham DY, Senior JR, Davies HW, Struthers BJ, Bittman RM, et al. Misoprostol reduces serious gastrointestinal complications in patients with rheumatoid arthritis receiving nonsteroidal anti-inflammatory drugs. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 1995;123:241-49.
24. Harirforoosh S, West K, Murrell D, Denham J, Panus P, Hanley G. Assessment of celecoxib poly (lactic-co-glycolic) acid nanoformulation on drug pharmacodynamics and pharmacokinetics in rats. Eur Rev Med Pharmacol Sci 2016;20:4818-29.
25. Kim T-H, Jeong Y-I, Jin S-G, Pei J, Jung T-Y, Moon K-S, et al. Preparation of polylactide-co-glycolide nanoparticles incorporating celecoxib and their antitumor activity against brain tumor cells. Int J Nanomedicine 2011;6:2621-31.

Original Article

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Genotype-phenotype Correlation in Pelizaeus Merzbacher Disease and Pelizaeus Merzbacher-like Disease

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ABSTRACT

Objective: Among the hypomyelinating diseases of childhood, Pelizaeus Merzbacher disease (PMD) is caused by X-linked proteolipid protein (PLP) gene mutations, whereas patients without mutations of PLP gene-called Pelizaues Merzbacher-like disease (PMLD) have recessive gap junction protein α12 (gap junction alpha-12/gap junction gamma-2) gene mutations. The aim of this study was to evaluate clinical severity and progression in time in patients with PMD and PMLD.

Methods: The motor developmental stages of the patients were reviewed; disease severity was classified according to the walking ability they were able to achieve. Progression pattern was determined according to comparison of neurological findings at the time of the study and at follow-up visits. Patients with PMD and PMLD were compared in terms of disease severity and progression rates as well as patient groups with a unique causative mutation were analyzed individually.

Results: There were 9 patients with PMD (mean age 15.2±3.1) and 11 patients with PMLD (mean age 12.4±1.9). The presence of severe disease was more common in patients with PMD when compared to PMLD. In X-linked PMD, missense mutations were associated with the most severe disease and rapid progression, while deletion mutations were associated with mild disease severity and slow progression. Disease severity and progression patterns seemed to be heterogenous in different causative mutations of PMLD.

Conclusion: Although PMLD might have milder disease phenotype when compared to PMD, certain causative mutations in different genetic traits may cause different disease severity and progression patterns.

Keywords: Pelizaues-Merzbacher disease, Pelizaues-Merzbacher-like Disease, gap junction protein α12, genotype, phenotype

Introduction

Pelizaeus-Merzbacher disease (PMD) is the prototype of hypomyelinating diseases of childhood which was firstly described as an X-linked disorder caused by mutations or rearrangements in the *proteolipid protein 1 (PLP1)* gene. Causative mutations of the *PLP1* gene are-in declining order of frequency-duplications, point mutations, insertions, and deletions (1). Clinical features include nystagmus (typically occurs in the first months of life and resolves within 2-5 years of age), dysarthria, ataxia, hypotonia and developmental delay evolving into spastic quadriplegia in the first years of life (2). The clinical severity and rate of progression vary widely, probably depending on the variability of the causative mutation (1). Although, it was firstly described as a X-linked disorder, patients with PMD phenotype but without mutations of *PLP1* gene were shown to have an autosomal recessive (OR) trait. One of the causative mutations in these patients -called

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Pelizaeus-Merzbacher-like disease (PMLD)- is in gap junction protein α 12 gene/ gap junction gamma (GJA12/GJC2) coding for Connexin 47 (3). Although GJA12/GJC12 mutations are found in a minority of PMLD, its frequency is higher in Turkish patients (4). The data about which phenotypical differences occur in these different types of genetic traits are limited. In this study, we aimed to compare the age of onset, clinical severity and progression rates in PMD and PMLD.

Methods

The data of this study were obtained from 20 patients who had been followed between 1995-2011 in the İstanbul Medical Faculty, Department of Neurology. All genetic analysis of patients and families were performed in the Department of Molecular Biology and Genetics in Bogaiçi University. Only patients who had a causative mutation were included into the study. Screening tests for metabolic diseases were performed. All patients underwent brain magnetic resonance imaging (MRI). Exclusion criteria were as following: a) the presence of any other congenital, metabolic or developmental brain diseases, b) the absence of follow-up visits, c) rejection to give written informed consent for the study.

The history of birth and motor developmental stages of the patients were learned from parents as well as from clinical charts. Detailed neurological examination was performed in all patients and compared with the clinical findings at follow-up visits. Moreover, functional independence measurement (FIM/ weeFIM), scale for the assessment and rating of ataxia (SARA) and Modified Achworth Spasticity scale (MAS) were also evaluated in all patients. FIM is an 18-item motor and cognitive function test that is used to assess a patient's level of functionality (5). weeFIM is, on the other hand, used to assess this functional independence in children (6). The total score for the FIM/weeFIM instrument (the sum of the motor and cognition subscale scores) will be a value between 18 and 126. The higher the score, the more independent the patient is according to the FIM/weeFIM scores. SARA is a clinical scale to assess ataxia (7). It has 8 categories with accumulative score ranging from 0 (no ataxia) to 40 (most severe ataxia). The MAS is the most widely used clinical scale to measure the increase of muscle tone (8). It has 5 categories ranging between 0 (no increase in muscle tone) and 4 (affected parts rigid in extension or flexion). In the study, the MAS score was scored according to the tone of lower extremities.

According to the obtained data, the patients were grouped according to the development of walking ability as follows: a) group 1 (severe disease) included patients who never had the ability to walk, b) group 2 (moderate disease) included patients who had the ability to walk with assistance, c) group 3 (mild disease) included patients who had the ability to walk without assistance during the developmental stages. Groups were compared in terms of age, sex, clinical scores, genetic traits and causative mutations.

progression-which was accepted if the patient lost the major activities that he/she was able to do (walking, sitting and speech) during the follow-up- was evaluated in every patient. Categorical variables were presented as percentage (%) and continuous variables as mean ± SD or median [interquartile range, (IQR)], as appropriate. The study approved by İstanbul University, İstanbul Medical Faculty Ethics Comittee (2011/807-567).

Statistical Analysis

Categorical variables were analyzed using pearson chi-square or Fisher's Exact test, and continuous variables using the independent-sample t-test (for normal distributions) and Kruskal-Wallis test (for non-normal distributions). Statistical assessments were performed using SPSS software pack (Statistical Package for Social Sciences for Windows Version 23 software). A p value of less than 0.05 was considered statistically significant.

Results

A total of 20 patients (14 female, 6 male) with a mean age of 13.7±7.84 years (minimum-maximum: 4-30) were included in the study. Follow-up period was 3-12 years. According to the genetic analysis, 9 patients had X-linked PMD and the remaining 11 patients had OR PMLD trait. The mean age between X-linked and OR patients was not different (15.2±3.1 vs 12.4±1.9, p=0.448). Detailed information about demographics, causative mutation and developmental stages according to the walking ability are given in Table 1.

There was no difference between 3 groups in terms of age (p=0.905), FIM/weeFIM score (p=0.085), SARA score (p=0.313) and MAS (p=0.305). Of patients with X-linked PMD, 33.3% were in the severe, 33.3% in moderate and the remaining 33.3% were in mild disease group. Of PMLD, 18.2% were in the severe, 45.2% in moderate and the remaining 36.4% were in mild disease group.

In 5 patients with a duplication in *PLP1* gene, 3 patients never had the ability to walk, 2 patients, other hand, had at once the ability to walk with assistance. Of these patients, case 5 (29 years of age) was still able to walk with assistance and he had only mild ataxia. When compared with the neurological examination performed 5 years before, he had a very slow progression. The other patient in moderate disease group was patient 1 who was 7 years of age. According to the follow-up visits, this patient had lost the walking ability with a progression in ataxia. The 2 patients with comparative genomic hybridization duplication had also apparently progressed during follow-up period. The 2 patients with GJA12/GJC2 duplications had the ability to walk with assistance, but both lost this ability during the 8-year follow-up period. Patient 7 (17 years of age) with a deletion in *PLP1* gene had the ability to walk but it was late according to normal motor developmental stages of childhood. Progression of the disease was slow in early follow-up period, but he was not able to walk at the time of study. Of 4 patients with a deletion in *GJA12/GJC2* gene, two patients never had the ability to walk, the other two patients, on the other hand, had the ability to walk

Table 1. Demographics, genetic characteristics and motor development stages of all patients

Patient	Age	Sex	Genetic mutation	Causative mutation	Development of sitting ability	Development of speech ability	Development of walking ability	Groups according to walking ability
1	7	M	*PLP1 gene*	Duplication	6 months of age	2 years of age	2 years of age with assistance	Moderate disease
2	8	M	*PLP1 gene*	Duplication	2 years of age	1 years of age	-	Severe disease
3	5	M	*PLP1 gene*	Duplication	-	2 years of age	-	Severe disease
4	17	M	*PLP1 gene*	Duplication	-	1.5 years of age	-	Severe disease
5	29	M	*PLP1 gene*	Duplication	8 months of age	1 years of age	3 years of age	Mild disease
6	7	M	*PLP1 gene*	Missense	8 months of age with assistance	3 years of age	3 years of age with assistance	Moderate disease
7	17	M	*PLP1 gene*	Deletion	6 months of age	2 years of age	3 years of age	Mild disease
8	17	M	*PLP1 gene*	CGH duplication	6 months of age	2 years of age	3 years of age with assistance	Moderate disease
9	30	M	*PLP1 gene*	CGH duplication	6 months of age	1 years of age	1.5 years of age	Mild disease
10	9	F	GJA12/GJC2	Duplication	5 years of age With assistance	3 years of age	5 years of age with assistance	Moderate disease
11	21	F	GJA12/GJC2	Duplication	8 months of age	2 years of age	2 years of age with assistance	Moderate disease
12	7	F	GJA12/GJC2	Insertion	1 years of age	1 years of age	2 years of age	Mild disease
13	5	F	GJA12/GJC2	Missense	6 months of age	1 years of age	2 years of age	Mild disease
14	18	F	GJA12/GJC2	Missense	6 months of age	1 years of age	2 years of age with assistance	Moderate disease
15	4	F	GJA12/GJC2	Missense	1 years of age	1 years of age	1.5 years of age	Mild disease
16	21	M	GJA12/GJC2	Missense	1 years of age	1 years of age	4 years of age	Mild disease
17	14	M	GJA12/GJC2	Deletion	1 years of age	1 years of age	1 years of age with assistance	Moderate disease
18	6	M	GJA12/GJC2	Deletion	8 months of age	1 years of age	2 years of age with assistance	Moderate disease
19	18	M	GJA12/GJC2	Deletion	1 years of age	1 years of age	-	Severe disease
20	14	M	GJA12/GJC2	Deletion	-	1 years of age	-	Severe disease

PLP: Proteolipid protein, GJA12: Gap junction alpha12, GJC2: Gap junction gamma2, CGH: Comporotive genomic hybridizotion

Table 2. Comparison of clinical scales between patients with PMD and PMLD

Clinical scales	PMD	PMLD	p
SARA, median (IQR)	35 (29.5-39)	37 (15-40)	0.788
FIM/weeFIM, median (IQR)	46 (36-57.5)	45 (36-83)	0.592
Ashworth spasticity score, median (IQR)	2 (1.5-3.5)	3 (0-4)	0.816

PMD: Pelizaeus Merzbacher Disease, PMLD: Pelizaeus Merzbacher Disease, SARA: Scale for the assessment and rating of ataxia FIM: Functional independence measurement, IQR: Interquartile range

with assistance, one of whom (6 years of age) was still able to walk at the time of study. The patient 6 (7 years of age) with a missense mutation in *PLP1* gene had the ability to walk with assistance however he lost this ability in a short time period. All of 4 patients with missense mutation in *GJA12/GJC2* gene had the ability to walk with assistance. Of these, 2 patients who were in the age of 18 and 21 lost this ability during the recent 5 -year follow-up period. The only patient with an insertion in *GJA12/GJC2* gene (patient 12; 7 years of age) had completed motor developmental stages in time. He was able to walk without assistance at the time of the study.

Regarding to clinical scales, there were no difference in terms of FIM/weeFIM, SARA and MAS scores between PMD and PMLD (Table 2). Age did not correlate in any of these scales.

Discussion

To our knowledge, this study has the largest series of Turkish patients and evaluates the association of phenotypic characteristics in the patients with PMD and PMLD. Both PMD and PMLD are hypomyelinating disorders with similar clinical and neuroradiological phenotypes. The patients present in infancy with nystagmus, jerky head movements, hypotonia, ataxia and developmental delay evolving into spastic paraplegia (9).

There have been numerous genetical and molecular studies that investigate the nature of the PMD and other hypomyelinating disorders (10-12). However, data about the clinical features and prognosis of the patients with genetically confirmed PMD and PMLD are limited. In a study in which types in patients having point mutations in *PLP1* gene were investigated, clinical severity was found to be correlated with the nature of the mutation. Single amino-acid mutations in highly-conserved region of PLP caused the most severe forms of PMD (13). Another study that investigated the phenotype-genotype correlation in 5 patients with PMD with duplication mutation, did not find any correlation with the extent of the duplicated genomic segments and the clinical severity (14). In a previous report in our patient population carrying duplication mutation (4), the severity of the disease did not show any correlation with the duplication size in contrary to previously reported studies (15,16). Our study did not focus on genetic basis of the disease, instead we compared clinical features and prognosis in two different traits and mutations in PMD and PMLD. Although PMD is known as the prototype of hypomyelinating diseases, PMLD caused by OR genetic trait has been reported to be seen in high frequency in Turkey as well as in other Eastern countries, probably depending on high rate of consanguineous marriages (4,17,18).

In a study that investigated clinical features of 16 patients with PMLD according to developmental scores, the majority of the patients were found to have ambulation capacities, speech and good cognitive functions (3). Our results also support that PMLD has better clinical severity and prognosis when compared to X-linked PMD. When we analyzed different types of causative mutations in all patients, patients with X-linked duplications constituted the most heterogenous patient group in terms of clinical severity and progression. OR duplications, on the other hand, had a moderate clinical severity and progression was not severe and these patients had milder ataxia and hypotonia even in late stages of the disease. In patients with X-linked deletion mutation, clinical findings were milder and progression rate was slower compared with other causative mutations, similar to previous publications (19). Clinical findings both in early and late stages of the disease were severe in patients with X-linked missense mutations, as previously reported (13). On the other hand, OR missense mutations had moderate severity and the progression was slower. The only patient with insertion in GJA12/CJG2 had moderate severity and slow progression rate. We did not find any differences in terms of clinical scores between PMD and PMLD, probable due to the small size of the cohort as well as the absence of age-matching PMD and PMLD patient groups. Although the mean age between PMD and PMLD was not different, we used two different scale formats according to the age (FIM vs weeFIM) for functional independency. Ataxia rating scales are reported to be age-dependent even in healthy children (20). Nevertheless, clinical scales enhance the communication between health professionals and they increase the objectivity when used to compare the patients with similar clinical findings.

There are limitations of the study. Despite we performed detailed neurological examinations and interviews with the families to identify the severity of the disease, the evaluation of progression was based on the retrospective review of clinical charts. Moreover, clinical scales we used at the time of present study were not be performed at the previous follow-up visits. Previous studies reported similar neuroradiological findings in patients with PMD and PMLD (21). We did not report MRI findings in patient groups, however MRI results were very similar in our patient series based on an unpublished data.

In a study comparing neurophysiologic findings between PMD and PMLD reported a clear difference in brain stem evoked potentials (BAEP) (22). However, BAEP studies were not performed in our patients.

Conclusions

With the increase in the use of MRI as well as the availability of genetical analysis, the number of patients receiving a diagnosis of PMD and PMLD have been increasing. However, the similarities in clinical features between them may complicate the decision of the appropriate genetic testing particularly if there is no evidence of genetic transformation pattern based on patient's pedigree. It might be helpful to know that X-linked PMD patients may have more severe disease when compared to OR PMLD patients. Certain causative mutations in different genetic traits differ in terms of clinical severity and progression rates. These data can be used when informing the family members about the possible course of the disease according to the existing causative mutation. Further prospective studies are needed in larger patient series.

Ethics

Ethics Committee Approval: The study approved by İstanbul University, İstanbul Medical Faculty Ethical Comittee (2011/807-567) .

Informed Consent: Written informed consent was obtained from all participants and/or their parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Z.Y., R.A., Design: E.G., R.A., Z.Y., Data Collection or Processing: E.G., Z.Y., Analysis or Interpretation: E.G., R.A., B.B., E.B., Literature Search: E.G., Z.Y., Writing: E.G., Z.Y.

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References

1. Koeppen AH, Robitaille Y. Pelizaeus-Merzbacher disease. J Neuropathol Exp Neurol 2002;61:747-59.
2. Boulloche J, Aicardi J. Pelizaeus-Merzbacher disease: clinical and nosological study. J Child Neurol 1986;1:233-9.
3. Henneke M, Combes P, Diekmann S, Bertini E, Brockmann K, Burlina AP. GJA12 mutations are a rare cause of Pelizaeus-Merzbacher-like disease. Neurology 2008;70:748-54.
4. Bilir B, Yapici Z, Yalcinkaya C, Baris I, Carvalho CM, Bartnik M, et al. High frequency of GJA12/GJC2 mutations in Turkish patients with Pelizaeus-Merzbacher disease. Clin Genet 2013;83:66-72.
5. Linacre JM, Heinemann AW, Wright BD, Granger CV, Hamilton BB. The structure and stability of the Functional Independence Measure. Arch Phys Med Rehabil 1994;75:127-32.
6. Msall ME, DiGaudio K, Duffy LC, LaForest S, Braun S, Granger CV. WeeFIM: Normative Sample of an Instrument for Tracking Functional Independence in Children. Clin Pediatr (Phila) 1994;33:431-8.
7. Schmitz-Hübsch T, Du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006;66:1717-20.
8. Meseguer-Henarejos AB, Sánchez-Meca J, López-Pina JA, Carles-Hernández R. Inter- and intra-rater reliability of the Modified Ashworth Scale: a systematic review and meta-analysis. Eur J Phys Rehabil Med 2018;54:576-90.
9. Hobson GM, Garbern JY. Pelizaeus-Merzbacher disease, Pelizaeus-Merzbacher-like disease 1, and related hypomyelinating disorders. Semin Neurol 2012;32:62-7.
10. Southwood CM, Garbern J, Jiang W, Gow A. The unfolded protein response modulates disease severity in pelizaeus-merzbacher disease. Neuron 2002;36:585-96.
11. Gow A, Southwood CM, Lazzarini RA. Disrupted proteolipid protein trafficking results in oligodendrocyte apoptosis in an animal model of pelizaeus-merzbacher disease. J Cell Biol 1998;140:925-34.
12. Mimault C, Giraud G, Courtois V, Cailloux F, Boire JY, Dastugue B, et al. Proteolipoprotein gene analysis in 82 patients with sporadic pelizaeus-merzbacher disease: duplications, the major cause of the disease, originate more frequently in male germ cells, but point mutations do not. Am J Hum Genet 1999;65:360-9.
13. Cailloux F, Gauthier-Barichard F, Mimault C, Isabelle V, Courtois V, Giraud G, et al. Genotype–phenotype correlation in inherited brain myelination defects due to proteolipid protein gene mutations. Clinical European Network on Brain Dysmyelinating Disease. Eur J Hum Genet 2000;8:837-45.
14. Regis S, Biancheri R, Bertini E, Burlina A, Lualdi S, Bianco MG, et al. Genotype–phenotype correlation in five Pelizaeus-Merzbacher disease patients with PLP1 gene duplications. Clin Genet 2008;73:279-87.
15. Inoue K, Osaka H, Imaizumi K, Nezu A, Takanashi J-I, Arii J, et al. Proteolipid protein gene duplications causing Pelizaeus-Merzbacher disease: Molecular mechanism and phenotypic manifestations. Ann Neurol 1999;45:624-32.
16. Woodward K, Kendall E, Vetrie D, Malcolm S. Pelizaeus-Merzbacher disease: identification of Xq22 proteolipid-protein duplications and characterization of breakpoints by interphase FISH. Am J Hum Genet 1998;63:207-17.
17. Bugiani M, Al Shahwan S, Lamantea E, Bizzi A, Bakhsh E, Moroni I, et al. GJA12 mutations in children with recessive hypomyelinating leukoencephalopathy. Neurology 2006;67:273-9.
18. Karimzadeh P, Ahmadabadi F, Aryani O, Houshmand M, Khatami A. New Mutation of Pelizaeus-Merzbacher-Like Disease; A Report from Iran. Iran J Radiol 2014;11:e6913.
19. Nevin ZS, Factor DC, Karl RT, Douvaras P, Laukka J, Windrem MS, et al. Modeling the Mutational and Phenotypic Landscapes of Pelizaeus-Merzbacher Disease with Human iPSC-Derived Oligodendrocytes. Am J Hum Genet 2017;100:617-34.
20. Brandsma R, Spits AH, Kuiper MJ, Lunsing RJ, Burger H, Kremer HP, et al. Ataxia rating scales are age-dependent in healthy children. Dev Med Child Neurol 2014;56:556-63.

