ISSN: 2619-9793 • E-ISSN: 2148-094X

istanbul MEDICAL JOURNAL

İSTANBUL TIP DERGİSİ

VOLUME 23 • ISSUE 3 • August 2022

Original Articles

istanbulmedicaljournal.org

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İstanbul MEDICAL JOURNAL

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Publisher Certificate Number: 14521

Publication Date: August 2022
ISSN: 2619-9793 **E-ISSN:** 2148-094X
International periodical journal published three times in a year.

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CONTENTS

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İstanbul Med J 2022; 23(3): 159-63
DOI: 10.4274/imj.galenos.2022.73444

Correlation Between *GSH-Px Pro198Leu, CAT-262C/T, MnSOD Ala16Val* Gene Polymorphisms and Allergic Rhinitis

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ABSTRACT

Introduction: In this study, we investigated the etiopathogenesis of allergic rhinitis by analyzing the polymorphisms including *GPx-1 Pro198Leu, CAT-262 C/T,* and *MnSOD Ala16Val*.

Methods: The diagnosis of allergic rhinitis was diagnosed by clinical history, examination, serum total immunoglobulin E levels and skin prick test. Five mL of peripheral blood from patients and individuals constituting the control group was taken into EDTA tubes. DNA isolation from whole blood samples was performed according to the Poncz method.

Results: Because of this study; for the Pro198Leu polymorphism of the GPX1; was concluded with 95% confidence that the presence of the Leu allele increased the susceptibility to allergic rhinitis 1.092 times. However, this increase was not found to be statistically significant. For the -262 C/T polymorphism of the *CAT* gene; was concluded with 95% confidence that the presence of the T-allele increased the susceptibility to allergic rhinitis 27,064 times. This increase was found to be statistically significant. For Ala16Val polymorphism of the *Mn-SOD* gene; was concluded with 95% confidence that the presence of the Ala allele increased the susceptibility to allergic rhinitis 25,791 times. This increase was found to be statistically significant.

Conclusion: A significant relationship was found between allergic rhinitis and the genotypes and the frequencies of alleles in the polymorphisms of the *MnSOD* and *CAT* genes. However, no significant relationship was found between allergic rhinitis and the polymorphisms of the *GPx-1* gene.

Keywords: Allergic rhinitis, *MnSOD, GPx-1, CAT,* polymorphism

Introduction

Allergic rhinitis is an inflammatory disease of the nasal mucosa characterized by nasal congestion, runny nose, nasal itching, frequent sneezing and conjunctival irritation, and it occurs due to an immunoglobulin E (IgE)-dependent type-1 hypersensitivity reaction (1-3). Allergic rhinitis typically begins in early childhood. An increase in symptoms is observed in the 20s, 30s, and 40s (4). It affects 10-40% of the entire population. Prospective studies have shown that its prevalence has been increasing (5). The dominant mechanism in the pathophysiology of allergic rhinitis is the IgE-dependent type-1 hypersensitivity reaction. Allergens in contact with the respiratory mucosa are taken in by antigen presenting cells (APC) in the nasal mucosa and broken down by proteolytic enzymes into peptides of 4-7 amino acids length. The broken down peptides are then expressed by MHC Class II molecules on the surface of APCs and presented to CD4+ (T-helper) lymphocytes. In people with atopic diathesis, CD4+ (T-helper) MCH II forms the Th2 cell because of the interaction between CD 28-B7. This Th2 cell then not only allows the Th2 cells in its own colon to proliferate, but also begins secreting its own characteristic cytokines such as interleukin-3 (IL-3), IL-4, IL-5, IL-13, and granulocyte macrophage colony-stimulating factor (GM-CSF) (6,7). IL-4 and IL-13 stimulate B-lymphocytes in circulation and cause their transformation into plasma cells. These plasma cells secrete allergen-specific IgE to which they are sensitized. These specific IgE antibodies bind to high affinity IgE receptors on circulating basophils and mast cells in tissues. IgE-bound mast cells that increase because of continuous allergen exposure pass into the epithelium and are degranulated by recognizing mucosal allergens (8). The products of this degranulation are Ready to Act mediators such as histamine, tryptase, chymase, quinogenase, heparin and other enzymes. Additionally, mast cells secrete new inflammatory mediators such as PGD_2, tumor necrosis factor-alpha, sulfidopeptidyl leukotrienes LTC_4, LTD_4, and LTE_4. These mediators lead to increased permeability and mucosal edema. These events occur within 1-2 minutes of allergen exposure and are called early phase allergic response (6). Late phase reactions occur because of infiltration with mast cells, basophils, neutrophils, eosinophils and mononuclear cells