21. Steenweg ME, Vanderver A, Blaser S, Bizzi A, de Koning TJ, Mancini GM, et al. Magnetic resonance imaging pattern recognition in hypomyelinating disorders. Brain 2010;133:2971-82.

22. Henneke M, Gegner S, Hahn A, Plecko-Startinig B, Weschke B, Gärtner J, et al. Clinical neurophysiology in GJA12-related hypomyelination vs Pelizaeus-Merzbacher disease. Neurology 2010;74:1785-9.

Original Article

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Investigation and Clinical Importance of Obsessive and Compulsive Signs Among Patients with Restless Legs Syndrome

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ABSTRACT

Objective: The purpose of this study was to examine obsessive and compulsive signs among patients with Restless Legs syndrome (RLS), to compare the results with healthy controls and to investigate clinic importance of those signs.

Methods: Thirty nine patients with RLS and 40 age-, sex- and education- matched healthy controls were assessed in Bezmialem Foundation University Medical Faculty Hospital Neurology and Psychiatry Clinics Hospital. Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders-IV axis-1 disorders severe combined immune deficiency-1, socio-demographic data form and Maudsley obsessive compulsive inventory (MOCI) were applied to both patients and controls. Patient group was also classified according to the International RLS study group RLS severity scale.

Results: Patient group had higher MOCI total scores and doubting and rumination subscale scores than the control group and the difference was statistically significant ($p<0.05$). No statistically significant relationship was found between RLS severity score and MOCI total and subscale scores.

Conclusion: Patients with RLS were generally found to have more obsessive and compulsive signs than healthy controls. In view of the fact that RLS is often a late-diagnosed syndrome, searching for concomitant RLS among young people who have obsessive and compulsive signs may be helpful for early diagnosis of RLS.

Keywords: Restless legs syndrome, alexithymia, depression, anxiety

Introduction

Restless Legs syndrome (RLS) is a common neurological syndrome which is sometimes diagnosed incorrectly or with delay and sometimes can not be diagnosed. It is classified in sleep-related movement disorders. The main symptoms are unpleasant sensations such as tingling and rubbing in the legs. It also presents with insomnia, fatigue, pain and depressive symptoms (1,2). Although the pathophysiology of RLS is not fully understood, it has been reported that it may be associated with disorders in dopamine metabolism (3,4).

The criteria for diagnosis of RLS were determined by the International RLS Study Group (IRLSSG) (1). By the same group, a scale was developed to determine the severity of the disease for use in clinical trials (IRLSSG RLS severity scale) (2). RLS may be idiopathic (primary) or may develop secondary to situations such as iron deficiency anemia, renal failure and pregnancy (5-7).

It is known that psychiatric disorders can also be seen in patients with RLS. Although there are few studies investigating the existence of this comorbidity in the last 20 years, there has been an increase in interest in this area in recent years. The most common

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psychiatric symptoms in patients with RLS are depression and anxiety (8,9). The association of RLS with other psychiatric conditions such as attention deficit and hyperactivity disorder (ADHD) and obsessive compulsive disorder (OCD), which are disorders of dopaminergic and serotonergic neurotransmitter systems, has also been investigated (10,11).

Although iron is an important modulator in neurotransmission, it is not yet clear how the disturbances in iron homeostasis in the brain disrupts neurotransmission and leads to the development of RLS (12). There are literature showing that iron deficiency changes monoaminergic activity in the central nervous system. In another study, repeated blood intake from guinea pigs showed increased levodopa (L-DOPA) levels in the caudat nuclei and in the prefrontal cortex, decreased L-DOPA levels in the anterior horn of the spinal cord, decreased serotonin levels in caudat nucleus, substantia nigra and putamen. In the same study, extracellular monoamine concentrations were measured and there was a significant increase in dopamine and serotonin levels in the striatum and a tendency of increase in leukotomotor activity, which was measured both day and night, as the number of blood intakes was increased (13). In a study, it was suggested that OCD and ADHD were included in clinical manifestations of RLS and that comorbid psychiatric conditions such as OCD and ADHD could be occult in patients with RLS, especially with marked iron deficiency anemia (14).

According to the criteria of the diagnostic and statistical manual of mental disorders (DSM-5); repetitive and persistent thoughts, impulses, or images that come to mind involuntarily and by force and that cause marked anxiety or distress in most people, are called obsessions and thinking new ideas or performing certain actions for the purpose of reducing the anxiety of these thoughts are called compulsions (15,16).

Classical knowledge of the disturbance in the monoaminergic transmission in the central nervous system on the ground of obsessions and compulsions is more focused on serotonin and dopamine. In patients with OCD, there is an increase in 5-hydroxyindoleacetic acid (5-HIAA) level, which is a serotonin metabolite, in cerebrospinal fluid (CSF). OCD responds significantly to treatment with clomipramine, a tricyclic antidepressant. A significant decrease in the level of 5-HIAA in CSF in patients with OCD treated with clomipramine was reported in a study (17). It is known that a positive correlation is found between the response level to clomipramine and serum concentration of clomipramine. However, no such correlation is found with desmetylclomipramine, an active metabolite of clomipramine preventing noradrenergic uptake. These data support that serotonin may be considered more important than noradrenaline in the etiology of OCD (18).

It is known that combination of low-dose antipsychotic drugs with selective serotonin reuptake inhibitors increases treatment response in OCD. This may be considered as a clinical evidence demonstrating the importance of dopamine in the etiology of OCD. It has been reported that the balance between dopaminergic and serotonergic activities in the central nervous system is also found on the background of some types of OCD (19). These data show that the disturbances in serotonergic mechanism as well as in the dopaminergic mechanism are also involved in the etiology of OCD.

There are a small number of studies in the literature referring to the relationship between obsessive compulsive symptoms and RLS. In a study comparing healthy volunteers with patients with RLS by using software collection (SCL)-90, the scores of, especially, somatization and obsessive compulsive subscales in patients with RLS were found to be significantly higher than healthy volunteers (20). In another study comparing healthy volunteers with patients with RLS, SCL-90 test was used. In untreated patients with RLS, especially somatization, compulsivity, anxiety and depression subscale scores were higher, while in treated patients with RLS, difference in terms of somatization subscale score disappeared, but still compulsivity depression and anxiety subscale scores were higher in treated patients with RLS (21).

In this study, we aimed to investigate obsessive-compulsive symptoms in patients with RLS and compare them with healthy volunteers. In addition to the previously reported relationship between RLS and obsessive compulsive symptoms and psychosomatic diseases, we aimed to examine this relationship in more detail as disturbances in the activity of serotonergic, dopaminergic and other neurotransmitter systems are proposed for obsessions and compulsions in the etiopathogenesis of RLS.

Methods

Our study was carried out in Bezmialem Vakıf University Faculty of Medicine Psychiatry and Neurology Services. Study protocol was approved by the Bezmialem Vakıf University Ethics Committee of non-invasive studies. Bezmialem Vakıf University was approved by the Ethics Committee of non-interventional studies in an acceptable condition (19.08.2017, 16/3).

Participants

Thirty nine patients diagnosed as having RLS according to the diagnosis criteria of the IRLSSG and, age, education and gender-matched forty healthy control subjects were included in the study. All subjects were 18 years of age or older. Patients with RLS symptoms due to comorbid conditions such as pregnancy, kidney failure, endocrine disorders, subjects with alcohol and substance use disorder, and with active major psychiatric disorders such as major depressive disorder, bipolar disorder and schizophrenia and were excluded from the study. By making necessary explanations about the study, all volunteers who agreed to participate in the study signed informed consent forms.

Measurement Tools

Patients diagnosed as having RLS and control subjects underwent Turkish forms of the Structured Clinical Interview for the DSM-IV Axis I Disorders (SCID-1), the socio-demographic data form and the Maudsley Obsessive Compulsive Inventory. In addition, the patient group underwent the RLS severity scale created by the IRLSSG.

The Structured Clinical Interview for the DSM-IV Axis I Disorders (SCID-1): It is a structured clinical interview formed by First et al. (22) for DSM-IV axis I disorders. Validity and reliability of its Turkish form was made by Özkürkçügil et al. (23).

IRLSSG RLS Severity Scale: This scale developed by the IRLSSG is a 10-question self-declaration measure, which is rated by 0-4 points according to the severity of the typical and common symptoms of the disease within the last 1 week before the test date. A total score between 0-10 suggests mild RLS, between 11-20 moderate, between 21-30 severe and between 31-40 very severe RLS (2).

The Maudsley Obsessive Compulsive Inventory (MOCI): It is used to measure the type and severity of obsessive compulsive symptoms. It is a self-declaration scale developed by Hodgson and Rachman (24). As the scale scores increase, it is assumed that the severity of obsessive compulsive symptoms increases. The adaptation of the scale to Turkish, as well as the validity and reliability of the Turkish form were done by Erol and Savasir (25). The scale can be applied to healthy subjects and psychiatric patients. The original scale is consisted of 30 items and contains 4 sub-scales which are called checking, cleaning, slowness and doubting sub-scales. Seven items from Minnesota Multiple Personality Inventory (MMPI) and one sub-scale called rumination are added to the Turkish form of Maudsley obsessive compulsive inventory (MOCI) and as a result it contains 37 items and 5 sub-scales. Measurement is done based on "true" or "wrong" responses that participants give.

Statistical Analysis

Statistical analysis of the data of our study which was planned as a cross-sectional study, was performed using SPSS 24.0 package program. All numerical variables were expressed as mean ± standard deviation, and categorical variables were represented by frequency and probability tables.

Kolmogorov Smirnov and Shapiro-Wilk tests were performed to test the compatibility of the sample with normal distribution in terms of age and educational level. It was shown that it had normal distribution.

Due to the compatibility with the normal distribution, independent samples t-test (Student t-test) was used for comparison of numerical data and the chi-square test was used for comparison of categorical variables. Pearson correlation test was applied to investigate the correlation between numerical data. A p value <0.05 was accepted as statistically significant in all statistical analyses.

Results

The study was planned to be completed with forty-four patients but it completed with 39 patients. One patient was excluded due to being feeble-mindedness, one due to having chronic renal failure, one due to having iron deficiency anemia, two due to meeting the criteria of having a major psychiatric disorder (major depressive disorder in one and alcohol abuse in another) after performing SCID-1, although they reached testing phase. The control group was composed of 40 healthy volunteers involving the staff of Bezmialem Vakıf University Center and their families. The mean age of the patients and control group was 43.4±8.5 years and 39.7±11.7 years, respectively, and no significant differences were found between them. There was no significant difference between the groups in terms of the marital status and educational level (Table 1).

Patient and control groups were compared in terms of MOCI total score and 5 sub-scale mean score. Total MOCI score and mean scores of doubting and rumination sub-scales were significantly higher in the patient group compared with the control group ($p<0.05$). Similarly, mean scores of checking, cleaning and slowness sub-scales were higher in the patient group compared with the control group, without reaching statistical significance (Table 2).

Table 1. Comparison of sociodemographic data of the patient and control groups

	Patient group (n=39)	Control group (n=40)	p
Age	43.4±8.5	39.7±11.7	0.11
Gender			
Female	29 (74.4%)	28 (70.0%)	0.67
Male	10 (25.0%)	12 (30.0%)	
Marital status			
Married	28 (71.8%)	23 (57.5%)	0.35
Single	8 (20.5%)	14 (35.0%)	
Divorced. widow	3 (7.7%)	3 (7.5%)	
Education			
Literate	1 (2.6%)	2 (5.0%)	
Primary school	17 (43.6%)	10 25.0%)	0.32
Secondary school	7 (17.9%)	9 (22.5%)	
Undergraduate	3 (7.7%)	8 (20.0%)	
Postgraduate	11 (28.2%)	11 (27.5%)	

n= Sample size, p= p value

Table 2. Total and sub-scale scores of MOCI of the patient and control groups

	Patient (n=39)	Control (n=40)	p
Checking	2.4±2.5	1.6±1.8	0.097
Cleaning	4.0±2.4	3.0±2.4	0.079
Slowness	1.9±1.5	1.4±1.3	0.175
Doubting	3.3±1.5	2.4±1.7	0.013
Rumination	1.9±1.3	1.2±1.1	0.011
MOCI total	13.4±7.0	9.4±6.2	0.008

n= Sample size, p= p value, Checking= Mean score of checking sub-scale, Cleaning: Mean score of cleaning sub-scale, Slowness= Mean score of slowness sub-scale, Doubting= Mean score of doubting sub-scale, Rumination= Mean score of rumination sub-scale, MOCI-total: Mean of total score of the Maudsley Obsessive Compulsive Inventory

Table 3. Correlation values of RLS severity and MOCI total and sub-scale scores in terms of p value in the patient group

	RLS severity	Checking	Cleaning	Slowness	Doubting	Rumination	MOCI-Total
RLS severity		0.810	0.458	0.460	0.479	0.792	0.500
Checking	0.810		0.01*	<0.01*	<0.01*	0.07	<0.01*
Cleaning	0.458	0.01*		0.013*	0.036*	0.388	<0.01*
Slowness	0.460	<0.01*	0.013*		<0.01*	0.050	<0.01*
Doubting	0.479	<0.01*	0.036*	<0.01*		0.01*	<0.01*
Rumination	0.792	0.07	0.388	0.050	0.01		<0.01*
MOCI-Total	0.500	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	

RLS severity: Mean of total score of restless legs syndrome severity, Checking: Mean score of checking sub-scale, Cleaning: Mean score of cleaning sub-scale, Slowness: Mean score of slowness sub-scale, Doubting: Mean score of doubting sub-scale, Rumination: Mean score of rumination sub-scale, MOCI-total: Mean of total score of the Maudsley Obsessive Compulsive Inventory
Note: Significant correlations were identified with the * sign

There was no statistically significant relationship between RLS severity score and MOCI total and sub-scale scores in the patient group (Table 3). There was positive correlation between mean MOCI total score and all sub-scale scores. MOCI sub-scale scores were found to be correlated with each other except rumination sub-scale. No relation was found between rumination sub-scale and checking, cleaning and slowness sub-scales. Only doubting sub-scale was found to be positively correlated with rumination sub-scale.

Discussion

The main result of our study was that obsessive compulsive symptoms were found more in patients with RLS than in healthy volunteers.

There may be delay in diagnosis of RLS in some cases. Because most of the patients with RLS have difficulty in recognizing and expressing sensory symptoms. Pain which can be easily understood and expressed, is found in about half of the patients with RLS, and is particularly noticeable in aircraft, cinema and similar environments where patients' movements are restricted (26). Since early recognition of RLS and early initiation of treatment will significantly increase the success of treatment, it may be helpful to investigate the possible diagnosis of RLS that accompanies comorbid conditions other than sensory symptoms.

The first study to investigate the comorbidity of psychiatric disorders in patients with RLS was published in 1965. In that study, the MMPI was applied to a group of patients who were admitted to the hospital. Depression and psychasthenia scores in the MMPI were found to be higher in patients with RLS than general patient population and than those who were admitted to psychiatry and (27). Psychiatric comorbidity in RLS has become more interesting especially in the last twenty years. In a study, RLS was shown to be associated with major depressive disorder and panic disorder (28). In another study, it was found that psychiatric disorders, such as major depressive disorder, panic disorder, post-traumatic stress disorder, were associated with RLS according to the DSM-IV criteria (29).

Besides dopaminergic mechanism, glutamaterjic (30), serotoninergic (31) and opioid (32) neurotransmitter mechanisms have also been proposed in the etiology of RLS. Although in obsessions and compulsions, serotonergic and dopaminergic neurotransmitter mechanisms are generally focused on, research is ongoing on other neurotransmitter systems, especially due to high treatment-resistant patients. Various studies have been conducted in OCD examining the disturbances of glutamatergic system (33). A study reported that drugs that are effective over glutamatergic system might be useful in the augmentation treatment of OCD (34). In a recent study published in our country, it was noted that n-methyl d-aspartic acid receptor modulators were effective in rapid treatment of OCD (35).

Reports have been made on the role of opioid system in OCD (36). In a recent study, it was shown that opioid system might play a common role in pathophysiology and treatment of OCD, impulse control disorders and drug addiction and that drugs effecting opioid receptors such as naltrexone and buprenorphine might be effective in some of OCD forms (37).

Although RLS is a neurological syndrome, psychosomatic symptoms often accompany this picture (38). It is also shown that psychosomatic symptoms accompanies OCD. In one study, it was found that body dysmorphic disorder was relatively common in patients with OCD (39). The relationship between OCD and different psychosomatic diseases is remarkable. In particular, there are numerous reports that OCD spectrum disorders are associated with dermatological diseases of psychosomatic origin (40,41).

There are associations in the neurotransmitter mechanisms of RLS and OCD, as well as psychiatric conditions such as impulse control disorders, psychosomatic disorders, depression and anxiety disorders that can accompany both RLS and OCD.

Based on these data, we can explain the frequent occurrence of obsessive compulsive symptoms in patients with RLS in our study for the following reasons:

a) Dysfunctions in serotonine, dopamine and other neurotransmitter systems accompany both RLS and OCD.

b) Both RLS and OCD are frequently associated with psychosomatic disorders.

c) Both RLS and OCD are frequently associated with depression and anxiety.

There are studies indicating that as age progresses, cortical dopamine D2 receptor density decreases (42). As a result of our partial correlation analysis, it was found that the relationship between OCD and RLS was not affected by age. Studies indicate that cortical D2 receptor density varies according to gender (43). Although the number of male patients was significantly lower than the number of female patients in our study, partial correlation analysis showed that the relationship between OCD and RLS was not affected by gender.

There was no significant correlation between RLS severity and MOCI scores in patients. We can link this finding to the excess number of factors affecting both RLS and OCD severity, the presence of roles of other neurotransmitters other than dopamine in both disorders, and the diversity of sub-types reported to be present in both disorders.

One limitation of our study was that the number of male patients (n=10, 25.6%) was significantly lower than the number of female patients (n=29, 74.4%). The number of females who are diagnosed as having RLS is higher than the number of males who are diagnosed as having RLS, which may be a reason for this. However, by doing partial correlation analysis, we found that the relationship between RLS and OCD was not affected by gender. Another limitation was that; we could not directly explain why the mean score of rumination sub-scale was not correlated with the mean score of sub-scales other than doubting subscale, while the mean scores of MOCI sub-scales other than rumination sub-scale were positively correlated with each other. Since rumination and doubting can also be seen in other psychiatric disorders other than OCD such as major depression and paranoid disorders, their correlation with each other as OCD sub-scales and lack of correlation between them and other sub-scales are expected. These data may indicate that the rumination sub-scale, which is not found in the original scale, but is added to the Turkish form during the validity and reliability study, needs further studies in our country. Another limitation of our study was that patients with impulse control disorders or with major psychiatric disorders, such as alcohol and substance abuse disorders were not included in the study. As the presence of these disorders might be a confusing factor in testing our main hypothesis, we excluded them.

Conclusion

But this situation eliminated the possibility of associating the obsessive and compulsive symptoms with impulsive features in patients with RLS and the possibility of differentiating them into subtypes accordingly. In addition, we did not have a chance to determine whether accompanying psychiatric disorders had an impact on OCD symptoms in patients with severity and course of RLS.

As a result, we suggest that the relationship between obsessive compulsive symptoms and RLS should be investigated by further studies searching neurotransmitter mechanisms, especially dopamine and serotonin, accompanied by neuroimaging methods which may also be associated with obsessive compulsive symptoms in young patients. In addition, we suggest that bringing RLS to mind that may accompany young patients with obsessive compulsive symptoms may help increase the early diagnosis of RLS.

Ethics

Ethics Committee Approval: Bezmialem Vakif University was approved by the Ethics Committee of non-interventional studies in an acceptable condition (19.08.2017, 16/3).

Informed Consent: Taken from the patient.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Consept: Y.Ş., O.Y., Design: H.S.Ş., F.B.P., Data Collection or Processing: Y.Ş., O.Y., F.B.P., Analysis or Interpretation: Y.Ş., O.Y., A.Ö., Literature Search: Y.Ş., O.Y., F.B.P., Writing: D.Y., A.Ö.

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References

1. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisi J, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med 2003;4:101-19.
2. Horiguchi J, Hornyak M, Voderholzer U, Kryger M, Skomrow R, Lipinski JF, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med 2003;4:121-32.
3. Cervenka S, Pålhagen SE, Comley RA, Panagiotidis G, Cselényi Z, Matthews JC, et al. Support for dopaminergic hypoactivity in restless legs syndrome: a PET study on D2-receptor binding. Brain 2006; 129:2017-28.
4. Connor JR, Wang X-S, Allen RP, Beard JL, Wiesinger JA, Felt BT, et al. Altered dopaminergic profile in the putamen and substantia nigra in restless leg syndrome. Brain 2009;132:2403-12.
5. Allen RP, Auerbach S, Bahrain H, Auerbach M, Earley C. The prevalence and impact of restless legs syndrome on patients with iron deficiency anemia. Am J Hematol 2013;88:261-4.
6. Merlino G, Lorenzut S, Gigli GL, Romano G, Montanaro D, Moro A. et al. A case-control study on restless legs syndrome in nondialyzed patients with chronic renal failure. Mov Disord. 2010;25:1019-25.

7. Manconi M, Govoni V, De Vito A, Economou NT, Cesnik E, Mollica G et al. Pregnancy as a risk factor for restless legs syndrome. Sleep Med 2004;5:305-8.
8. Trenkwalder C, Paulus W, Walters AS. The restless legs syndrome. Lancet Neurol 2005;4:465-75.
9. Sevim S, Doğu O, Kaleağası H, Aral M, Metin O, Çamdeviren H. Correlation of anxiety and depression symptoms in patients with restless legs syndrome: a population based survey. J Neurol Neurosurg Psychiatry 2004;75:226-30.
10. Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. Behav Brain Res 1998;94:127-52.
11. Stein DJ. Neurobiology of the obsessive–compulsive spectrum disorders. Biol Psychiatry 2000;47:296-304.
12. Earley CJ, Connor J, Garcia-Borreguero D, Jenner P, Winkelman J, Zee PC, et al. Altered brain iron homeostasis and dopaminergic function in Restless Legs Syndrome (Willis-Ekbom Disease). Sleep Med 2014;15:1288-301.
13. Hyacinthe C, De Deurwaerdere P, Thiollier T, Li Q, Bezard E, Ghorayeb I. Blood withdrawal affects iron store dynamics in primates with consequences on monoaminergic system function. Neuroscience 2015;290:621-35.
14. Ghorayeb I, Gamas A, Mazurie Z, Mayo W. Attention-Deficit Hyperactivity and Obsessive-Compulsive Symptoms in Adult Patients With Primary Restless Legs Syndrome: Different Phenotypes of the Same Disease? Behav Sleep Med 2017;17:1-8.
15. American Psychiatric Association, American Psychiatric Association. DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5. American Psychiatric Association; 2013. p 947.
16. Van Ameringen M, Patterson B, Simpson W. DSM-5 obsessive-compulsive and related disorders: clinical implications of new criteria. Depress Anxiety 2014;31:487-93.
17. Altemus M, Swedo SE, Leonard HL, Richter D, Rubinow DR, Potter WZ, et al. Changes in cerebrospinal fluid neurochemistry during treatment of obsessive-compulsive disorder with clomipramine. Arch Gen Psychiatry 1994;51:794-803.
18. Kelly MW, Myers CW. Clomipramine: A tricyclic antidepressant effective in obsessive compulsive disorder. DICP 1990;24:739-44.
19. Goodman WK, McDougle CJ, Price LH, Riddle MA, Pauls DL, Leckman JF. Beyond the serotonin hypothesis: A role for dopamine in some forms of obsessive compulsive disorder? J Clin Psychiatry 1990;51:36-43.
20. Kim J Bin, Koo YS, Eun M-Y, Park K-W, Jung K-Y. Psychosomatic symptom profiles in patients with restless legs syndrome. Sleep Breath 2013;17:1055-61.
21. Scholz H, Benes H, Happe S, Bengel J, Kohnen R, Hornyak M. Psychological distress of patients suffering from restless legs syndrome: a cross-sectional study. Health and quality of life outcomes 2011;9:73.
22. First MB, Spitzer R, Giboobn M, Williams J. Structed Clinical Interview for DSM IV Axis Disorders (SCID-1) Clinical Version. Washington DC and London: American Psychiatric Press;1997.
23. Özkürkçügil A, Aydemir Ö, Yılmaz M, Esen Danacı A, Köroğlu E. DSM-IV Eksen I bozuklukları için yapılandırılmış klinik görüşmenin Türkçeye uyarlanması ve güvenilirlik çalışması. İlaç ve Tedavi Derg 1999;12:233-6.
24. Hodgson RJ, Rachman S. Obsessional-compulsive complaints. Behav Res Ther 1977;15:389-95.
25. Erol N, Savaşır I. Maudsley Obsessif-Kompulsif Soru Listesinin Türkiye Uyarlaması. In: XXIV Ulusal Psikiyatri ve Nörolojik Bilimler Kongresi Bildiri Kitabı. Ankara: Gata Basımevi: 1988.p.107-14.
26. Merlino G, Valente M, Serafini A, Gigli GL. Restless legs syndrome: diagnosis, epidemiology, classification and consequences. Neurol Sci 2007;28:37-46.
27. Gorman CA, Dyck PJ, Pearson JS. Symptom of Restless Legs. Arch Intern Med 1965;115:155-60.
28. Lee HB, Hening WA, Allen RP, Kalaydjian AE, Earley CJ, Eaton WW, et al. Restless Legs Syndrome is Associated with DSM-IV Major Depressive Disorder and Panic Disorder in the Community. J Neuropsychiatry Clin Neurosci 2008;20:101-5.
29. Cho S-J, Hong JP, Hahm B-J, Jeon HJ, Chang SM, Cho MJ, et al. Restless Legs Syndrome in a Community Sample of Korean Adults: Prevalence, Impact on Quality of Life, and Association with DSM-IV Psychiatric Disorders. Sleep 2009;32:1069-76.
30. Allen RP, Barker PB, Horská A, Earley CJ. Thalamic glutamate/glutamine in restless legs syndrome: increased and related to disturbed sleep. Neurology 2013;80:2028-34.
31. Jhoo JH, Yoon I., Kim YK, Chung S, Kim JM, Lee SB, et al. Availability of brain serotonin transporters in patients with restless legs syndrome. Neurology 2010;74:513-8.
32. Walters AS, Ondo WG, Zhu W, Le W. Does the endogenous opiate system play a role in the Restless Legs Syndrome? A pilot post-mortem study. J Neurol Sci 2009;279:62-5.
33. Pittenger C, Bloch MH, Williams K. Glutamate abnormalities in obsessive compulsive disorder: Neurobiology, pathophysiology, and treatment. Pharmacol Ther 2011;132:314-32.
34. Marinova Z, Chuang D-M, Fineberg N. Glutamate-Modulating Drugs as a Potential Therapeutic Strategy in Obsessive-Compulsive Disorder. Curr Neuropharmacol 2017;15:977-95.
35. Köse S, Çetin M. Ketamine and rapastinel: NMDA receptor modulators in the rapid treatment of obsessive compulsive disorder. Psychiatry Clin Psychopharmacol 2017;27:213-4.
36. Urraca N, Camarena B, Gómez-Caudillo L, Esmer MC, Nicolini H. μ opioid receptor gene as a candidate for the study of obsessive compulsive disorder with and without tics. Am J Med Genet Part B Neuropsychiatr Genet 2004;127:94-6.
37. Fontenelle LF, Oostermeijer S, Harrison BJ, Pantelis C, Yücel M. Obsessive-compulsive disorder, impulse control disorders and drug addiction. Drugs 2011;71:827-40.
38. Kim JB, Koo YS, Eun MY, Park KW, Jung KY. Psychosomatic symptom profiles in patients with restless legs syndrome. Sleep Breath 2013;17:1055-61.

39. Phillips KA, Gunderson CG, Mallya G, McElroy SL, Carter W. A comparison study of body dysmorphic disorder and obsessive-compulsive disorder. J Clin Psychiatry 1998;59:568-75.

40. Folks DG, Warnock JK. Psychocutaneous disorders. Curr Psychiatry Rep 2001;3:219-25.

41. Zhu TH, Nakamura M, Farahnik B, Abrouk M, Reichenberg J, Bhutani T, et al. Obsessive-compulsive skin disorders: a novel classification based on degree of insight. J Dermatolog Treat 2017;28:342-6.

42. Kaasinen V, Vilkman H, Hietala J, Någren K, Helenius H, Olsson H, et al. Age-related dopamine D2/D3 receptor loss in extrastriatal regions of the human brain. Neurobiol Aging 2000;21:683-8.

43. Kaasinen V, Någren K, Hietala J, Farde L, Rinne JO. Sex Differences in Extrastriatal Dopamine D 2 -Like Receptors in the Human Brain. Am J Psychiatry 2001;158:308-11.

Original Article

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Malaria Prophylaxis and Vaccinations Among Turkish International Travelers: National Data, 2011-2016

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ABSTRACT

Objective: The number of international travelers is increasing steadily. As a result, there is also an increase in travel-related infections. There is not enough data on travel vaccinations and prophylaxis in Turkey. The aim of this study is to create awareness and to guide national politics by presenting national data of Turkey.

Methods: Travelers who took travel health services (THS) at travel health centers (THC) during 2011-2016 were included in the study. The data in the THC's record system were examined for age, sex, purpose of travel, duration of stay, country of destination, learning source of THS, vaccinations and malaria prophylaxis.

Results: During the study period, 162,023 people took THS. There was a 57% increase in the number of travelers who took THS in 2016 compared to 2011. There was no significant increase in the incidence of THS in Turkish travelers going abroad (0.33% and 0.43%, respectively in 2011 and 2016) (rs=0.36 p=0.47). The most common reason for traveling was business trips (79%), the most visited region was Africa (65.7%). The percentages of yellow fever vaccine, typhoid vaccine and malaria prophylaxis were 86.7%, 28.3%, and 44.7%, respectively.

Conclusion: The main reason for travel was business and the most frequent destination was Africa. The number of travelers who took THS in travelers going abroad was very low in our country. It was concluded that awareness of travel-related health risks and preventive measures in the community should be increased.

Keywords: Travel health, malaria prophylaxis, travel vaccination

Introduction

In the world, millions of people travel every day for various reasons. Despite the threats emerging due to the outbreaks such as influenza and severe acute respiratory syndrome , there has been an on-going growth in tourism during the last six decades (1). Destinations around the world welcomed about one billion international tourists in 2016 (2). Travelers are exposed to a variety of health risks in unfamiliar areas and are involved in the spread of global infections (3). Health risks that travelers may face are determined by the level of development of the destination, season, duration, purpose of travel (trip, to work in rural areas, visit friends etc.), hygiene standards of accommodation, activities (camping, diving, hunting), age and health condition of travelers (4,5). Malaria, dengue, enteric fever, chikungunya, non-specific viral syndromes, rickettsioses are the most common infectious diseases among international travelers (6) Most of travel-related health risks can be prevented or minimized by health measures to be taken before, during and after travel. Travel health services (THS) are important for travel-related diseases, because they provide vaccines, pre-travel advice and other preventive measures such as information on preventing insect bites, food and water safety precautions (7).

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In Turkey, Ministry of Health, Travel Health Centers (THC) have provided THS for many years. There is not enough data in the literature about travel vaccinations and prophylaxis in Turkey. The aim of this study is to discuss the topic with regard to possible improvements, to create awareness and to guide national politics by presenting national data of Turkey.

Methods

In Turkey, THSs (pre-travel advice, vaccinations and malaria prophylaxis) are provided by THC run by the Ministry of Health, Turkey Directorate General of Health for Borders and Coastal Areas. THCs are also authorized centers for issuing an "International Vaccination and Prophylaxis Certificate" in Turkey. Pre-travel advice, vaccinations and malaria prophylaxis are provided in line with the national, US Centers for Disease Control and Prevention and World Health Organization guidelines. Travel health call center connected to the General Directorate also make counseling and travelers directed to THCs, if required.

All international travelers who attended THCs during 1 January 2011-31 December 2016 were included in this study. The following data about international travelers were collected retrospectively from the Directory's THCs record system: Age, sex, purpose of travel, duration of stay, and country of destination, recommendation of vaccines and malaria prophylaxis and learning sources of THCs. The age of travelers categorized into 3 groups (<15 years, 15-45 years,> 45 years). Time of travel was defined as less than 10 days and more than 10 days. Purposes of travel were divided into four groups (Business, holiday, residency and others). All variables were categorical and descriptive statistics were defined as percentages and numbers. Data processing was performed in Microsoft Office Excel 2007 and SPSS (version 16.0). Pearson correlation test was used for correlation of incidence of THS by years.

The study was approved by the Ethics Committee of Bezmialem Foundation University (decision date/number: 16-01-2018 / 2/10). Written consent was not obtained from patients because the research was done retrospectively.

Results

During the study period, THS were provided for 162,023 people. The number of travelers who took THS was shown in Figure 1. The number of total international Turkish travelers increased by 57% and 19.4%, respectively in 2016 compared to 2011 (8). The incidence of THS for total Turkish travelers was found to be 0.33% and 0.43%, respectively in 2011 to 2016. There was not a significant correlation between the incidence of THS and years (rs=0.36 p=0.47).

Demographic and travel characteristics of the travelers who took THS are shown in Table 1. Of all the travelers; 82.8% were male, 77.8% were between 15-45 years old, and 1.6% were under 15 years old. Of all the travelers; 91 (0.06%) were pregnant, 92 (0.06%) were nursing mothers, 16.9% were seafarers and 8.9% were flight staff travelling internationally due to their occupational status.

In most of these travels, the travel purpose was business (79%). The second most common travel purpose was holiday (11.6%). When the duration of travel was evaluated, most of trips lasted more than 10 days (61%). When we assessed the travelers according to the regions of destination, African countries were at the top (65.7%). Distribution of the first six most frequently visited regions are shown in Table 1.

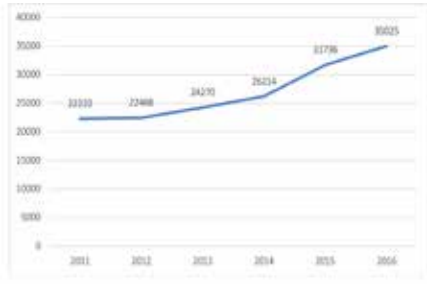

Figure 1. The number of travelers who took travel health services by years

Table 1. Demographic and travel characteristics of the travelers (2011-2016)

Features	n (%)
Sex	
Male	134,113 (82.8)
Age	
<15	2,598 (1.6)
15-45	126,084 (77.8)
>45	33,341 (20.6)
Pregnant	91 (0.06)
Nursing	92 (0.06)
Seafarers	27,436 (16.9)
Flight staff	14,557 (8.9)
Destination	
East Africa	39,158 (24)
West Africa	39,354 (24)
Central Africa	19,083 (12)
South and Southeast Asia	8,565 (5.3)
South Africa	7,534 (4.6)
South America	4,648 (2.9)
Purpose of travel	
Busines	128,117 (79)
Holiday	18,881 (11.6)
Other	15,025 (9.4)
Duration of stay (day)	
<10	62,729 (39)
>10	99,294 (61)
Chronic diseases	
Yes	3,924 (2.4)

Rates of vaccinations and malaria prophylaxis given are shown in Table 2. Of travelers, 86.7% were vaccinated with yellow fever vaccine. Typhoid vaccine and malaria prophylaxis rates were 28.3% and 44.7%, respectively during study period.

The distribution of the drugs for malaria prophylaxis is shown in Table 3. Doxycycline was most frequently (41.2%) used drug for malaria prophylaxis during study period. Atovaquone-proguanil and Mefloquine were used in 35.4% and 23.4% of the travelers, respectively.

Resources from which THS were learned are shown in Table 4. Most of the travelers were directed to the THCs by their companies (31.4%). Of travelers, 13.4% came on the advice of their friends and 6.9% of them learned the THCs through the internet.

Discussion

The number of international travelers in Turkey have been increasing day by day like the rest of the world. Traveling from Turkey to different regions for various reasons such as business, holiday, congress, reached up to eight million in 2016 (8). Health risks during international travel are determined by many factors like destination, region, age and health status of the traveler, duration and type of the travel and the planned activities (4). According to the data of the GeoSentinel Surveillance Network, the region where international travelers encounter diseases most frequently is Sub-Saharan Africa (23%) and the most frequent diseases are acute diarrhea (22%), inflammatory/systemic diseases (14%) and dermatological diseases (12%) (9). People travelling to tropical and subtropical countries may face different infectious diseases and may cause transmission of these diseases to the countries where they are not endemic (10,11). Due to its extensive geographical distribution and potential fatal results, malaria is one of the most important travel-related diseases. Malaria is diagnosed in 29% of returnees with fever and seen mostly in passengers returning from Africa (12,13). Most of these cases are related to Sub-Saharan Africa and in 60% of them, the agent is *Plasmodium falciparum* (14). Vaccine-preventable diseases also take an important place among travel-related infections (9). Vaccine-preventable diseases (enteric fever, acute viral hepatitis and influenza most frequently) are seen in 1.5% of returnees (9).

Table 2. Number of people to whom vaccine and malaria prophylaxis were given (2011-2016)

Vaccine and chemoprophylaxis	n (%)
Yellow fever	140,423 (86.7)
Typhoid	45,954 (28.3)
Malaria prophylaxis*	72,481 (44.7)

*Malaria prophylaxis: Mefloquine, atovaquone-proguanil, doxycycline

Table 3. Distribution of malaria prophylaxis medications (2011-2016)

Malaria prophylaxis medications	n (%)
Doxycycline	29,883 (41.2)
Atovaquone-proguanil	25,701 (35.4)
Mefloquine	16,897 (23.4)
Total	72,481 (100)

Table 4. Resources from which travel health services were learned (2011-2016)

Sources	n (%)
Company	50.709 (31.4)
Friends	21.335 (13.3)
Internet	11.222 (6.9)
Travel health call center	3.463 (2.2)
Other	16.616 (10)
No data	58.678 (36.2)

THS should be received before travel in order to take necessary advice for these health risks, to be vaccinated and to begin to use protective medications (15). Ideally, the most convenient time to do this is 4-6 weeks before travel, but it is important to benefit from this service even if it is the last week. In pre-travel consultation, all factors should be assessed and personal advice should be given. Vaccination is the most important component of this consultation (4).

In Turkey, Ministry of Health, Turkey Directorate General of Health for Borders and Coastal Areas is giving THS by THCs, travel health call center and official travel health website (www.seyahatsagligi.gov.tr). Travel health website give information about infectious diseases seen in countries, required and recommended vaccinations, malaria prophylaxis, and other travel related health risks. When the number of people travelling internationally (eight million) every year in Turkey is considered, it is seen that the number of people for whom we provide THS is quite low. Although the number of travelers receiving THS has increased over the years (57%), there was not a significant correlation in the incidence of THSs by years (rs=0.36 p=0.47). In the studies performed before, it was also reported that most of the travelers did not receive consultation before travel, and most of those who receive consultation, did this through friends, travel agencies, pharmacies and internet and they rarely visit doctors (15-17). In a study on travelers going to Africa, it was shown that only half of the passengers received pre-travel consultancy (18).

Evaluation of our study group for demographic features indicated that similar to other studies, most of the travelers were male and young/middle-aged (16-18). While in our study, coexisting diseases were detected in 2.4% of the travelers, in the reports of Global TravEpiNet, coexisting health problems were notified in 53% of travelers (19). When we assessed the travelers for travel purposes, we concluded that in comparison to similar studies, the travel rates for business purposes were high whereas for holiday purposes were low (18,20,21). Global TravEpiNet has also declared that according to the collected data, most frequently reported travel purpose is holiday (49%) and the others are business (15%), service work (15%) and visiting relatives (11%) (19).

In our study, yellow fever vaccination rate (86.7%) was higher in comparison to other studies (5,18,20,22). This outcome was thought to be the result of THCs' being the only authorized ones in the country for yellow fever vaccination and yellow fever vaccine is an obligatory vaccine. When we consider that most of travelers went to typhoid endemic regions for business purposes and for long durations, typhoid vaccination rate (28.3%) was lower than expected (16,23). In Turkey, for other routine vaccinations, travelers are directed to family physicians. It has been evaluated that providing these vaccinations in THCs is important for increasing and pursuing the other vaccination rates.

The most frequent travel destinations of Turkish travelers were African countries (61.4%). It is thought that the most important reason for such a high rates for travelling to African countries is the increase in business relations between our country and African countries in recent years. Another reason is that the African countries are endemic for yellow fewer and vaccination is required for entering these countries. On the website of Global TravEpiNet, it has been reported that more than 80% of travelers visit the countries with less resources and that Africa is the most frequently visited region (20). In many other studies, Africa has also been reported as the most visited region (22). It has been understood that travelers who contact us are mostly directed by companies or are recommended by their friends and that their awareness about travel health is low.