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Cite this article as: Kundi P, Bozan N, Berkoz M, Çankaya H. Correlation Between *GSH-Px Pro198Leu*, CAT-262C/T, *MnSOD Ala16Val* Gene Polymorphisms and Allergic Rhinitis. İstanbul Med J 2022; 23(3): 159-63.

Received: 20.12.2021
Accepted: 09.06.2022

2-4 hours after allergen exposure. The cells that play an important role in the late phase reaction are eosinophils. Cytokines and chemokines released from activated eosinophils cause tissue damage on the one hand, and increase inflammation via autocrine and paracrine pathways on the other hand (9). Th2 cells release IL-3, IL-4, IL-5, and other cytokines that facilitate IgE production, eosinophil chemoattraction, and eosinophil delivery, resulting in more eosinophils in the environment. Cysteinyl leukotrienes, eosinophilic cationic protein, major basic protein which are proinflammatory mediators released from eosinophils, cause epithelial damage and a late phase response occurs via GM-CSF (10). As a response to this situation, we encounter thickening of the nasal mucosa (hypertrophy), increased resistance to nasal flow and ultimately nasal obstruction in the clinic.

Cytokines released from eosinophils play a fundamental role in the generation of free oxygen radicals (FOR) in late phase reactions. Oxidative stress is thought to play an effective role in maintaining and increasing inflammation after this stage (1,11). Therefore, this suggests that free radicals may be effective in the pathophysiology of allergic rhinitis. Overproduction of free radicals and/or inability to neutralize them by antioxidant systems causes the oxidant/antioxidant balance in the cell to deteriorate and lead to "oxidative stress". Antioxidant systems play a critical role in maintaining this balance and preventing oxidative damage (12,13). In this study, we hypothesized that glutathione peroxidase (GSH-Px), catalase (CAT) and cytochrome oxidase enzymes, which are part of the antioxidant defense system in our body, may be effective in this mechanism, and investigated the relationship between gene polymorphisms of these enzymes.

Methods

In this study, the relationship between allergic rhinitis and *GPX1 Pro198Leu*, *CAT-262C/T,* and *Mn-SOD Ala16Val* gene polymorphisms was investigated. For this purpose, the control group of our study consisted of 236 healthy individuals, while the patient group consisted of 214 individuals diagnosed with allergic rhinitis. Five mL of peripheral blood from both the patients diagnosed with allergic rhinitis and the individuals in the control group were collected in EDTA tubes and stored at + 4 °C until the study day. Additionally, a patient follow-up form was created to determine the laboratory and clinical data of patients with allergic rhinitis, and these forms were completed in accordance with the outpatient clinic and service files of the patients. Each sample amplified by PCR was cut using *ApaI*, *SmaI* and *BsaWI* enzymes to determine the Pro → Leu change at position 198 of the *GPX1* gene, C → T change at position -262 of *CAT* gene and Ala → Val change at position 16 of the *Mn-SOD* gene. *GPX1 Pro198Leu*, *CAT -262 C/T,* and *Mn-SOD Ala16Val* polymorphisms of 450 individuals, including 236 healthy volunteers in the control group and 214 patients diagnosed with allergic rhinitis, were examined.

The study was approved by the Ethics Committee of Van Yüzüncü Yıl University Clinical Research Ethics Committee (approval number: 10, date: 27.10.2015).

Statistical Analysis

The SPSS 21 (SPP Inc., Chicago. IL., USA) program was used for statistical analysis of the data. Frequencies and percentage values were calculated for all parameters. The difference between the frequency of the allergic rhinitis patients and control groups was analyzed with the chi-square test. The cut off of p-value was determined as 0.05 in 95% confidence interval.

Results

Because of this study; for the Pro198Leu polymorphism of the *GPX1* gene, the total number of Pro alleles in the control group was 392, the allele frequency was 83.05%, the total Leu allele number was 80, and the allele frequency was found to be 16.95%. In the allergic rhinitis group, the total number of Pro alleles was 350, the allele frequency was 81.78%, the total Leu allele number was 78, and the allele frequency was found to be 18.22%. There was no significant difference between allergic rhinitis and control groups (p=0.62) (Table 1). For the -262 C/T polymorphism of the *CAT* gene; in the control group, the total number of C alleles was 412, the allele frequency was 87.29%, the total T allele number was 60, and the allele frequency was 12.71%. In the allergic rhinitis group, the total number of C alleles was 307, the allele frequency was 71.73%, the total T allele number was 121, and the allele frequency was 28.27%. This difference was found to be statistically significant between the allergic rhinitis and control groups (p<0.05) (Table 2). For Ala16Val polymorphism of the *Mn-SOD* gene; in the control group, the total number of Val alleles was 358, the allele frequency was 75.85%, the total Ala allele number was 114, and the allele frequency was found to be 24.15%. In the allergic rhinitis group, the total number of Val alleles was 235, the allele frequency was 54.91%, the total Ala allele number was 193, and the allele frequency was 45.09%. This difference was found to be statistically significant between the allergic rhinitis and control groups (p<0.05) (Table 3).

Discussion

The etiopathogenesis of allergic diseases have not been fully elucidated. For this reason, this study we conducted to elucidate the etiopathogenesis of allergic rhinitis is of great importance. Eosinophils are the cells that play a major role in late phase reactions in the pathophysiology of

Table 1. GPX1 Pro198Leu polymorphism allele frequency in allergic rhinitis patients and control groups

Alleles	Allergic rhinitis patient (n=428), n (%)	Control (n=472), n (%)	p-value
Pro	350 (81.78%)	392 (3.05%)	p=0.62
Leu	78 (18.22%)	80 (16.95%)	

n: Number of alleles. Allele frequencies of this polymorphism was evaluated by chi-square analysis

Table 2. CAT-262 C/T polymorphism allele frequency in allergic rhinitis patient and control groups

Alleles	Allergic rhinitis patient (n=428), n (%)	Control group, (n=472) n (%)	p-value
C	307 (71.73%)	412 (87.29%)	p<0.0001
T	121 (28.27%)	60 (12.71%)	

n: Number of alleles. Allele frequencies of this polymorphism was evaluated by chi-square analysis.

Table 3. Mn-SOD Ala16Val polymorphism allele frequency in patients with allergic rhinitis and control groups

Alleles	Allergic rhinitis patient (n=428), n (%)	Control group (n=472), n (%)	p-value
Val	235 (54.91%)	358 (75.85%)	p<0.0001
Ala	193 (45.09%)	114 (24.15%)	

n: number of alleles. Allele frequencies of this polymorphism was evaluated by chi-square analysis