Conclusion

The number of travelers going abroad who take THS are very low in our country. We conclude that, we should reach out more people in order to access the purpose of THS. The awareness of travel-related health risks and preventive measures in the community should be increased. For this purpose, it can be planned to inform people who plan to travel abroad about THS through travel agencies and aviation companies and to raise awareness by preparing informative publications in the national press.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Bezmialem Foundation University (decision date/number: 16-01-2018 / 2/10).

Informed Consent: Written consent was not obtained from patients because the research was done retrospectively.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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References

1. Keystone JS, Kozarsky PE, Freedman DO, Connor BA, Nothdurft HD. eds. Travel Medicine, 3rd Edition. Saunders, Edinburgh: Elsevier 2013.
2. Close to one billion international tourists in the first nine months of 2016 07-Nov-2016 [Available from: http://media.unwto.org/press-release/2016-11-07/close-one-billion-international-tourists-first-nine-months-2016.
3. Gautret P, Schlagenhauf P, Gaudart J, Castelli F, Brouqui P, von Sonnenburg F, et al. Multicenter EuroTravNet/GeoSentinel study of travel-related infectious diseases in Europe. Emerging Infect Dis 2009;15:1783-90.
4. Chen L. The Pre-travel Consultation. Brunette G, editor. New York: Oxford University Press; 2014.
5. LaRocque RC, Rao SR, Lee J, Ansdell V, Yates JA, Schwartz BS, et al. Global TravEpiNet: a national consortium of clinics providing care to international travelers--analysis of demographic characteristics, travel destinations, and pretravel healthcare of high-risk US international travelers, 2009-2011. Clinical Infectious Dis 2012;54:455-62.
6. Sotir MJ LR. Travel epidemiology. GW B, editor. New York: Oxford University Press; 2016. 11-4p.
7. Wieten RW, van der Schalie M, Visser BJ, Grobusch MP, van Vugt M. Risk factors and pre-travel healthcare of international travellers attending a Dutch travel clinic: a cross-sectional analysis. Travel Med Infect Dis 2014;12:511-24.
8. Our citizens traveling abroad and tourism expenses. Number of citizens going abroad by years [Available from: http://www.tuik.gov.tr/jsp/duyuru/upload/yayinrapor/Vatandas_Giris_Anketi_Raporu_2013.pdf.
9. Boggild AK, Castelli F, Gautret P, Torresi J, von Sonnenburg F, Barnett ED, et al. Vaccine preventable diseases in returned international travelers: results from the GeoSentinel Surveillance Network. Vaccine 2010;28:7389-95.
10. Khan K, Arino J, Hu W, Raposo P, Sears J, Calderon F, et al. Spread of a novel influenza A (H1N1) virus via global airline transportation. N Engl J Med 2009;361:212-4.
11. Freedman DO, Weld LH, Kozarsky PE, Fisk T, Robins R, von Sonnenburg F, et al. Spectrum of disease and relation to place of exposure among ill returned travelers. N Eng J Med 2006;354:119-30.
12. Harvey K, Esposito DH, Han P, Kozarsky P, Freedman DO, Plier DA, et al. Surveillance for travel-related disease--GeoSentinel Surveillance System, United States, 1997-2011. MMWR Surveill Summ 2013;62:1-23.
13. Leder K, Torresi J, Libman MD, Cramer JP, Castelli F, Schlagenhauf P, et al. GeoSentinel surveillance of illness in returned travelers, 2007-2011. Ann Intern Med 2013;158:456-68.
14. Leder K, Black J, O'Brien D, Greenwood Z, Kain KC, Schwartz E, et al. Malaria in travelers: a review of the GeoSentinel surveillance network. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;39(8):1104-12.

15. Gherardin T. The pre-travel consultation-an overview. Australian family physician 2007;36:300-3.
16. Van Herck K, Zuckerman J, Castelli F, Van Damme P, Walker E, Steffen R. Travelers' knowledge, attitudes, and practices on prevention of infectious diseases: results from a pilot study. J Travel Med 2003;10:75-8.
17. Wilder-Smith A, Khairullah NS, Song JH, Chen CY, Torresi J. Travel health knowledge, attitudes and practices among Australasian travelers. J Travel Med 2004;11(:9-15.
18. Pavli A, Spilioti A, Smeti P, Patrinos S, Maltezou HC. Vaccination and Malaria Prevention among International Travelers Departing from Athens International Airport to African Destinations. J Trop Med 2014;2014:563-30.
19. Regina C L, David O. Freedman, Mark J. Sotir. Travel Medicine Data Collection: Geosentinel and Global Travepinet. Brunette GW, editor. New York: Oxford University press;2014.
20. LaRocque RC, Rao SR, Tsibris A, Lawton T, Barry MA, Marano N, et al. Pre-travel health advice-seeking behavior among US international travelers departing from Boston Logan International Airport. J Travel Med 2010;17:387-91.
21. Heywood AE, Watkins RE, Iamsirithaworn S, Nilvarangkul K, MacIntyre CR. A cross-sectional study of pre-travel health-seeking practices among travelers departing Sydney and Bangkok airports. BMC public health 2012;12:321.
22. Heudorf U, Tiarks-Jungk P, Stark S. [Travel medicine and vaccination as a task of infection prevention--data of the special consultation hours of the public health department Frankfurt on the Main, Germany, 2002-2004]. Gesundheitswesen 2006;68:316-22.
23. Lopez-Velez R, Bayas JM. Spanish travelers to high-risk areas in the tropics: airport survey of travel health knowledge, attitudes, and practices in vaccination and malaria prevention. J Travel Med 2007;14:297-305.

Original Article

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Measurement of Impedance Values of Different Erythrocyte Suspensions

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ABSTRACT

Objective: The study was aimed to determine whether the impedance measurements of erythrocyte suspensions can be used with a different calculation method in determining the degree of erythrocyte aggregation.

Methods: Impedance measurements of different erythrocyte suspensions were recorded in horizontal glass capillaries with a haematocrit value of 40% with inductance-capacitance-resistance meter after a stop flow generated by the injector pump.

Results: As a result of calculating the impedance values of the samples for which the aggregation in phosphate-buffered saline didn't occur, the impedance values of the samples that aggregation at different grades took place were calculated as in the previous measurements of erythrocyte suspensions time course was significantly decreased for in diluted plasmas and was increased for in the 1% dextran 500 solution. It is obvious that aggregation index (AI) was calculated, using Z data exhibited a similar trend for the diluted plasma and plasma with 1% dextran 500, with a rank order of Dextran 500 >whole blood >1/2 diluted plasma.

Conclusion: Although impedance measurements of erythrocyte suspensions don't allow for the calculation of erythrocyte aggregation kinetics, it is thought that they can be used as AI which indicates the degree of erythrocyte aggregation.

Keywords: Electrical measurements, impedance, capacitance, red blood cell aggregation

Introduction

Aggregation give rise to erythrocyte surfaces parallel to each other and clustering in a special way. Erythrocyte aggregation occurs only in the suspension phase in the presence of a certain size and structure of macromolecules. This clustering does not occur when the same cells suspended in simple salt solutions. Factors affecting red blood cell (RBC) aggregation can be divided into intrinsic and extrinsic factors: (1) Extrinsic factors include levels of plasma proteins [exempli gratia (e.g.), fibrinogen, macroglobulins], hematocrit and shear rate; (2) Intrinsic factors include RBC shape, deformability and membrane surface properties (1-3)

Aggregation can be altered during pathophysiological processes due to modifications of both plasma composition (e.g., in acute phase reactions) and cellular factors (e.g., increased oxidant stress) (3-5) RBC aggregation affects the flow properties of blood, especially at low shear rates, and therefore has the potential to influence blood flow in the circulatory system (6). The factors determining RBC aggregation could be classified in two groups, extracellular and cellular. The extracellular factors are: flow condition, concentration of plasma proteins (fibrinogen, immunoglobulins, albumin etc.), the presence of other macromolecules (e.g. dextrans), pH-value, osmotic pressure. The cellular factors are: the glycocalyx, determines kinetics, and extent of the contact area between the cells and so the rate and the strength of the aggregation. For example glutaraldehyde treatment of RBC results in intra- and intermolecular crosslinking membrane and cytosol constituents with drastically alteration of membrane viscoelastic and cytosol

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viscous properties with reduction in cell deformability and aggregation. Direct measurement of RBC aggregation has not been frequently used in routine clinical laboratories. The RBC aggregation characteristics can be analyzed using various methods and techniques which are described in the literature (7). Briefly, measurement of erythrocyte sedimentation rate (ESR) (8), low-shear viscometry (9), image analysis (10), dielectric analysis (11), ultrasound backscattering (12) electrical properties (13,14) and photometry (15) are among the methods which have been employed to quantify aggregation.

There are some reports assert that measuring the electrical properties of erythrocyte suspensions can be used for evaluation of erythrocyte aggregation (7,14,16,17). It was reported that electrical properties of erythrocyte suspensions showed a similar trend as photometric measurement and the parameters associated with aggregations could be calculated by using these records (13,16,18). In our previous study, we recorded the photometric and electrical properties of suspensions simultaneously during the erythrocyte aggregation through a glass capillary tube with a diameter of 800 μm. Recorded light transmittance-time curves (LT) showed a great similarity to the typical curves reflecting erythrocyte aggregation process (7,15,19,20).

This study was designed to compare the time course of electrical impedance and capacitance during RBC aggregation in a horizontal glass tube. RBC suspended in non-aggregating and aggregating media were investigated; comparisons between measured modalities were based on calculated aggregation indexes that are accepted to reflect RBC aggregation (16) and are used by commercial instruments (15). In this study, it is aimed to determine whether the impedance measurements of erythrocyte suspensions can be used with a different calculation method in determining the degree of erythrocyte aggregation.

Methods

Blood Samples and Preparation of Red Blood Cell Suspensions

Venous blood samples were obtained from 10 healthy male volunteers, aged between 20 to 25 years, following the guidelines for hemorheological laboratory methods (16). The study was approved by the Clinical Research Ethical Committee of Bezmialem Vakıf University (19.09.2018). Informed consent was obtained from all participants, and study was conducted according to the Declaration of Helsinki Principles. In addition, adult male Wistar albino rats weighing between 250-300 g were used in this study. Blood samples were taken from abdominal aorta under ketamine-xylazine anesthesia from ten animals into sodium heparin (15 IU/mL). All animal use procedures were approved by the Laboratory Animals Ethics Committee, Bezmialem Vakıf University. Blood was centrifuged at 1400 g for 5 min, and plasma was separated carefully and saved. RBC were washed twice with isotonic phosphate-buffered saline (PBS, pH=7.4). Samples were immediately evaluated, and studies on samples were completed within four hours following the blood drawing.

Modification of Red Blood Cell Aggregation: Red Blood Cell Suspended in Diluted Plasma

Plasma was diluted with PBS at ratios of 1/2 corresponding to 50% of the plasma components in undiluted plasma. Washed RBC from each donor were re-suspended either in autologous plasma (control) or in diluted plasma at 0.4 l/l hematocrit.

Modification of Red Blood Cell Aggregation: Red Blood Cell Suspended in 1% Dextran 500

Washed RBC from each donor were suspended in plasma containing 1% Dextran 500 (500 kDa, Sigma Chemical Company, St. Louis, MO, USA) at 0.4 l/l hematocrit.

Modification of Red Blood Cell Aggregation: Glutaraldehyde Treatment of Red Blood Cell

Washed RBC from each donor were re-suspended in PBS at 0.05 l/l hematocrit, following which glutaraldehyde (GA, Merck, Darmstadt, Germany) was added to achieve a final concentration of 0.003% and the suspension incubated at room temperature (22 °C) for 30 min. RBC were then washed three times with PBS after the incubation and re-suspended in autologous plasma at 0.4 l/l hematocrit.

Measurement System and Experimental Protocol

RBC aggregation in glass capillaries with internal diameters of 800 μm was assessed by monitoring electrical impedance during aggregation process. The measurement system is outlined in Figure 1. Electrical impedance of RBC suspensions were measured in horizontally-alligned, 75 mm long capillaries, across the two stainless steel electrodes attached to both ends of the capillary. The measurement chamber (glass capillary and electrodes) were placed in a temperature-controlled box and maintained at 37 °C. A computer controlled inductance-capacitance-resistance (LCR)-meter (Hioki, 3532 LCR HiTester, Nagano, Japan) was used for continuous monitoring of electrical impedance parameters between these electrodes, during the RBC aggregation process. The instrument was operated in series mode and a two-electrode configuration was applied. Briefly, the LCR meter applies a test signal across the electrodes at 100 kHz and 1 volt constant voltage (V), measures the resulting current (I) and phase angle (θ) between V and I.

RBC suspensions were pumped through the capillary with a syringe pump (World Precision Instruments, Aladdin-4000, Florida, USA) at a calculated flow rate to generate a wall shear rate of ~500 s-1, for 5 s, to obtain complete disaggregation, at the start of each measurement procedure. Electrical impedance parameters were recorded at 1 s intervals for the 120 s following the stoppage of flow in the capillary tube, via a RS-232 interface on a digital computer.

Calculations and Statistics

It is known that when erythrocyte aggregation is examined by its property of light reflection or transmittance, the course of time can be modelled by a curve with double-exponential Time courses of C and Z data recorded during the aggregation process

after abruptly stopping flow were used to calculate aggregation index (AI) which is equivalent to those reported by commercially-available RBC aggregometers (Figure 2). Calculations were conducted using a special software written in LabView 8.6 (National Instruments, Austin, Texas, USA). The results of the measurements on RBC suspensions with normal and modified aggregation are expressed as mean ± standard error. Statistical comparisons were done using one-way analysis of variance followed with Bonferroni's multiple comparison test.

Results

Time Course of C and Z During Red Blood Cell Aggregation

Figure 3a demonstrates the time course C, recorded using the same RBC suspensions prepared in 1/2 diluted plasma, dextran and whole plasma. There is a sharp decrease in C following the stoppage of flow, reaching to a minimum in a few second. This valley is followed by increased C with a characteristic time course. The initial phase of decreased C reflects the recovery of RBC morphology for cells initially deformed by shear forces during high-shear flow (corresponding to ~500 s-1 shear rate), with the shape changing from an elongated form to the normal biconcave-discoid shape. The later phase of gradual increases of C reflects RBC aggregate formation which follows a characteristic time course.

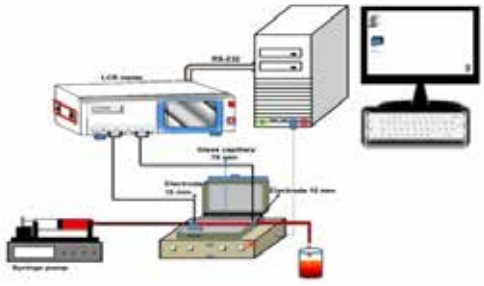

Figure 1. Measurement system
LCR: Inductance-capacitance-resistance

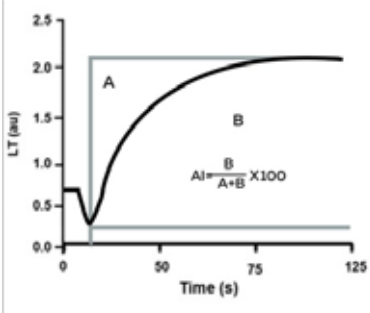

Figure 2. Calculation procedure for aggregation parameters, using light transmittance -time data. "A" and "B" are the areas of the surfaces above and below the curve, respectively. Aggregation index is equal to B/(A+B)
AI: Aggregation index, LT: Light transmittance

Capacitance recorded in RBC suspension in dextran, whole blood and 1/2 diluted plasma exhibited similar pattern, although the time course was significantly slower in 1/2 diluted plasma. In capacitance measurements, the changes of plasma samples diluted by PBS after the flow stopped showed a little peak than the plasma samples not diluted by PBS (Figure 3a). There are significant changes in the capacitance measurements in all samples.

The time course of the change in Z after reaching to the peak values were similar to each other for RBC suspensions in PBS, diluted and undiluted plasma (Figure 3b). A finding of this study was the time course of Z changes for non-aggregating suspensions of RBC in PBS. Z changes had the same pattern as those for aggregating suspensions, while such similarity between aggregating and non-aggregating suspensions was not observed for C.

Aggregation Characteristics of Red Blood Cell Suspensions

RBC aggregation parameters calculated using syllectogram recorded from different RBC suspensions were compared by analyzing data obtained using samples with whole blood, decreased (i.e., 1/2 diluted plasma), and enhanced (i.e., 1% Dextran 500) aggregation. Aggregation parameters for these samples, as measured by a widely used commercial erythrocyte aggregometer [i.e., Laser-assisted optical rotational cell analyzer (LORCA)], indicated the expected differences (Table 1) (14,18).

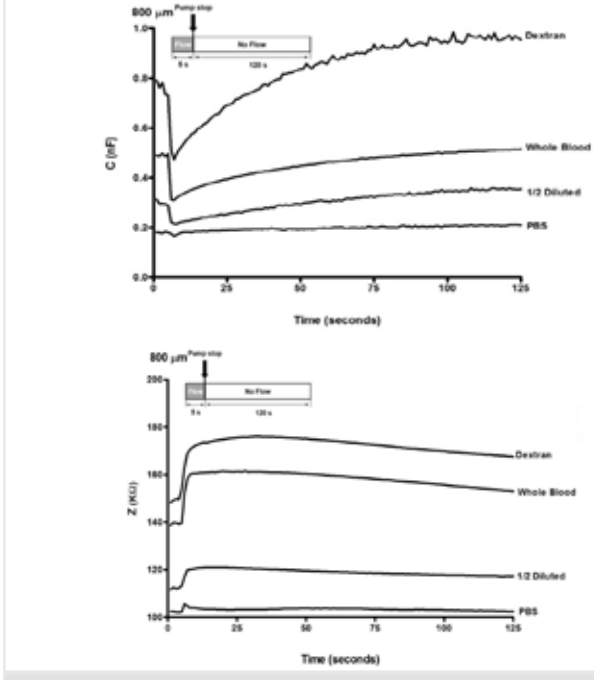

Figure 3. (a) Time course of series capacitance (C) and (b) Impedance (Z) across the glass capillary after a sudden stop of flow of red blood cell suspended in dextran, whole blood and 1/2 diluted plasma. Capacitance and impedance were monitored at 100 KHz frequency
PBS: Phosphate-buffered saline

The AI parameter was significantly decreased for RBC suspended in 1/2 diluted plasma and was increased for RBC suspended in the 1% dextran 500 solution. It is obvious that AI calculated using C data exhibited a similar trend for the diluted plasma and plasma with 1% dextran 500, with a rank order of plasma + D500 >undiluted plasma >1/2 diluted plasma. This trend was not seen when using *Z* data.

Electrical Characteristics of Red Blood Cell -plasma Suspensions and Red Blood Cell Aggregation Indexes Calculated Using Z Time Course

In Figure 4, it is seen that the impedance values of the blood samples prepared in the second minute after stopping the flow are increased. Impedance values in PBS and 40% hematocrit adjusted samples in plasma showed statistically significant increases in both PBS and plasma samples without erythrocytes ($p<0.001$). An interesting result is that although the hematocrit ratios are the same, the impedance value of erythrocyte suspension prepared in plasma is significantly different from the PBS sample ($p<0.001$). However, there was no significant difference between PBS and plasma in which there was no cell ($p>0.05$).

In Figure 5, the impedance values of the erythrocyte suspension in the plasma are increased due to the increase of the hematocrit value. Gradually increasing the hematocrit values from 20% to 40% to 60% by addition erythrocyte into the plasma significantly increases the impedance values ($p<0.001$). As shown in Figure 4 and Figure 5, there is a significant difference between the impedance values of erythrocyte suspensions in PBS and plasma with the same hematocrit value (40%). This shows us that the value of the impedance changes according to both the properties of the suspension environment and the number of cells it contains. The impedance values of samples with 40% hematocrit prepared in PBS and the impedance values of samples with 20% hematocrit in plasma shown in Figure 5 are very close to each other. This may also be related to the behavior of erythrocytes, besides the number of cells and suspension medium. It is known that erythrocyte aggregation does not occur in simple salt solutions in which there are no macromolecules. Higher values of impedance in samples prepared in plasma may be associated with erythrocyte aggregation. The rise in the impedance value with the increase in hematocrit also explains this case.

In simple salt solutions (eg PBS and 0.9% NaCl) in which no macromolecule is present, rouleaux formation does not occur. As shown in Figure 6, the impedance values of PBS and 0.9% NaCl are very close to each other. Erythrocytes in suspension are responsible for the increased impedance in 40% erythrocyte suspension prepared in PBS. When the PBS and/or 0.9% NaCl values are compared with the plasma, the impedance value of the plasma is higher. When the PBS and/or 0.9% NaCl values are compared with the plasma, the impedance value of the plasma is higher. Although this difference is not significant ($p>0.05$), it is thought that the macromolecules in the plasma may be

Table 1. Aggregation Index values by the commercial erythrocyte aggregometer (i.e., Laser-assisted optical rotational cell analyzer, Myrenne Aggregometer) (14,18) Impedance and Capacitance for red blood cell suspensions prepared in autologous plasma, ½ diluted autologous plasma with phosphate-buffered saline corresponding to the 50% of plasma components and autologous plasma with 1% dextran 500 in. Data are mean ± standard error (n=10)

Aggregation index (AI) (au)	LORCA (AI)	Myrenne (M index)	Capacitance (C)	Impedance (Z)
Whole blood	70.3±2.3	19.9±1.1	79.0±1.7	73.8±2.5
1/2 diluted	32.6±2.4***	2.5±1.0**	60.6±5.1**	59.6±1.5**
1% 500 kDa Dextran	82.1±1.5*	33.2±1.6**	84.4±1.8*	69.8±5.3
PBS	ND	ND	ND	34.5±2.3**

Difference from "whole blood"; *: p<0.05; **: p<0.01, ***: p<0.001. au: arbitrary unit ND: Not determined, AI: Aggregation index, PBS: Phosphate-buffered saline, LORCA: Laser-assisted optical rotational cell analyzer

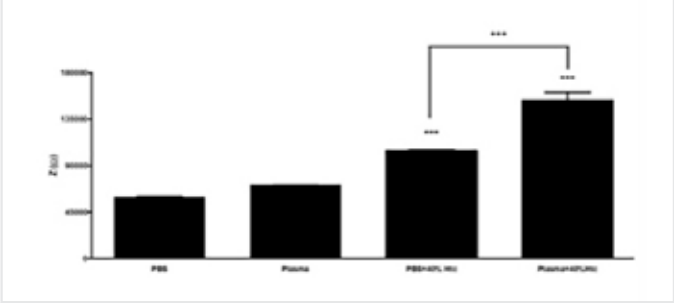

Figure 4. Impedance values of phosphate-buffered saline and plasma samples adjusted to 40% hematocrit. Data are mean ± standard error (n=10). Difference from "phosphate-buffered saline and plasma"; ***: p<0.001, +++: p<0.001

PBS: Phosphate-buffered saline

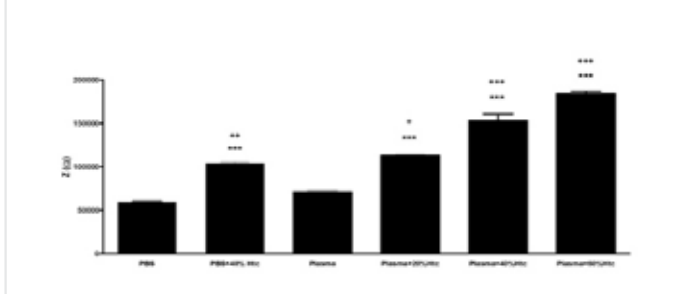

Figure 5. Effect of hematocrit ratio on impedance value. Data are mean ± standard error (n=10). Difference from "phosphate-buffered saline", ***: p<0.001. Difference from "Plasma", +: p<0.05, +++: p<0.01, +++: p<0.001

PBS: Phosphate-buffered saline

responsible for this increment (eg, fibrinogen, etc.). It is assumed that this significant difference (p<0.001) between the two samples that only the suspension medium is different with the same hematocrit represents erythrocyte aggregation.

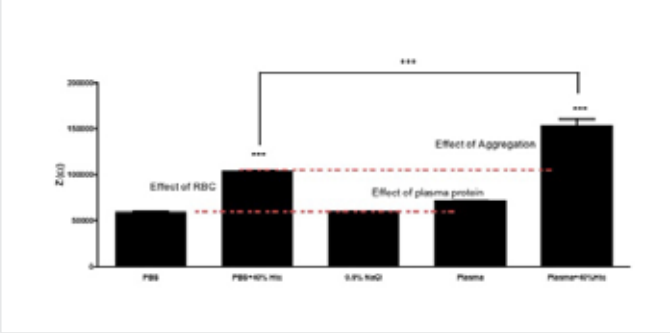

Figure 6. Comparison of phosphate-buffered saline and plasma impedance values. Data are mean ± standard error (n=10). Difference from "phosphate-buffered saline, 0.9% NaCl and plasma", ***: p<0.001, +++: p<0.001

PBS: Phosphate-buffered saline, RBC: Red blood cell

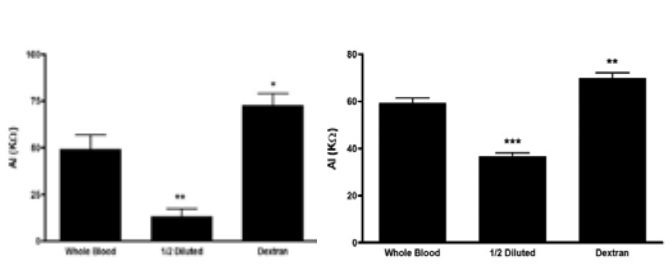

Figure 7. Comparison of calculated impedance values for (a) Human and (b) Rat erythrocyte suspensions. Data are mean ± standard error (n=10). Difference from "whole blood"; *: p<0.05; **: p<0.01, ***: p<0.001

AI: Aggregation index

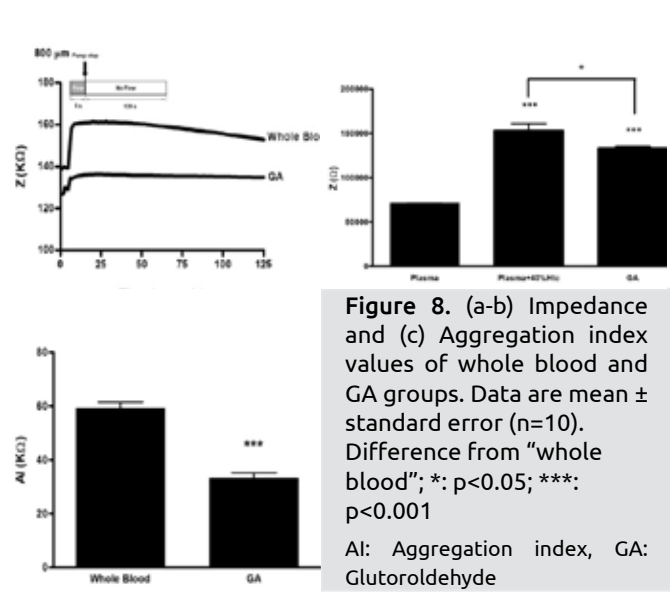

Figure 8. (a-b) Impedance and (c) Aggregation index values of whole blood and GA groups. Data are mean ± standard error (n=10). Difference from "whole blood"; *: p<0.05; ***: p<0.001

AI: Aggregation index, GA: Glutoroldehyde

Figure 7 shows that when it presented in terms of kilo ohm there is a significant difference (p<0.001) between the impedance value of the erythrocyte suspension in the hematocrit adjusted to 40% and the impedance value of the erythrocyte suspension in the plasma with the hematocrit of 40%. Figure 7a shows that the value of dilution prepared with human blood decreased significantly (p<0.01) compared to the whole blood group and increased in the dextran group (p<0.05). When the same study was repeated with rat blood (Figure 7b), the same changes were observed. In dilution and dextran samples where aggregation levels have been modified, a similar trend is seen with photometric devices commonly used for measuring erythrocyte aggregation (Dextran>Whole Blood>Dilution).

Figures 8a and 8b show impedance values for whole blood and GA groups. The impedance value of the erythrocytes treated with GA and suspension prepared with 40% hematocrit with its own plasma was found to be lower than the whole blood impedance value (p<0.05). These results show that in erythrocytes prepared with the same plasma with the same hematocrit ratio, only the GA-induced structural change resulted in reduced measured impedance values. Similarly, the calculated AI value was significantly reduced compared to the whole blood group (Figure 8c, p<0.001).

Discussion

Aggregation denotes the formation of reversible clumps of RBC under sufficiently low shear stresses (21,22). It has been reported that electrical properties of red cell suspensions also change during aggregation (11,13,14,23-27). The electrical properties of blood plasma and blood cells differ from each other. The plasma and cell interior consist of conducting fluids with certain electrical resistivities and can be simulated by resistors, whereas the cell membranes consist of phospholipids and proteins with dielectric properties and can be simulated by capacitors. Hence, the electrical impedance of blood is primarily characterized by three parameters: plasma resistance Rp, cell interior resistance R; and cell membrane capacitance C (28).

Electrical properties of erythrocyte suspensions are determined by various factors. They include the suspension characteristics (suspension medium, hematocrit and RBC properties), the hydrodynamic conditions (shear forces), the measurement conditions (geometry of the measuring system, the electrodes and measuring the frequency characteristics). Prior studies have suggested that electrical properties of the suspensions may reflect the kinetics of RBC aggregation if monitored during the aggregation process.

In our previous studies, C recordings were also obtained using RBC suspended in isotonic PBS, a suspending medium which did not contain proteins or macromolecules. RBC do not aggregate if they are suspended in such media, and this lack of aggregation was confirmed by "zero" aggregation indexes provided by the photometric aggregometer. The time course of C after the sudden stop exhibited a different pattern than Z. There was no detectable change of C for the nonaggregating RBC suspension in PBS. An expected finding of this study was the time course of Z changes

for non-aggregating suspensions of RBC in PBS (Table 1). Z changes had the same pattern as those for aggregating suspensions, while such similarity between aggregating and nonaggregating suspensions was not observed for C.

Impedance values in PBS and 40% hematocrit adjusted samples in plasma showed statistically significant increases in both PBS and plasma samples without erythrocytes (Figure 4). An interesting result is that although the hematocrit ratios are the same, the impedance value of erythrocyte suspension prepared in plasma is significantly different from the PBS sample ($p<0.001$). However, there was no significant difference between PBS and plasma in which there was no cell ($p>0.05$).

It was reported that measuring the electrical properties of erythrocyte suspensions can be one of the important methods which evaluate the erythrocyte aggregation (11,25-27). In these reports, the electrical properties of plasma and erythrocyte suspensions were evaluated by impedance and dielectric spectroscopy (11,19,26,29). Zhao and Jacopson (29) showed that the values of electrical impedance and capacitance were increased by fibrinogen concentration in suspensions. They also report that ESR was correlated with capacitance and capacitance was very sensitive to erythrocyte sedimentation rate. Pribush et al. (11,26,27) demonstrated that electrical properties of erythrocyte suspensions changed during aggregation. They speculated that monitoring of the electrical properties of these suspensions could give information about the process of aggregations. Capacitance values measured after the sudden cessation of blood flow in the system has changed and this change caused by the RBC aggregation were reported in studies using whole blood and erythrocyte suspensions (11). Electrical properties of erythrocyte suspensions showed a similar trend as photometric measurement and the parameters associated with aggregations could be calculated by using these records were reported (13,14,25).

The LORCA records reflected light (LR) from RBC suspensions while the Myrenne aggregometer record transmitted light (LT). The three types of RBC suspensions (i.e., suspensions in 1/2 diluted plasma, whole blood and plasma containing 1% dextran 500) exhibited different aggregation characteristics, judged by LORCA and Myrenne aggregometers (Table 1). RBC suspended in 1/2 diluted plasma (50% of original plasma components) represent decreased aggregation, while RBC suspended in 1% dextran 500 represent markedly increased aggregation; both changes of aggregation are due to alterations of suspending phase composition. The decreased concentrations of plasma components due to dilution resulted in significant and progressive changes of AI as measured by the three methods (LT, LR and C).

In our previous studies (13,17), we recorded the photometric and electrical properties of suspensions simultaneously during the erythrocyte aggregation of glass capillary tubes with a diameter of 1000 μm. We evaluated the correlations of AI of erythrocyte suspensions measured by photometric aggregometer and AI obtained from calculating the area under curve of light transmission, impedance and capacitance measurements and found that there was a positive and statistically significant correlation between capacitance value/light transmission and AI. There was no significantly correlation between impedance and AI value. According to results of this pre study, it was suggested that capacitance values monitored during erythrocyte aggregation in capillary tubes were reflected the aggregation time period. This suggestion was stated by comparisons using simultaneously records of LT.

When the PBS and/or 0.9% NaCl values are compared with the plasma, the impedance value of the plasma is higher. Although this difference is not significant ($p>0.05$), it is thought that the macromolecules in the plasma may be responsible for this increment (eg, fibrinogen, etc.). It is assumed that this significant difference ($p<0.001$) between the two samples that only the suspension medium is different with the same hematocrit represents erythrocyte aggregation (Figure 6 and 7). Only, the GA-induced structural change resulted in reduced measured impedance values in erythrocytes prepared with the same plasma with the same hematocrit ratio Similarly, the calculated AI value was significantly reduced compared to the whole blood group (Figure 8) It was obvious from all these results, the impedance value of the erythrocyte suspensions is influenced by the composition of the suspension medium, the number of erythrocytes in suspension (hematocrit) and the structural properties of erythrocytes.

Conclusions

We aimed in this study to determine whether the impedance measurements of erythrocyte suspensions can be used with a different calculation method in determining the degree of erythrocyte aggregation. Although impedance measurements of erythrocyte suspensions do not allow for the calculation of erythrocyte aggregation kinetics (T fast and t1/2), it is thought that they can be used as "AI" which indicates the degree of erythrocyte aggregation. In this way, AI can be measured in a simpler and repeatable way.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Bezmialem Vakıf University (19.09.2018).

Informed Consent: Informed consent was obtained from all participants, and study was conducted according to the Declaration of Helsinki Principles.

Peer-review: Externally peer-reviewed.

References

1. Baskurt OK. In vivo correlates of altered blood rheology. Biorheology 2008;45:629-38.
2. Meiselman HJ. Red blood cell aggregation: 45 years being curious. Biorheology 2009;46:1-19.
3. Rampling MW, Meiselman HJ, Neu B, Baskurt OK. Influence of cell-specific factors on red blood cell aggregation. Biorheology 2004;41:91-112.
4. Rampling MW. Haemorheology and the inflammatory process. Clin Hemorheol Microcirc 1998;19:129-32.

5. Baskurt OK, Temiz A, Meiselman HJ. Red blood cell aggregation in experimental sepsis. J Lab Clin Med 1997;130:183-90.
6. O.K.Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman. In vivo hemorheology iHoHaH, IOS Press, Amsterdam 2007;322-38.
7. Baskurt OK, Meiselman HJ, Kayar E. Measurement of red blood cell aggregation in a „plate-plate" shearing system by analysis of light transmission. Clin Hemorheol Microcirc 1998;19:307-14.
8. Houbouyan LL, Delamaire M, Beauchet A, Gentil M, Cauchois G, Taccoen A, et al. Multicenter study of an erythro-aggregometer: quality control and standardization. Clin Hemorheol Microcirc 1997;17:299-306.
9. Baskurt OK, Meiselman HJ. Cellular determinants of low-shear blood viscosity. Biorheology 1997;34:235-47.
10. Berliner S, Zeltser D, Shapira I, Assayag EB, Mardi T, Serov J, et al. A simple biomarker to exclude the presence of low grade inflammation in apparently healthy individuals. J Cardiovasc Risk 2002;9:281-6.
11. Pribush A, Meiselman HJ, Meyerstein D, Meyerstein N. Dielectric approach to the investigation of erythrocyte aggregation: I. Experimental basis of the method. Biorheology 1999;36:411-23.
12. Rouffiac V, Peronneau P, Hadengue A, Barbet A, Delouche P, Dantan P, et al. A new ultrasound principle for characterizing erythrocyte aggregation: in vitro reproducibility and validation. Invest Radiol 2002;37:413-20.
13. Baskurt OK, Uyuklu M, Meiselman HJ. Simultaneous monitoring of electrical conductance and light transmittance during red blood cell aggregation. Biorheology 2009;46:239-49.
14. Baskurt OK, Uyuklu M, Meiselman HJ. Time course of electrical impedance during red blood cell aggregation in a glass tube: comparison with light transmittance. IEEE Trans Biomed Eng 2010;57:969-78.
15. Hardeman MR, Dobbe JG, Ince C. The Laser-assisted Optical Rotational Cell Analyzer (LORCA) as red blood cell aggregometer. Clin Hemorheol Microcirc 2001;25:1-11.
16. Baskurt OK, Boynard M, Cokelet GC, Connes P, Cooke BM, Forconi S, et al. New guidelines for hemorheological laboratory techniques. Clin Hemorheol Microcirc 2009;42:75-97.
17. Baskurt OK, Uyuklu M, Hardeman MR, Meiselman HJ. Photometric measurements of red blood cell aggregation: light transmission versus light reflectance. J Biomed Opt 2009;14:540-44.
18. Baskurt OK, Uyuklu M, Ulker P, Cengiz M, Nemeth N, Alexy T, et al. Comparison of three instruments for measuring red blood cell aggregation. Clin Hemorheol Microcirc 2009;43:283-98.
19. Gaw RL, Cornish BH, Thomas BJ. The electrical impedance of pulsatile blood flowing through rigid tubes: a theoretical investigation. IEEE Trans Biomed Eng 2008;55:721-7.
20. Kiesewetter H, Radtke H, Schneider R, Mussler K, Scheffler A, Schmid-Schonbein H. [The mini-erythrocyte aggregometer: a new apparatus for the rapid quantification of the extent of erythrocyte aggregation]. Biomed Tech (Berl) 1982;27:209-13.
21. M.W. Rampling. Red cell aggregation and yield stress. In: Clinical blood rheology; Lowe GDO ECP, Inc Florida 1988;45-64.
22. H.J. Meiselman. Red blood cell role in RBC aggregation: 1963-1993 and beyond. Clin Hemorheol Microcirc 1993;13:575-92.
23. Antonova N, Riha P, Ivanov I. Time dependent variation of human blood conductivity as a method for an estimation of RBC aggregation. Clin Hemorheol Microcirc 2008;39:69-78
24. Balan C, Balut C, Gheorghe L, Gheorghe C, Gheorghiu E, Ursu G. Experimental determination of blood permittivity and conductivity in simple shear flow. Clin Hemorheol Microcirc 2004;30:359-64.
25. Pribush A, Hatzkelson L, Meyerstein D, Meyerstein N. A novel technique for quantification of erythrocyte aggregation abnormalities in pathophysiological situations. Clin Hemorheol Microcirc 2007;36:121-32.
26. Pribush A, Meiselman HJ, Meyerstein D, Meyerstein N. Dielectric approach to investigation of erythrocyte aggregation. II. Kinetics of erythrocyte aggregation-disaggregation in quiescent and flowing blood. Biorheology 2000;37:429-41.
27. Pribush A, Meyerstein D, Meyerstein N. Conductometric study of shear-dependent processes in red cell suspensions. I. Effect of red blood cell aggregate morphology on blood conductance. Biorheology 2004;41:13-28.
28. Fricke H, Morse S. The Electric Resistance and Capacity of Blood for Frequencies between 800 and 4(1/2) Million Cycles. J Gen Physiol 1925;9:153-67.
29. Zhao TX, Jacobson B. Quantitative correlations among fibrinogen concentration, sedimentation rate and electrical impedance of blood. Med Biol Eng Comput 1997;35:181-5.

Review

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Management of Patients Using Oral Anticoagulant Agent in Dental Practice

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ABSTRACT

An increasing number of patients in our country use oral anticoagulants for the prophylaxis and treatment of thromboembolic events. The cornerstone of these groups of agents is warfarin, a vitamin K antagonist, which has been the single alternative used by oral route for several years. However, due to warfarin's late onset and long lasting action and the intense interactions with food and drugs, newer oral anticoagulants have emerged in the market in recent years. Dabigatran, rivaroxaban and apixaban are the novel agents used in our country.

Those drugs should be regulated in the perioperative period when patients receiving oral anticoagulants are referred for dental interventions. The interruption of agents may result in lethal consequences of thromboembolic events, while continuing raises the risk of bleeding. This review outlines the various properties of the oral anticoagulants and the most recent recommendations and guidelines regarding the management of dental patients taking these medications.

Keywords: Warfarin, dental aproach, novel, bleeding risk, thromboembolism, dabigatran, rivaroxaban, apixaban

Introduction

Oral anticoagulant agents are used for the treatment of arterial and venous thromboembolism (VTE) or prophylaxis. Patients using these agents frequently refer to clinics for dental interventions. Withdrawing oral anticoagulants during the perioperative period increases the risk of thromboembolism and maintenance of them is associated with the risk of bleeding. Dentists should consider the risk of thromboembolism and bleeding under the guidance of a cardiologist and choose the most appropriate option among discontinuation, continuation or bridging.