allergic rhinitis. Cytokines released from eosinophils play a fundamental role in the production of FORs. Additionally, immunological or non-immunological stimulation of basophils, eosinophils and mast cells increased in the nasal mucosa results in the production of FORs such as superoxide anion, hydrogen peroxide (H_2O_2), or hydroxyl radicals. Oxidative stress is thought to play an effective role in maintaining and increasing inflammation after this stage. Based on this information, we investigated gene polymorphisms in GSH-Px, CAT and MnSOD, which serve as antioxidant enzymes in our body, to investigate the effect of FORs in the etiopathogenesis of allergic rhinitis. In the literature, GSH-Px, CAT and superoxide dismutase (SOD) gene polymorphisms decrease the normal activity of these enzymes. As a result, free radicals accumulate in our body and the oxidant/antioxidant balance in the cell is disrupted, which results in oxidative stress. FOR play a role in the etiopathogenesis of many diseases, and *GSH-Px, CAT* and *SOD* gene polymorphisms increase susceptibility to many other chronic diseases. No other study investigating these gene polymorphisms in allergic rhinitis has been found in the literature. However, some studies have shown that FORs may be effective in the etiopathogenesis of allergic rhinitis. A disruption in the oxidant/antioxidant balance in favor of oxidants directly causes damage to the upper and lower airway epithelial cells. The most important mechanism in the formation of tissue damage due to FOR is the peroxidation of lipids in the cell membrane. The increase in lipid peroxidation can be used as an indicator of tissue damage caused by free radicals. One of the lipid peroxidation degradation products is malondialdehyde (MDA) (14). This molecule causes the formation of superoxide anion and H_2O_2 by reducing oxygen, and these products damage cells and tissues. In a previous study, Akbay (15) compared the MDA level in allergic rhinitis patients with a control group. In this study, the patient group was between the ages of 4-63, the control group was between the ages of 5-56, 13 of the patients were male and 27 were female, and the control group consisted of 20 male and 20 female subjects. In this study, a statistically significant increase in MDA levels was detected in the patient group compared with the control group. Additionally, the same study found that the antioxidant enzymes myeloperoxidase and CAT levels were low in patients with allergic rhinitis. The author concluded that this strengthens the view that oxidants play a role in the pathogenesis of allergic rhinitis. In the same study, a statistically significant decrease was observed in vitamin A and E levels in patients with allergic rhinitis compared with the control group, but the author did not provide treatment and evaluate the results (15). Emin et al. (16) measured the total serum IgE levels and eosinophil count, total antioxidant status and its relation with oxidative stress in children with allergic rhinitis. This study included 106 patients and 70 controls. When the patient and control groups were compared, no significant difference was found in terms of age, gender and body mass index. However, there was a significant increase in serum total IgE and eosinophil counts in the patient group compared with the control group. Furthermore, it was shown that there was a significant increase in plasma oxidative stress level and a significant decrease in the antioxidant defense system in the patients (16). These studies show that oxidative stress mechanism plays an important role in allergic rhinitis. CAT is a critical endogenous antioxidant enzyme that detoxifies H_2O_2 into water and oxygen, thus protecting the body from the damaging effects of reactive oxygen species. The *CAT* gene is located on the 11p13 chromosome and contains 12 introns and 13 exons. There are different regions of polymorphism in the *CAT* gene. In *CAT-262 C/T* gene polymorphisms, T allele diversity was associated with lower enzyme activity compared to the C allele (17). This in turn increases the level of SOR in the body. Hu et al. (18) conducted a study to determine whether the *CAT-262 C/T* gene polymorphism increases the risk of prostate cancer. Based on the results, they found that *CAT -262 C/T* gene polymorphism significantly increased the risk of prostate cancer (18). In this study, we found that *CAT-262 C/T* gene polymorphism significantly increased susceptibility to allergic rhinitis. Zarafshan et al. (19) investigated the *CAT-262 C/T* gene polymorphism in women with endometriosis. By definition, endometriosis is the presence of the endometrial gland and stroma outside the uterine cavity. Recent studies have shown that this disease may be associated with oxidative stress. Based on the results of this study, the frequency of CAT-262 C/T CC/CT/TT genotypes in patients with endometriosis was 67.5%, 32.5%, and 0%, respectively, while it was 12%, 68%, and 20% in the healthy control group. In other words, a statistically significant difference was found in the genotype and allele distribution of *CAT-262 C/T* gene polymorphism in endometriosis compared with the control group. It was found that *CAT-262 C/T* gene polymorphism increases susceptibility to endometriosis (19). Wang et al. (20) investigated the relationship between survival and risk and *CAT-262 C/T* gene polymorphism in patients with cancer. Based on the results, they found a significant relationship between cancer risk and *CAT-262 C/T* gene polymorphism. In their subgroup analysis, they found that it especially increased the risk of prostate cancer. In the survival analysis, they showed that there was no significant relationship between *CAT-262 C/T* gene polymorphism and survival in patients with prostate cancer. The results of this study showed that the *CAT-262 C/T* gene polymorphism can be used as a marker for some specific types of cancer with geographic localization, but cannot be used as a good prognostic factor for survival in cancer patients (20). In this study, while the frequency of CAT-262 C/T CC/CT/TT genotypes in allergic rhinitis patients was 63.08%, 17.29% and 19.63%, respectively, the frequency was 82.63%, 9.32%, and 8.05% in the control group, respectively. Based on these results, we found a statistically significant relationship between allergic rhinitis and *CAT-262 C/T* gene polymorphism genotypes. This shows that as it plays a role in the etiopathogenesis of many other diseases, *CAT-262 C/T* gene polymorphism is also significant in allergic rhinitis and antioxidants can be used for treatment. GSH-Px is an endogenous enzyme that acts as an antioxidant in our body. There are at least 4 different GPX isoenzymes in mammals. *GSH-Px1* gene is located on chromosome 3p21.3 (21). GSH-Px 1 can metabolize organic peroxides including cholesterol and long chain fatty acid peroxides and H_2O_2.