Heart valve prosthesis, atrial fibrillation (AF) and VTE/pulmonary embolism history are the most important risk factors for thromboembolism. Thrombophilia tendency and some systemic diseases also increase the risk of thromboembolism (Table 1).

The risk of bleeding (no risk of bleeding, low or high risk of bleeding) is also affected by factors such as hypertension, liver and kidney failure, old age, predisposition to bleeding, and other drugs and alcohol use that increase bleeding

Warfarin and Bridging

The prototype of oral anticoagulant agents is warfarin, which is an antagonist of vitamin K-dependent factors (FII, FVII, FIX, FX). The therapeutic effect of warfarin is monitorized by prothrombin time and international normalized ratio (INR) (therapeutic level INR: 2.5-3.5±0.5). However, the effectiveness of the agent should be checked frequently, due to its interactions with food, differences of the patients in response to the drug and narrow therapeutic range of the drug (1,2).

Discontinuation of warfarin during the perioperative period is not preferred in terms of risk of thromboembolism. Warfarin is continued or replaced by another anticoagulant agent. "Bridging" means replacing an agent with another drug that has similar effects. Since warfarin has a long half-life, it is necessary

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to interrupt taking warfarin 5 days prior to the preoperative period to reduce bleeding. Patients are exposed to the risk of thromboembolism in a large window as a result of slow reach to the therapeutic level when the drug is started again during the postoperative period. It is thought that discontinuation of warfarin for a while and bridging with another short-and fast-acting agent (heparin derivatives) is both protective and reduces the risk of bleeding. It is aimed to eliminate the effect of heparin in the morning of surgery by skipping the morning dose and to maintain thromboembolism prophylaxis after homeostasis is achieved in postoperative period by re-starting heparin. Bridging is recommended in patients with high thromboembolism risk who will undergo interventions with major bleeding risk. In patients with moderate risk of thrombosis, patient-based decisions are made by taking into account the risk of bleeding (3).

Varfarin is most commonly bridged by low molecular weight heparin (LMWH). Since this method is generally preferred in patients at high risk of thromboembolism, it is recommended that heparin be administered in a therapeutic dose (Enoxaparin 2x1mg/kg, therapeutic dose: 2x80 mg for a patient weighing 80 kg, Clexane 2x0.8 mL). In the bridging protocol, warfarin is stopped 5 days before the surgery and LMWH is initiated 3 days before the surgery. INR follow-up is required to maintain a value of 1.4 or lower before the surgery. Patients should continue with LMWH for 5-7 days after the onset of warfarin in the postoperative period, which is necessary to achieve therapeutic INR level. After the decision is made that there is no risk of bleeding, 48-72 hours after major surgeries, and 24 hours after minor surgeries, LMWH is initiated. Warfarin is also initiated with the dose used in preoperative period, with LMWH and when INR reaches therapeutic level (2-3), LMWH is stopped (3).

Recent studies show that bridging with LMWH does not affect the frequency of thromboembolism but increases bleeding dramatically (3-5). When all studies are evaluated together, the rate of perioperative bleeding is 13/1 with bridging and 5/1 without bridging (6). Although it is known that thromboembolic events may be more vital than bleeding, such a high risk of bleeding is also a major drawback.

Beyer-Westendorf et al. (7) reported that major surgery and bridging with LMWH were the factors that increased the risk of bleeding in perioperative period in the Dresden study on new generation oral anticoagulants (drugs were continued, stopped or bridged with LMWH). Douketis et al. (8) stopped warfarin in patients with AF with low risk of thromboembolism and treated one group with placebo and treated other group with 100 IU/kg dalteparin bridging treatment. The incidence of thromboembolism was found to be 0.3% and 0.4% in the groups, while the incidence of major and minor bleeding was significantly higher in the bridging group with dalteparin (8). The most common reason for the increase in bleeding with bridging was early onset of LMWH in postoperative period and not skipping the evening dose of LMWH which was given in therapeutic doses (2x1), in preoperative period (3).

New Generation Oral Anticoagulant Agents

In recent years, new-generation oral anticoagulant agents (NOACs), which are fast acting, have short half-life, have less drug and food interaction, have more predictable effects and do not need to be followed by laboratory tests, have been introduced. These are dabigatran, a direct thrombin inhibitor (Pradaxa, Boehringer Ingelheim, İstanbul, Turkey); rivaroxaban, a factor Xa inhibitor (Xarelto, Bayer, İstanbul, Turkey); and apixaban, a factor Xa inhibitor (Eliquis, Bristol Myers Squibb, İstanbul, Turkey). Features of NOACs are summarized in Table 2 (9-12).

Dabigatran (Pradaxa): It is a thrombin inhibitor and is often used in a dose of 2x150 mg. Thrombin time (TT) and ecarin clotting time (ECT) are tests that measure the efficacy of dabigatran, and minimal prolongation is observed in activated partial thromboplastin time (aPTT). In the case of renal failure, its effect is prolonged and dose adjustment is required, since it is excreted from the kidney.

Rivaroxaban (Xarelto): It is a factor Xa inhibitor and is often used in a dose of 1x15-20 mg. Its efficacy is measured with anti-factor Xa level.

Table 1. Classification of thromboembolism risk

Risk	Mechanical valve	Atrial fibrillation	Venous thromboembolism
High	Mitral valve prosthesis Caged ball, tilting-disk aortic valve prosthesis CVA/TIA <6 months	$CHADS_2$ score 5-6 CVA/TIA <3 months Rheumatic valvular disease	VTE <3 months Severe thrombophilia (+)
Moderate	Aortic valve bioprosthesis and other risk factors (*)	$CHADS_2$ score 3-4	VTE 3-12 months Recurrent VTE Active cancer Mild thrombophilia
Low	Aortic valve bioprosthesis	$CHADS_2$ score 1-2 No history of CVA/TIA	VTE >12 months without other risk factors

CVA: Cerebrovascular accident, TIA: Transient ischemic attack, $CHADS_2$ score: A scoring scale to determine thromboembolism risk in atrial fibrillation. (C: Congestive heart failure, H: Hypertension, A: Age>75 years, D: Diabetes mellitus, S:Stroke-2 points), (*) Other risk factors: Congestive heart failure, hypertension, age>75 years, diabetes mellitus, AF, history of CVA/TIA, (+) Severe thrombophilia: Protein C and Protein S deficiency, antithrombin, anti-phospholipid antibodies, (&) Hafif trombofili: Heterozigot faktör V Leiden, protrombin gen mutasyonu, VTE: Venous thromboembolism

Apixaban (Eliquis): It is a factor Xa inhibitor and is often used in a dose of 2x5 mg. Its efficacy is measured with anti-factor Xa level.

The most important disadvantage of NOACs is the problem faced in reversing their effects. While the effect of varfarin can be eliminated with proper doses of vitamin K and fresh frozen plasma infusion, idarucizumab which is the antidote of dabigatran, a NOAC, is not available in our country. Protrombin complex concentrate is recommended for bleeding due to these agents (10).

Dental Management in Patients Using Oral Anticoagulants

Dentists should determine the treatment approach by evaluating the risk of bleeding in dental interventions in patients using oral anticoagulants perioperatively. The risk of bleeding in dental procedures is classified in different ways in different sources (1,2,13,14). Table 3 shows the classification of bleeding risk according to the guidelines of the Scottish Dental Clinical Effectiveness Programme (SDCEP) (1).

When the literature is examined, there are highly reliable studies searching the use of warfarin in dental interventions, but there are no evidence-based data for NOAC use. However, most studies support the idea of continuing anticoagulant treatment in patients undergoing outpatient dental surgery, including tooth extraction (15,16)

Dental interventions are usually evaluated in the group of operations with minor bleeding risk and in patients with INR level below 4, it is recommended to continue warfarin (1,2,11,15-20). In patients with stable INR levels, the INR level obtained in the last 72 hours may be acceptable, but in patients with labil INR levels, the INR level obtained in the last 24 hours should be evaluated. If the patient's INR level is 4 or higher, the patient should be consulted to his/her physician and dental treatments should be postponed until the INR level falls below 4. For emergency treatment, the patient should be directed to an upper level dental treatment center.

Broekema et al. reported that dental procedures can be performed in patients using warfarin with INR level of 3.5 or lower (checked in the last 24 hours) without discontinuing treatment (20). These operations include simultaneous extraction of no more than 3 teeth, surgical removal of buried teeth, periodontal treatment, apical resection, abscess drainage or dentoalveolar surgery performed by placing up to 3 implants. It is recommended that the operations be performed atraumatically, that the

Table 2. Features of new generation oral anticoagulant agents

	Dabigatran	Rivaroxaban	Apixaban
Implementation	PO, 2x1	PO, 1x1	PO, 2x1
Peak affect	1-3 h	2-4 h	1-3 h
Half-life	12-14 h	5-13 h	8-15 h
Elimination	80% renal, 20% feces	65% renal, 35% hepatic	25% renal, 70% hepatic
PT	+/-	+/-	+/-
aPTT	+/-	+/-	+/-
TT	+++	-	-
Anti-Xa level	-	+++	+++
Antagonization	Idarucizumab	4 PCCs (Cofact)	4 PCCs (Cofact)

PT: Prothrombin time, aPTT: Activated partial thromboplastin time, TT: Thrombin time, PCC: Prothrombin complex concantrate

Table 3. Risk of postoperative bleeding in dental interventions

Dental interventions that are not expected to cause bleeding	Dental interventions that may cause bleeding	
	Bleeding complication with low risk	Bleeding complication with high risk
Local anesthesia (infiltration, intraligamenter, mental nerve block, inferior dental block or other regional nerve block)	Simple extractions (1-3 teeth) Incision and intraoral drainage Root surface straightening and subgingival curettage Direct or indirect restorations in the subgingival margin	Complicated extractions (large wound surface or extraction of more than 3 teeth) Interventions with flap removal: -Elective surgical teeth extraction -Periodontal surgery -Preprosthetic surgery -Periradicular surgery -Surgical crown lengthening -Dental implant surgery -Gingival contouring biopsy
Basic periodontal examination		
Removal of supragingival plaque, stone and discoloration		
Direct or indirect restorations in supragingival margin		
Endodontic interventions		
Prosthetic interventions		
Ortodontic interventions		

suction socket is sutured close to the mouth, that the patient is discharged after the bleeding is stopped and information is given and that the mouth is rinsed with 5% tranexamic acid during the postoperative 5 days (20).

Recommendations on the use of new generation oral anticoagulants are based on clinical experience, pharmacodynamics, and expert opinions. According to the SDCEP (1), in patients taking NOACs with low risk of bleeding, dental treatment should be performed without discontinuation of the drug. Many national guidelines and reviews including expert opinions recommend that many attempts at dentistry should be made without discontinuing NOACs (2,9-11,17-19,21-23). In this group of patients using NOACs, it is emphasized that there is no place to bridge with LMWH during the perioperative period (18,19,24).

New-generation oral anticoagulant drugs are generally preferred in patients with lower risk of thrombosis (such as non-valvular AF), so they can be discontinued when there is a need for more major surgery. Dabigatran and apixaban are stopped 12 hours before, whereas rivaroxaban is stopped 24 hours before a dental intervention with high risk of bleeding by skipping the dose on the morning of the intervention. Rivaroxaban should be started four hours after the bleeding is under control and dabigatran and apixaban could be initiated by giving the evening dose (25).

Conclusion

In conclusion, it is observed that there is no need to bridge with LMWH routinely in perioperative period in patients who are currently using oral anticoagulants and who will undergo dental intervention. In line with this data, we believe that a large number of dental interventions can be performed using local hemostatic precautions without stopping oral anticoagulants and warfarin.

Authorship Contributions

Concept: A.A., Ö.D., Design: Ö.D., T.Y., Literature Search: T.Y., Writing: Ö.D.

Peer-review: Externally peer-reviewed.

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

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References

1. http://www.sdcep.org.uk/wp-content/uploads/2015/09/SDCEP-Anticoagulants-Guidance.pdf
2. http://www.sigwales.org/wp-content/uploads/dental-management-of-patients-taking-anticoagulant-drugs-outside-a-general-hospital-setting.pdf
3. Siegal D, Yudin J, Kaatz S, Douketis JD, Lim W, Spyropoulos AC. Periprocedural heparin bridging in patients receiving vitamin K antagonists: systematic review and meta-analysis of bleeding and thromboembolic rates. Circulation 2012;126:1630-9.
4. Douketis JD, Healey JS, Brueckmann M, Eikelboom JW, Ezekowitz MD, Fraessdorf M, et al. Perioperative bridging anticoagulation during dabigatran or warfarin interruption among patients who had an elective surgery or procedure. Substudy of the RELY trial. Thromb Haemost 2015;113:625-32.
5. Steinberg BA, Peterson ED, Kim S, Thomas L, Gersh BJ, Fonarow GC, et al. Outcomes Registry for Better Informed Treatment of Atrial Fibrillation Investigators and Patients. Use and outcomes associated with bridging during anticoagulation interruptionsin patients with atrial fibrillation: findings from the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF). Circulation 2015;131:488-94.
6. Rechenmacher SJ, Fang JC. Bridging Anticoagulation: Primum Non Nocere. J Am Coll Cardiol 2015;66:1392-403.
7. Beyer-Westendorf J, Gelbricht V, Förster K, Ebertz F, Köhler C, Werth S, et al. Peri-interventional management of novel oral anticoagulants in daily care: results from the prospective Dresden NOAC registry. Eur Heart J 2014;35:1888-96.
8. Douketis JD, Spyropoulos AC, Kaatz S, Becker RC, Caprini JA, Dunn AS, et al. Perioperative Bridging Anticoagulation in Patients with Atrial Fibrillation. N Engl J Med 2015;373:823-33.
9. Firriolo FJ, Hupp WS. Beyond warfarin: the new generation of oral anticoagulants and their implicationsfor the management of dental patients. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:431-41.
10. Elad S, Marshall J, Meyerowitz C, Connolly G. Novel anticoagulants: general overview and practical considerations for dental practitioners. Oral Dis 2016;22:23-32.
11. Little JW. New oral anticoagulants: will they replace warfarin? Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:575-80.
12. Breuer G, Weiss DR, Ringwald J. 'New' direct oral anticoagulants in the perioperative setting. Curr Opin Anaesthesiol 2014;27:409-19.
13. Hong CH and Intekhab I. Anti-Thrombotic Therapy: Implications for Invasive Outpatient Procedures in Dentistry. J Blood Disorders Transf 2013, 4:6. http://dx.doi.org/10.4172/2155-9864.1000166
14. Mingarro-de-León A, Chaveli-López B. Alternative to oral dicoumarin anticoagulants: Considerations in dental care. J Clin Exp Dent 2013;5:e273-8.
15. Perry DJ, Noakes TJ, Helliwell PS; British Dental Society. Guidelines for the management of patients on oral anticoagulants requiring dental surgery. Br Dent J 2007;203:389-93.
16. Richards D. Guidelines for the management of patients who are taking oral anticoagulants and who require dental surgery. Evid Based Dent 2008;9:5-6.
17. O'Connell JE, Stassen LF. New oral anticoagulants and their implications for dental patients. J Ir Dent Assoc 2014;60:137-43.
18. Spyropoulos AC, Douketis JD, Gerotziafas G, Kaatz S, Ortel TL, Schulman S; Subcommittee on Control of Anticoagulation of the SSC of the ISTH. Periprocedural antithrombotic and bridging therapy: recommendations for standardized reporting in patients with arterial indications for chronic oral anticoagulant therapy. Thromb Haemost 2012;10:692-4.
19. Breik O, Tadros R, Devitt P. Thrombin inhibitors: surgical considerations and pharmacology. ANZ J Surg 2013;83:215-21.

20. Broekema FI, van Minnen B, Jansma J, Bos RR. Risk of bleeding after dentoalveolar surgery in patients taking anticoagulants. Br J Oral Maxillofac Surg 2014;52:e15-9.

21. Van Diermen DE, van der Waal I, Hoogstraten J. Management recommendations for invasive dental treatment in patients using oralantithrombotic medication, including novel oral anticoagulants. Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:709-16.

22. Costantinides F, Rizzo R, Pascazio L, Maglione M. Managing patients taking novel (NOAs) in dentistry: a discussion paper on clinical implications. BMC Oral Health 2016;28:16:5.

23. Heidbuchel H, Verhamme P, Alings M, Antz M, Diener HC, Hacke W, et al. Updated European Heart Rhythm Association practical guide on the use of non-vitamin-K antagonist anticoagulants in patients with non-valvular atrial fibrillation: Executive summary. Eur Heart J 2017;38:2137-49.

24. Turpie AG, Kreutz R, Llau J, Norrving B, Haas S. Management consensus guidance for the use of rivaroxaban--an oral, direct factorXa inhibitor. Thromb Haemost 2012;108:876-86.

25. Johnston S. An evidence summary of the management of patients taking direct oral anticoagulants (DOACs) undergoing dental surgery, Int J Oral Maxillofac Surg 2016;45:618-30.

Case Report

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The Value of Magnetic Resonances Imaging in Localized Lipoatrophy

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ABSTRACT

Localized lipoatrophy usually presents as isolated or multiple, atrophic, depressed areas which are commonly found in the proximal part of extremities. We report a case with idiopathic localized lipoatrophy and want to emphasize the role of magnetic resonance imaging in the evaluation of atrophic lesions.

Keywords: Lipoatrophy, idiopatic localized, magnetic resonance imaging

Case Report

Here, we report a case who had idiopathic localized lipoatrophy and want to emphasize the role of magnetic resonance imaging (MRI) in the evaluation of atrophic lesions. Informed consent was taken from the patient.

A 48-year-old female who was a physical education teacher was admitted to our clinic with a 16-year history of slowly progressive atrophy on her anterolateral part of right tight. She described a tingling-like sensation on the atrophic area at the beginning. She also realized a slight atrophy on her left tight in addition to the sensation for the last one year. She did not have an occult trauma or injection history to this atrophic area. Her physical examination revealed only an artophic area on her right tight without any skin coloration pathology. There was no muscle weakness and sensory deficit. Her electromyography (EMG) test was also normal both in upper and lower extremities. Lumbar MRI did not reveal any pathology. Lower extremity MRI (Figure 1 and 2) showed localized atrophy in only adipose tissue on the anterolateral part of her right tight. There was no muscle pathology in the neighborhood of the atrophy. Her blood tests including complete blood count, thyroid functions, infection markers and vasculitic screen were all normal.

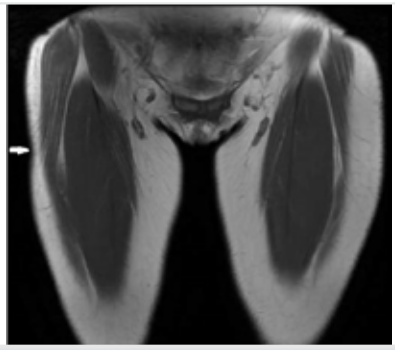

Figure 1. Lower extremity magnetic rezonans imaging, coronal T2 weighted sequences showed localized atrophy in adipose tissue on the anterolateral part of right tight

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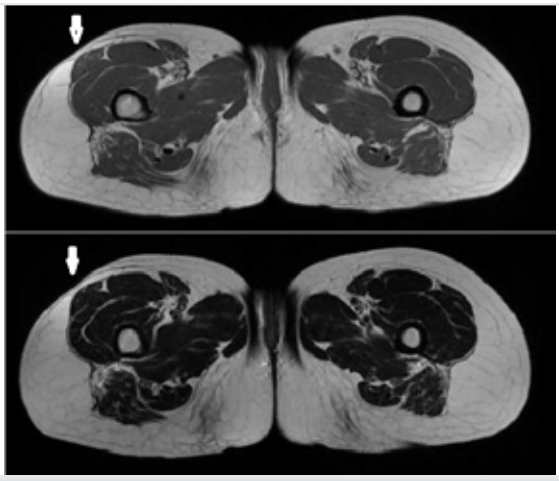

Figure 2. Lower extremity magnetic rezonans imaging, axial T2 weighted sequences showed localized atrophy in adipose tissue on the anterolateral part of right tight

Localized lipoatrophy usually presents as isolated or multiple, atrophic, depressed areas which are commonly found in the proximal part of extremities. Lesions usually have no overlying skin changes but secondary skin hypopigmentation may also accompany. The idiopathic localized lipoatrophy is a rare syndrome and its cause is unknown. Localized lipoatrophic syndromes may be associated with subcutaneous and intradermal injections (1,2). Also some authors reported co-occurrence with connective tissue disease (3). Additionaly, in the recent years, researches have emphasized the role of recurrent microtraumas in semicircular localized lipoatrophy (4).

There was no occult trauma, injection history in our patient and we excluded all the systemic conditions that may have caused lipoatrophy. But, our patient was a physical education teacher and she made intense exercises for 35 years. We can speculate that the microtraumas may have caused lipoatrophy. However, there is no explanation to why some people are affected by microtraumas and others are not affected.

Lipoatrophy in the extremities may be misinterpreted as neuromuscular disorders Detailed neurological examination and EMG test are helpful to discriminate a neuromuscular disorder from a lipoatrophy.

Additionaly, in recent years, MRI has commonly been used in diagnosis of the neuromuscular disorders and it may also help the evaluation of adipose tissue (5). MRI of lower extremities, a non-invasive method, showed us that the atrophy was only in adipose tissue under the depressed areas in our patient. MRI can help us to evaluate not only the depressed area, but also all the extremity, the contralateral side and all the body.

Ethics

Informed Consent: Informed consent was taken from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.E., P.H.Y., Design: A.E., P.H.Y., A.F.K., Data Collection or Processing: A.E., P.H.Y., A.F.K., Analysis or Interpretation: A.E., P.H.Y., A.F.K., Literature Search: A.E., Writing: A.E., P.H.Y., A.F.K.

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

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References

1. Dahl PR, Zalla MJ, Winkelmann RK. Localized involutional lipoatrophy: a clinicopathologic study of 16 patients. J Am Acad Dermatol 1996;35:523-8.
2. Yamamoto T, Yokozeki H, Nishioka K. Localized involutional lipoatrophy: report of six cases. J Dermatol 2002;29:638-43.
3. Winkelmann RK. Panniculitis in connective tissue disease. Arch Dermatol 1983;119:336-44.
4. Herane MI, Urbina F, Sudy E. Lipoatrophia semicircularis: a compressive lipoatrophy consecutive to persistent mechanical pressure. J Dermatol 2007;34:390-3.
5. Ogino J, Saga K, Tamagawa M, Akutsu Y. Magnetic Resonance Imaging of Semicircular Lipoatrophy. Dermatology 2004;209:340-1

Case Report

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Bilateral Phrenic Nerve Block For the Treatment of Intractable Hiccup in a Palliative Care Patient: A Case Report

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ABSTRACT

Hiccup is the characteristic voice caused by the sudden closure of the glottis during the contraction of the muscles of respiration especially diaphragm. It usually ends spontaneously in a short time. If it lasts more than one month, it is called as intractable hiccup. Intractable hiccups may lead to malnutrition, hypoxia, arrhythmia, dehydration, depression, tiredness and sleep disorder. Those can affect the quality of life of the patient. It is reported that pharmacologic agents such as gamma-aminobutyric acid receptor agonists, dopamine antagonists, antipsychotics and baclofen may be beneficial in the treatment of intractable hiccups. If success is not achieved with these methods, invasive methods such as phrenic nerve block, regional anesthesia, and phrenic nerve pulse radiofrequency may be applied as alternative therapies. In this case report, it is aimed to present the medical management of a palliative care patient with intractable hiccup. A 55-year-old male patient underwent gastrectomy and distal esophagectomy due to esophagogastric junction tumor. He suffered from intractable hiccup after esophageal hematoma. Bilateral phrenic nerve block was performed; hence no recovery was achieved after five weeks of medical treatment. Most of his complaint about hiccup was recovered after phrenic nerve block.

Keywords: Hiccup, phrenic nerve block, esophageal diseases

Introduction

In babies in mother's womb, hiccups are frequent and are considered as physiological. This is thought to be due to the preparation of the infant's inspiratory respiratory muscles for respiration after birth. However, hiccups do not serve a physiological purpose in adults; they disturb the humans and the environment due to the sound emitted. Hiccup is a symptom accompanied by a sound which results from sudden and involuntary contractions of accessory respiratory muscles and diaphragm together with the simultaneous closure of the glottis. Hiccups are mostly benign and their frequency can range from 4 to 60 per minute and heal spontaneously in a short time (1). Hiccups are classified into 3 types: Hiccups ending up in 48 hours, persistent hiccups lasting 48 hours-1 month and intractable hiccups lasting more than 1 month (2). Persistent hiccups can cause extremely vital problems such as malnutrition, exhaustion, fatigue, weight loss, hypoxia, bradycardia, arrhythmia, heart block, disturbed sleep patterns, speech disorders, depression, weakness, and dehydration (3). Hiccup reflex arch is composed of afferent pathway consisting of sympathetic nervous system, phrenic and vagus nerves; and efferent pathway connected to glottis and accessory respiratory muscles; and central mediators.

Cerebral events which cause deterioration in reflex arch, neck-related diseases, esophageal, stomach and other gastrointestinal diseases, thoracic diseases such as pneumonia and tumor, cardiovascular diseases, metabolic causes such as alcohol and hypocapnia, pharmacological agents such as carboplatin and cyclophosphamide are among the causes of resistant and persistent hiccups (Table 1) (1,4).

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Hiccups are usually relieved with physical maneuvers, such as breathing by closing the nose, increasing carbon dioxide by holding the breath, nasopharyngeal irritation and drinking water slowly for a long time. Patients whose symptoms are not relieved by physical maneuvers can be treated mostly by noninvasive pharmacological methods such as chlorpromazine, gabapentin, metoclopramide, baclofen and proton pump inhibitors. In cases where conservative or pharmacological treatment fails, phrenic nerve block or regional anesthesia may be applied. In cases where these treatments are inadequate, studies have shown that pulsed radiofrequency to the phrenic nerve can be performed (4,5).

Case Report

A 55-year-old male who was followed up in the surgical oncology service had complaints of loss of appetite, vomiting, malnutrition and insomnia due to hiccups lasting for five weeks. Four months ago, due to gastroesophageal junction tumor, total gastrectomy and distal esophagectomy were performed; two months after surgery, stent was placed due to anastomotic leak. The stent was removed back when it was determined that the stent was migrated. In the computerized tomography of the patient after severe hiccups started, hematoma in the area of anastomosis and empyema in the right lung were detected, and a chest tube was inserted to drain the empyema. The patient who did not respond to conservative treatment, including chlorpromazine, was scheduled for bilateral phrenic nerve block. After completing 5 mL 0.5% bupivacaine and 4 mg dexamethasone to 10 mL with saline, this mixture was applied to both sides with 22 G stimulator needle (Stimuplex, Braun) between sternocleidomastoid and anterior scalene muscles after contraction of diaphragma was detected (Figure 1). In the five-week follow-up of the patient after the phrenic nerve block, the patient had a 70% decrease in hiccup frequency and severity, and consequently, the severity of the symptom cluster of loss of appetite, insomnia and fatigue was observed to be significantly reduced. After 5 weeks of phrenic nerve block, the patient's hiccups and symptoms of loss of appetite, sleeplessness and fatigue recurred, and in the computerized tomography, widespread involvement of periton and tumor recurrence at anastomosis side were observed. Bilateral phrenic nerve blocks were applied to the patient. Fourteen hours after procedure, first hiccup attack occured and lasted 10 minutes. Second hiccup attack occured 3.5 hours after first attack and lasted 15 minutes. On the 3rd day of the procedure, 910 hiccup attacks occured and each lasted 2-3 minutes. The frequency and severity of hiccup reduced by 70% at the 6th week of the procedure. Symptoms of malnutrition, vomiting, loss of appetite, and sleep deprivation were observed to improve nearly completely. The patient was followed by palliative care unit for 5 months after the last phrenic block. During this time, hiccups were rarely seen. The patient died in the palliative care unit. Oral and written consents were obtained from the patient during all the procedures that were planned to be performed.

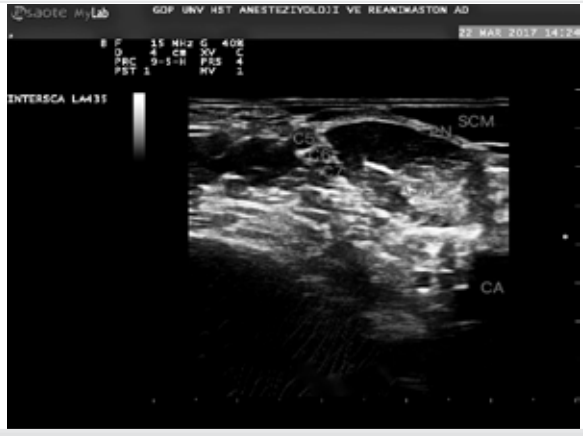

Figure 1. Ultrasound image of phrenic nerve block (SCM: Sternocleiodomastoid muscle, CA: Carotid artery, ASM: Anterior scalen muscle, PN: Phrenic nerve, C5: Fifth cervical root, C6: Sixth cervical root, C7: Seventh cervical root)

Table 1. Causes of resistant and persistent hiccups

Postoperative	General anesthesia, intubation (glottic stimulation), neck extension (stretch of phrenic nerve root), pulling out of internal organs
Central nervous system diseases	Vascular diseases, infections, structural disturbances such as head trauma
Irritation of vagal and phrenic nerves	A neck mass, goiter, pharyngitis, irritation of tympanic membrane
Gastrointestinal system diseases	Gastric distention, ulcer, pancreatic disease, gastric carcinoma, abdominal abscess, inflammatory bowel disease, hepatitis, aerophagia, etc.
Cardiovascular diseases	Myocardial infarction, pericarditis
Thoracic diseases	Enlarged lymph nodes, pneumonia, empyema, bronchitis, astma, pleuritis, aortic aneurysm, mediastinitis, mediastinal tumor, chest trauma, pulmonary embolism
Toxic-metabolic disorders	Alcohol, diabetes mellitus, herpes zoster, influenza, malaria, hypocalcemia, hypocapnia, hyponatremia, tuberculosis, uremia
Drugs	Alpha methyl dopa, short-acting barbiturates, chemotherapy drugs (carboplatin, cisplatin), dexamethasone, diazem
Psychological	Anorexia nevroza, konversiyon, excitement, pretending to be ill, stress

Discussion

The rate of admission to hospital with a resistant and persistent hiccup without any physical pathology complaint is 55/100.000. Recurrent hiccups can be seen in 20% of patients with Parkinson's disease and in 10% of patients with gastroesophageal reflux. In general, the prevalence of hiccups in advanced cancer patients has been reported as 3.9-4.8% (1). In a case series, more than a quarter of patients with esophageal carcinoma experienced hiccups lasting more than 48 hours at least once. The treatment options of this symptom, which deteriorates the quality of life and brings many sets of symptoms, are as follows: Non-pharmacological treatments including simple physical manoeuvres in the first step, pharmacological treatments in the second step and invasive interventions in the third step. In the treatment of persistent hiccups, one of three interventional methods, phrenic nerve block, regional anesthesia, pulsed radiofrequency to phrenic nerve, can be considered as the option of treatment (6).

The pathogenesis of hiccup is complex and has not been clearly explained. As a result of stimulation of vagal, phrenic and sympathetic afferent fibers, efferent nerve fibers result in contractions of diaphragm and intercostal muscles. Neurotransmitters affecting the hiccup reflex arch and which are thought to be responsible for the pathophysiology of hiccup, are gamma-aminobutyric acid and dopamine. It is known that many dopamine antagonists such as chlorpromazine and dopamine agonists such as pramipexole and amantadine have been used in the treatment of persistent hiccups by acting on this complex reflex arch (7). Interventional applications such as phrenic nerve block, regional anesthesia and pulsed radiofrequency to phrenic nerve may be considered in cases who are unresponsive to pharmacological treatments. In addition, in the treatment of patients with resistant hiccup; decompression of the vagus nerve and surgical interventions involving the phrenic nerve can be applied. The last choice is surgical treatment involving the denervation of the phrenic nerve. This method can cause permanent nerve damage and partial paralysis in the diaphragm. For this reason, as the procedure may affect normal breathing, it should only be used as the last option (8).

Kuusniemi et al. (9) reported a 72-year-old patient who developed persistent hiccups after L4-5 laminectomy under general anesthesia. They reported that the patient did not respond to medical and conservative treatment and that the right phrenic nerve block was applied with ultrasonography on the postoperative 17th day, and that ten minutes after the procedure hiccup disappeared and sensomotoric block developed on the right shoulder. They reported that hiccup did not recur in the follow-up and sensomotor block on right shoulder disappeared on the 3rd day of the procedure (9). Arsanious et al. (10) reported a 60-year-old patient who underwent esophageal stent due to tracheoesophageal fistula reported hiccups and developed hiccup on the day after the procedure, unresponsive to conservative treatment. With ultrasonography, right phrenic nerve block was applied and 40% relief was achieved in the patient. Three days after the operation, left phrenic nerve block was applied and 100% relief was achieved. Hiccups did not recur in the follow-up (10).

In additon, there are publications showing temporary relief due to single application of phrenic nerve block (9,10). Renes et al. (8) administered local anesthetic infusion with a catheter for 24 hours with phrenic nerve block. They reported that when the infusion was stopped, the hiccup re-occured and that local anesthetic infusion was re-administered for 24 hours, and that at the end of the infusion, complete relief was observed (8). In the study of Kang and his colleagues, it was observed that temporary relief was provided by performing phrenic nerve block and that pulsed radiofrequency was applied accompanied by ultrasonography after phrenic nerve block and complete relief was achieved (5).

Hiccups lasted more than five weeks in our patient. Loss of appetite, vomiting, nutritional disorders and sleep disorders due to long-lasting hiccups were seen and the patient's quality of life was corrupted. Because the patient was unable to respond to medical treatment, phrenic nerve block, a two-sided invasive treatment method, accompanied by ultrasonography and peripheral nerve stimulator, was applied to the patient. The severity and frequency of the hiccups of the patient decreased after the block. When hiccups started again after a rest period of about 1 month, the operation was repeated again and a significant improvement was observed in the quality of life of the patient. We thought that the hiccups were due to the recurrence of the tumor and the phrenic nerve irritation.

Conclusion

Persistent hiccup is not just a peculiar annoyance that the patient has caused; it also causes additional disturbances such as malnutrition, exhaustion, fatigue, weight loss, hypoxia, bradycardia, arrhythmia, heart block, disturbed sleep patterns, speech disorders, depression, weakness and dehydration. In cases of hiccup that does not respond to pharmacological treatment, bilateral phrenic nerve block provides permanent treatment. Phrenic nerve block can be considered in treatment of hiccups in patients with terminal cancer, especially with gastrointestinal tumors.

Ethics

Informed Consent: Written informed consent was obtained from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: MS., Design: S.D., Data Collection or Processing: A.A., Analysis or Interpretation: İ.O., Literature Search: M.S., Writing: V.K.

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

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

References

1. Steger M, Schneemann M, Fox M. Systemic review: the pathogenesis and pharmacological treatment of hiccups. Aliment Pharmacol Ther 2015;42:1037-50.
2. Marinella MA. Diagnosis and management of hiccups in the patient with advanced cancer. J Support Oncol 2009;7:122-7.
3. Lewis JH. Hiccups: causes and cures. J Clin Gastroenterol 1985;7:539-52.
4. Bagheri H, Cismondo S, Montastruc JL. Drug-induced hiccup: a review of the France pharmacologic vigilance database. Therapie 1999;54:35-9.
5. Kang KN, Park IK, Suh JH, Leem JG, Shin JW. Ultrasound-guided pulsed radiofrequency lesioning of the phrenic nerve in a patient with intractable hiccup. Korean J Pain 2010;23:198-201.
6. Campbell P, Janak S, Hilas O. Gabapentin for the treatment of persistent hiccups. Consult Pharm 2014;29:408-12.
7. Vaidya V. Sertraline in the Treatment of Hiccups. Psychosomatics 2000;41:353-5
8. Renes SH, van Geffen GJ, Rettig HC, Gielen MJ, Scheffer GJ. Ultrasound-guided continuous phrenic nerve block for persistent hiccups. Reg Anesth Pain Med 2010;35:455-7.
9. Kuusniemi K, Pyylampi V. Phrenic nerve block with ultrasound-guidance for treatment of hiccups: a case report. J Med Case Rep 2011;5:493.
10. Arsanious D, Khoury S, Martinez E, Nawras A, Filatoff G, Ajabnoor H et al. Ultrasound-Guided Phrenic Nerve Block for Intractable Hiccups following Placement of Esophageal Stent for Esophageal Squamous Cell Carcinoma. Pain Physician 2016;19:E653-6.

Case Report

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A Case with Laron Syndrome

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ABSTRACT

Laron syndrome (LS) is a rare disorder leading to short stature as a result of growth hormone (GH) insensitivity. It is caused by mutations in GH receptor gene and characterized by post-natal growth retardation, craniofacial abnormalities, high serum GH and low insulin-like growth factor-I (IGF-I) levels. Several different genetic mutations have been documented up to date. In this article, a patient with LS is reported.

A 2-year-old female patient was admitted to the hospital with the complaint of short stature. Her height and weight was 71.7 cm [<3 p., -4.09 standard deviations (SDS)] and 9.7 kg (<3 p., -2.2 SDS) respectively. She had dysmorphic features such as maxillary hypoplasia, blue sclera, small hands and feet, and extreme proportionate shortness. She had a high basal serum GH level (61.879 ng/mL), whereas serum IGF-I (<10 ng/mL) and IGF-binding protein 3 (<0.54 ng/mL) concentrations were significantly low. Both clinical and laboratory measurements were consistent with LS. A missense variation leading to a stop codon (W182X) was determined in GH receptor gene. Recombinant IGF-I therapy improved height z-score from -4.09 to -3.4 SDS after 24-month treatment.

In this report, we presented a case with LS. The description of a mutation in a specific region may be helpful in defining the genetic pattern of other patients with LS and in determining whether it is a mutation with a founder effect that is unique in the Turkish population.

Keywords: Growth hormone insensitivity, Laron syndrome, insulin-like growth factor-I therapy, short stature

Introduction

Along with autosomal recessive inheritance, the Laron syndrome (LS) is a congenital disorder, which is infrequent in prevalence. The disease was firstly diagnosed by Laron et al. (1) on several oriental Jewish families in 1966. Clinically, post-natal growth retardation is observed in patients with this syndrome. Adult length of these patients remain between -4 and -10 SDS unless they receive necessary treatment (2).

Growth hormone (GH) insensitivity occurs in these patients as a result of *GH receptor* (GHR) gene defects. Appropriate insulin-like growth factor-I (IGF-I) secretion in response to endogenous and exogenous GH does not occur and serum IGF-I value remains low while serum GH value is high. To date, around 99 mutations have been documented in *GHR* gene (chromosome 5p13-p12) in more than 300 cases (3).

In this article, a patient who had a mutation in her *GHR* gene and who received mecasermin (IGF-1) treatment for 24 months is reported.

Case Report

Two-year old female patient was admitted to the hospital with the complaint of short stature. She had a height of 71.7 cm (<3p., -4.09 SDS) and a weight of 9.7 kg (<3p., -2.26 SDS). Her birth weight, length and head circumference were 3420 gr (50-75 p.), 47 cm (10-25 p.) and 41cm (50-75 p.) respectively. Her parents were third-degree relatives. The height of the father and mother

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were 167 cm (3-10 p., 1.38 SDS) and 155 cm (10 p., -1.3 SDS) respectively. She had a healthy sibling. In her physical examination, a hypoplastic nasal bridge, frontal bossing, small mandible, blue sclera, small hands and feet, and small extremities were observed (Picture 1). Neurological development was suitable with her age. Abdominal ultrasonography, cardiac evaluation and her cranial magnetic resonance imaging were also normal.

Complete blood count and renal and liver function tests, fasting blood glucose, serum electrolytes, blood pH, thyroid function tests were normal. In accordance with X-ray of wrist, the bone age of the case was the same as a newborn. She had high basal serum GH levels (61.879 ng/mL), low serum IGF-I (<10 ng/mL; normal range for age, 16-143 ng/mL) and low insulin-like growth factor binding proteins -3 (<0.54ng/mL; normal range, 0.8-3.9 ng/ml). The IGF-I (somatomedin) generation test was performed with a dose of 0.1 mg/kg/day for four days. However, IGF-I value of the patient remained below 0.25 ng/mL after GH. Hereby, her clinic presentation and laboratory values were consistent with LS. In her genetic examination, homozygote W182X variation was detected in her *GHR* gene. Moreover, her parents, both her mother and father, were heterozygotes carrier for the same variation. (The variation was predicted to be pathogenic according to 3 modelling programs (scale-invariant feature transform, Polyphen 2, Muationtaster). Mecasermin (IGF-I) treatment with the dose of 0.12 mg/kg twice a day by subcutaneous injection was initiated to the case. At the end of 24 months, with IGF-I therapy, the height and height z-scores of the patient were improved from 71.7 cm to 85.1 cm and from -4.09 to -3.4, respectively, whereas her body mass index (BMI) z- score increased from 1.51 to 2.92. Her anthropometric development was given in Table 1.

Picture 1. Patient at the beginning of the therapy (two years old)

Picture 2. Patient at the 24th month of therapy (four years old)

Discussion

Our patient was a typical case of LS with a classical GH insensitivity syndrome (GHIS) phenotype and laboratory findings. Normally, patients with LS have a standard birth weight and length (4). Over time, short stature and obesity become evident. In the early stage, mild mental retardation, craniofacial anomalies (such as facial bone growth is particularly retarded and they exhibit frontal bossing and a saddle nose), blue sclera, defective and crowded teeth, sparse hair growth, small gonads and genitalia, small hands and feet (acrohypoplasia), obesity, retarded skeletal maturation, delayed puberty, and hypoglycemia can be observed. In the late stage, patients show very short stature (-4 to -10 height SDS), marked progressive obesity, muscle underdevelopment and weakness, osteoporosis, hypercholesterolemia, hyperinsulinemia, and various degrees of glucose intolerance (5-7). When the intellectual development of these patients is taken into consideration, it seems

Table 1. Anthropometric measure according to years

	At the beginning of therapy	At the 13rd month of therapy	At the 24th Month of therapy
Age	2 years old	3 years and 1month old	4 years old
Height (cm)	71.7	80.9	85.1
Height z-score	-4.09	-3.5	-3.4
Weight	9.7	13	16
Weight z-score	-2.2	-0.6	-0.39
BMI*	18.8	19.9	22.09
BMI z-score	1.51	2.38	2.92

*BMI: Body mass index

to vary in a wide range from normal intelligence to severe mental retardation in accordance with their psychological examination (5).

LS has one effective treatment: rIGF-I is administered on daily basis starting from early childhood and throughout whole life of the patient. Although these patients may not reach standard adult height, they can experience considerable increase in their height with the treatment when compared to the cases who do not receive the treatment (8). Previous data have demonstrated a good increment of growth rate during the first year of treatment. Growth velocity was 5.6 cm/year in the Ecuadorian study (9), and 4.3 cm/year in the European and North America studies (10) compared to baseline. Although, these three studies used the same amount of rhIGFI, with twice daily administrations, growth response to treatment was not similar. Many factors may affect growth response to treatment such as age of children or poor compliance to therapy. Moreover, it can be speculated that genotype may also affect growth response. However as the disease is rare, it is not possible to perform large studies about relationship between genotype and treatment response. So, the case reports including treatment results and genotypes may also serve to product meta-analysis on the issue about relationship between genotype and response to mecasermin treatment. The growth velocity of our patient was 8.49 cm/year with a 13-month treatment. However, in the second year of treatment her growth velocity was only 4.58 cm/year. The cases with LS, particularly when they are infant, can experience hypoglycemia, which is the most prevalent side effect, prior to or in the process of the therapy (2). However; no hypoglycemia is detected during the treatment process of our case. On the other hand, the BMI z-score increased rapidly. Obesity, another common symptom, is considered to be a compensatory mechanism aiming normalization of hypoglycemia (sex hormones, glucocorticoids, among others)

The main pathogenetic defect is in GH-IGF axis in which IGF-I is not synthesized due to the insensitivity of GH receptor. The *GHR* gene on the short arm of chromosome 5 includes 9 exons comprising 638 amino acid residues in its mature form. The mature GH receptor is consisted of an extracellular domain (encoded by exons 3-7), a single transmembrane domain (encoded by exon 8) and a cytoplasmic domain (encoded by exons 9 and 10) (1). GHR mutations are mainly detected in extracellular domain (3). The phenotype-genotype relationship in GHIS is widely variable. The same GHR mutation may be associated with a wide variation of phenotype. Although, severe phenotype is observed in homozygous cases, no clinical finding may be present in heterozygous individuals except compound heterozygous patients. Blanco et al. (11) described a homozygous non-sense mutation (with a potential lack of GHR protein) leading to a very severe LS phenotype (height from -5.4 to -7.04 SD) with their parents having a nearly normal phenotype. Similarly in our case, although the parents of patients with LS had heterozygous W182X alleles and normal phenotype, the patient had homozygous mutation.

Conclusion

A child with LS with a homozygous GHR W182X mutation is presented. The description of a mutation in a specific region may be helpful in defining the genetic pattern of other patients with LS and in determining whether it is a mutation with a founder effect that is unique in the Turkish population. Furthermore as the disease is rare, the case reports including treatment results may also serve to product meta-analysis on the issue about relationship between genotype and response to mecasermin treatment.

Ethics

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: İ.T.Ö., E.K., G.Y., Design: İ.T.Ö., Y.C., G.Y., Data Collection or Processing: İ.T.Ö., E.K., G.Y., Analysis or Interpretation: İ.T.Ö., E.K., Y.C., Literature Search: İ.T.Ö., E.K., Writing: İ.T.Ö., E.K.

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References

1. Laron Z, Pertzelan A, Mannheimer S. Genetic pituitary dwarfism with high serum concentration of growth hormone-a new inborn error of metabolism? Isr J Med Sci 1966;2:152-5.
2. Laron Z, Lilos P, Klinger B: Growth curves for Laron syndrome. Arch Dis Child 1993;68:768-70.
3. David A, Hwa V, Metherell LA, Netchine I, Camacho-Hubner C, Clark AJ et al. Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev 2011;32:472-97.
4. Laron Z, Lilos P, Klinger B. Growth curves for Laron syndrome. Arch Dis Child 1993;68:768-70.
5. Laron Z, Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958-2003. J Clin Endocrinol Metab 2004;89:1031-44.
6. Rosenbloom AL, Guevara Aguirre J, Rosenfeld RG, Fiedler PJ. The little women of Loja-- growth hormone-receptor deficiency in an inbred population of southern Ecuador. N Engl J Med 1990;323:1367-74.
7. Ayling RM, Ross R, Towner P, Von Laue S, Finidori J, Moutoussamy S et al. A dominant-negative mutation of the growth hormone receptor causes familial short stature. Nat Genet 1997;16:13-4.
8. Chernausek SD, Backeljauw PF, Frane J, Kuntze J, Underwood LE, GH Insensitivity Syndrome Collaborative Group: Long-term treatment with recombinant insulin-like growth factor (IGF)-I

in children with severe IGF-I deficiency due to growth hormone insensitivity. J Clin Endocrinol Metab 2007;3:902-10.

9. Guevara-Aguirre J, Rosenbloom AL, Vasconez O, Martinez V, Gargosky SE, Allen L et al. Two-year treatment of growth hormone (GH) receptor deficiency with recombinant insulin-like growth factor I in 22 children: comparison of two dosage levels and to GH-treated GH deficiency. J Clin Endocrinol Metab 1997;82:629-33.
10. Ranke MB, Savage MO, Chatelain PG, Preece MA, Rosenfeld RG, Wilton P. Long-term treatment of growth hormone insensitivity syndrome with IGF-I. Results of the European Multicentre Study. The Working Group on Growth Hormone Insensitivity Syndromes. Horm Res 1999;51:128-34.
11. Gorbenko del Blanco D, de Graaff LC, Visser TJ, Hokken-Koelega AC. Growth hormone insensivity syndrome caused by a heterozygous GHR mutation: Phenotypic variability owing to moderation by nonsense-mediated decay. Clin Endocrinol (Oxf) 2012 May;76:706-12.

Case Report

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Granulomatosis Polyangiitis Case that Mimics Henoch-Schönlein Purpura

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ABSTRACT

Granulomatosis polyangiitis (GPA) is a systemic, necrotizing, granulomatous, antineutrophil cytoplasmic antibody (ANCA) -associated vasculitis that affects small and medium arteries, mainly affecting the upper and lower respiratory tract and the kidneys. It is usually seen over 40 years old. Diagnosis is based on clinical findings, cytoplasmic- C-ANCA positivity and histological findings. Here we report a case of 21-year-old patient who presented with petechial purpuric lesions, abdominal pain, large joint arthritis and hematuria- proteinuria and to whom we started treatment for Henoch-Shönlein Purpura. But a chest imaging showed mass lesion and our final diagnosis was atypical GPA after excluding malignancy, cryoglobulinemia and ANCA related vasculitis in differantial diagnosis.

Keywords: Henoch-Schönlein purpura, atypical granulomatous polyangiitis, ANCA associated vasculitis

Introduction

Granulomatous polyangiitis (GPA), formerly known as Wegener granulomatosis, is an antinutrophil cytoplasmic antibody (ANCA) associated systemic necrotizing granulomatous vasculitis that involves small and medium diameter vessels, mainly affecting the upper and lower respiratory tract and kidneys (1). It has 2 types: Limited GPA and diffuse GPA. Limited GPA is the type of vasculitis that involves upper or lower respiratory tract or involves only eye (2). Proteinase-3 enzyme is the target antigen of C-ANCA and C-ANCA is considered very sensitive. C-ANCA is 90% positive in the active period and this rate decreases in remission (3). Henoch-Schonlein purpura (HSP) is a systemic leukocytoclastic vasculitis characterized by the storage of immunoglobulin A containing immune complexes and complement components in small vessel walls. Although skin, joint, gastrointestinal tract and kidney involvement are at the forefront, other organs such as brain, lung and scrotum can be involved during the course of HSP (4). We wanted to present a case presenting with palpable purpura, severe abdominal pain, arthritis in large joints and hematuria-proteinuria suggesting HSP, but who was diagnosed as having GPA based on the involvement pattern, kidney biopsy findings and C-ANCA positivity.

Case Report

A 21-year-old female patient admitted to our emergency service with throat sore, difficulty in swallowing, cough, bloody phlegm, abdominal pain, common joint pains, rash in the buttocks and legs starting three weeks ago. In physicial examination; fever was 39 °C, blood pressure 110/70 mmHg, heart rate 124/min. She had pale skin, 3x1 cm aphthous ulcer on the back wall of the pharynx, 2 cm splenomegaly on the costa broadcast and palpable purpura on both lower extremities. Perianal ulcers were present (Figure 1). She had proteinuria (+++) and hematuria in urine examination. Other laboratory data were given in Table 1. The amount of protein in 24-hour urine was 1380 mg. Neck computed tomography (CT) showed 23x13 mm lymphadenomegalies (LAM) in all zones, of which the largest ones were in posterior cervical triangle and the carotid space. Thorax CT showed consolidated areas in all zones of bilateral lungs which were diffuse, were in icy glass density, tended to merge and were suggestive of

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alveolitis. In the upper right lung, a 30x22 mm irregular soft tissue mass was observed and a 13x12 mm irregular bordered satellite nodule was observed near it (Figure 2). Diffuse wall thickening was observed in caecum, ascending colon, hepatic flexura, transverse and descending colons in abdominal CT. In the right iliac chain, heterogeneous contrasting soft tissue lesion approximately 30x20x48 mm in size was observed. Mesenteric LAMs in size of 17x12 mm were observed. Acid-resistant bacillus was found negative in the phlegm and no reproduction was observed in haemocultures. The Quantiferon test was negative. Immune globulins were detected in normal range. The result of cryoglobulin was negative. The skin biopsy showed intraepidermal pustular formation and leukocytoclastic vasculitis. Ig, complement, and fibrinogen accumulation were not observed by immunofluorescence method. P-ANCA was negative, whereas C-ANCA was positive (143 pg/mL). The kidney biopsy showed a crescentic glomerulonephritis (GN) without accumulation of immune complexes which was compatible with the diagnosis of GPA. For three days, 500 mg pulsed steroid and endoxan were given. The patient's steroid treatment was continued to be 1 mg/kg after pulse steroid treatment. After three days of treatment, the patient's oral ulcers disappeared and she began to eat, the rash of the patient improved, the findings of arthritis were lost and the patient was transferred to rheumatology and nephrology departments for follow-up and treatment.

Figure 1. Patient's rash screen

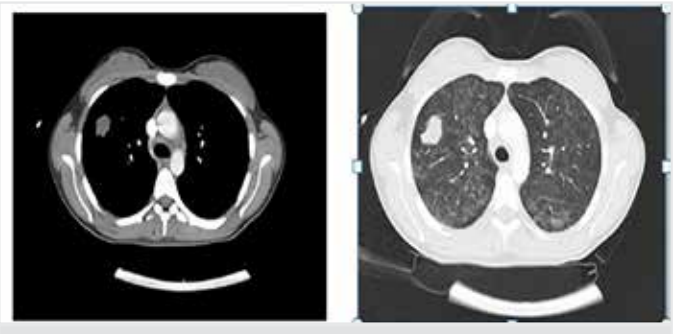

Figure 2. Tharax computed tomography image

Discussion

Vasculitis is a group of inflammatory diseases which have different clinical and pathological characteristics and lead to organ failure with inflammation, necrosis and damage to the walls of the vessels of various sizes and types. They can be fatal without early diagnosis and proper treatment. Immune deposits on the vessel wall are minimal or absent in the ANCA- associated vasculitis (pauci-immune vasculitis). ANCA-related vasculitis is classified as granomatous polyangiitis (Wegener's), eosinophilic granulomatous polyangiitis (Churg-Strauss), microscopic polyangiitis and one-organ limited vasculitis (for example renal limited vasculitis) (5). Lung involvement may be asymptomatic or acute and fulminant. It may present with alveolar haemorrhage causing respiratory insufficiency. Nodules, cavity formations, and infiltrations can be seen frequently in the lung X-ray or in the high-resolution computed tomography scan of the lungs (6). Fever, fatigue, sweating, weight loss (>10%), myalgia, arthralgia are general complaints. About half of the cases have vasculitic skin lesions. The most frequently observed skin symptom is palpable purpura, which shows pathology of leukocytoclastic vasculitis (7). Arthralgia, seen in approximately 70% of patients, usually affects the major joints of lower extremity. Arthritis is less common (8). GPA's gastro-intestinal (GI) involvement may be asymptomatic, as well as gastroenteritis, GI bleeding, ulcer, perforation, cholecystitis, acid, pancreatitis, pancreatic mass and perianal ulcer can be seen (9). In the literature, we found two cases with GPA who presented similar to our case with HSP (10). We know that ANCA is positive in 90% of patients with systemic GPA and 60% of patients with limited GPA. However, it is important to remember that ANCA positivity is not only seen in ANCA-associated vasculitis, but can also be seen in many other diseases and during drug treatments. Biopsy negativity or ANCA positivity does not always exclude the diagnosis of GPA. Appropriate biopsy sites for diagnosis of GPA are upper respiratory tract (especially sinuses), skin, lung and kidneys. Due to the insufficiency of wegener diagnostic criteria of the American College of Rheumatology in 1990, the following criteria were defined at the Chapel Hill Consensus Conference in 1992.

- Granulomatous inflammation of the respiratory tract
- Vasculitis involving small and medium diameter vessels,
- Necrotizing GN
- C-ANCA positivity

In our 21-year-old case; abdominal pain, iron deficiency anemia with fecal occult blood test positivity, microscopic hematuria, non-thrombocytopenic palpable purpura, arthritis and arthralgia suggested the diagnosis of HSP. Alveolar hemorrhage can be seen in HSP, but nodules and formation of cavity are not common in HSP. We evaluated ANCA-associated vasculits, tuberculosis, lymphoma, sarcoidosis, cryoglobulinemic vasculitis and viral infections in differential diagnosis due to oral ulcers, alveolar consolidation and nodules in the lungs in Thorax CT and widespread LAMs in the patient. The skin biopsy showed leukocytoclastic vasculitis, which helped us narrow the differential diagnosis.

Table 1. Laboratory findings of the patient

	28.04.2018	29.04.2018	1.05.2018	Normal range
Glucose	99		89	70-105 mg/dL
BUN	21		20	7-18 mg/dL
Creatinin	0.8		0.67	0.57-1.11 mg/dL
AST	26		26	5-34 U/L
ALT	28		62	0-55 U/L
LDH	247		355	125-220 U/L
Total protein		5.6	4.9	6.2-8.1 g/dL
Albumin		3.1	2.7	3.5-5 g/dL
Hb	7.3		7.4	12.2-16.2
Hct	23		23.9	35.5-48
MCV	70		72	80-97
WBC	14.100		13.100	4.6-10.2
PLT	495.000		369.000	142-424
Urinary analysis	Protein (+++), 34 erytrocytes			
CRP		11	11,9	<0.5 mg/dL
Procalsitonin		2.12	4.82	<0.5 ng/mL
Na	135		139	mmol/L
K	4.8		4.5	mmol/L
HBsAg	Negative			Negative
Anti-HBs	Negative			Negative
Anti-HCV	Negative			Negative
Anti-HIV	Negative			Negative
ANA	Negative			Negative
Anti-ds DNA	Negative			Negative
Anti ENA scl70	Negative			Negative
Anti Sm	Negative			Negative
Antiphospholipid	Negative			Negative
Anti-cardiolipin IgM	Negative			Negative
Anti-cardiolipin IgG	Negative			Negative
ANCA	Positive, 1:100			Negative <1:10
p-ANCA	Negative			Negative
c-ANCA	Positive, 161			Negative, <12 IU/mL
Complemant C3	107			89-193 mg/dL
Complemant C4	15			15-57 mg/dL
GBMA	Negative			
LA	Negative			
Quantiferon	Negative			
IgA	56			65-421 mg/dL
IgM	873			552-1631 mg/dL
IgG	32			33-293 mg/dL
Protein in 24-hour urine	1306		2416	-300 mg/gün

BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanin aminotransferaz, LDH: Laktat dehidrogenaz, MCV: Mean cell volume, WBC: White blood cell, PLT: Platelet, CRP: C-reactive protein, ANA: Nurses association, HIV: Human immmunodeficiency virus, IgM: Immunoglobulin M, IgG: Immunoglobulin G, GBMA: Generic Biosimilar Medicines Association, Hb: Hemoglobin, ANCA: Antinutrophil cytoplasmic antibody, IgA: Immunoglobulin A

C-ANCA positivity supported the diagnosis of GPA. Significant mucosal thickening of the sinuses in the paranasal sinus x-ray and large oral ulcers suggested the diagnosis of GPA. Urine analysis findings showed renal involvement. The kidney biopsy showed a crescentic GN without accumulation immune complexes which suggested the diagnosis of GPA. Perianal ulcers were also compatible with GPA as reported in the literature.

As a result, we could start treatment for HSP with the existing diagnostic criteria in our case with abdominal pain, rashes, joint involvement and appropriate age suggesting HSP. However, as in our case, taking into account several incompatible findings, we achieved a diagnosis that is more aggressive and can result in death if not treated quickly as a result of extensive research. We wanted to emphasize that the first diagnosis could be misleading and that even the smallest hint should be investigated.

Ethics

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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References

1. LutaloPM, D'Cruz DP. Diagnosis and classification of granulomatosis with polyangiitis (aka Wegener's granulomatosis). J Autoimmun 2014;48-49:94-8.
2. Holle JU, Gross WL, Holl-Ulrich K, Ambrosch P, Noelle B, Both M, et al. Prospective long-term follow-up of patients with localised Wegener's granulomatosis: does it occur as persistent disease stage? Ann Rheum Dis 2010;69:1934-9.
3. Csernok E. Antineutrophil cytoplasmic antibodies and pathogenesis of small vessel vasculitides. Autoimmun Rev 2003;2:158-164.
4. Koçak M, Büyükkaragöz B, Kuraş Can Y, Çelebi Tayfur A, Çaltık Yılmaz A, Günbey S. The Epidemiological, Clinical and Laboratory Features of 91 Children with Henoch-Schönlein Purpura. Abant Medical Journal 2015;4:134-40.
5. J. C. Jennette, R. J. Falk, P. A. Bacon, N. Basu, M. C. Cid, F. Ferrario ,et al. 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1-11.
6. Martinez F, Chung JH, Digumarthy SR, Kanne JP, Abbott GF, Shepard JA et al. Common and Uncommon Manifestations of Wegener Granulomatosis at Chest CT: Radiologic-Pathologic Correlation. RadioGraphics 2011; 32;51-69.
7. Daoud MS1, Gibson LE, DeRemee RA, Specks U, el-Azhary RA, Su WP. Cutaneous Wegener's granulomatosis: clinical, histopathologic, and immunopathologic features of thirty patients. J Am Acad Dermatol 1994;31:605-12.
8. Jeleniewicz R, Suszek D, Majdan M. Musculoskeletal symptoms in a group of granulomatosis with polyangiitispatients. Wiad Lek 2018;71:17-20.
9. Pagnoux C, Mahr A, Cohen P, Guillevin L. Presentation and outcome of gastrointestinal involvement in systemic necrotizing vasculitides: analysis of 62 patients with polyarteritis nodosa, microscopic polyangiitis, Wegener granulomatosis, Churg-Strauss syndrome, or rheumatoid arthritis-associated vasculitis. Medicine (Baltimore) 2005;84:115-28.
10. Bui T, Chandrakasan S, Poulik J, Fathalla BM. Granulomatosis with polyangiitis presenting as Henoch-Schönlein purpura in children. J Clin Rheumatol 2013;19:199-202.