Many studies have investigated *GSH-Px1 Pro198Leu* gene polymorphisms. Sousa et al. (22) investigated the relationship between chronic hepatitis C and *CAT* and *GSH-Px1* gene polymorphisms. Four hundred forty-five patients with chronic hepatitis C were included in this study. In this group, 139 patients had mild liver fibrosis (F0-F1), 200 patients had moderate liver fibrosis (F2-F4), and 106 patients had hepatocellular carcinoma (HCC). Because of this study, CT + TT genotypes and frequency of the T allele in the *CAT* gene was higher in patients with HCC compared to other patient groups (moderate and mild liver fibrosis). However, this value was not statistically significant. In terms of *GPx1 Pro198Leu* gene polymorphism, the frequency of pro/pro genotype and pro allele were found to be lower in patients with mild liver fibrosis compared with patients in other groups (HCC and moderate liver fibrosis). When the distribution of CT + TT genotypes in the *CAT* gene and pro/pro genotypes in *GSH-Px1* gene were evaluated together, a strong relationship was found with liver fibrosis grading and HCC (22). In this study, when the genotype frequency in the *GSH-Px1* gene of allergic rhinitis was compared with reference to the control group using multiple regression model, it was concluded with 95% confidence that Pro/Leu and Leu/Leu genotypes increased the susceptibility to allergic rhinitis by 0.9948 and 12,914 times, respectively. However, this increase was not statistically significant. When the genotype frequency of the *CAT* gene was compared using a multiple regression model, it was concluded with 95% confidence that the CT and TT genotypes increased susceptibility to allergic rhinitis by 3,044 and 3,193 times, respectively. This increase was found to be statistically significant. SOD catalyzes the superoxide radical to H_2O_2 and molecular oxygen. There are 3 forms of the SOD enzyme. SOD2, MnSOD is located in the mitochondria and this gene contains five exons and is located on chromosome 6q25 (23). Seçkin et al. (24) investigated the relationship between vitiligo disease and *Mn-SOD* and *GSH-Px1* gene polymorphisms in their study. *Mn-SOD Ala-9Val* and *GSH-Px 1 Pro-198Leu* gene polymorphisms were evaluated. Fifty-seven patients (32 female, 25 male) and 69 controls (40 female, 29 male) were included in this study. The frequencies of *Mn-SOD Ala-9Val* gene polymorphisms (Ala/Ala, Ala/Val, and Val/Val genotypes) in vitiligo patients were 19.3%, 49.1%, and 31.6%, respectively. In the control group, the frequency was 17.4%, 47.8%, and 34.8%, respectively. Additionally, the frequencies of Pro/Pro, Pro/Leu, and Leu/Leu genotypes of *GSH-Px1 Pro-198Leu* gene polymorphism in vitiligo patients were 38.6%, 49.1%, and 12.3%, respectively, whereas the frequency was 42.0%, 39.1%, and 18.8% in the control group, respectively. Based on these results, the authors could not find a significant difference between susceptibility to vitiligo disease with respect to *GSH-Px1 Pro-198Leu* and *Mn-SOD Ala-9Val* gene polymorphisms (24). In this study, we investigated whether there is a relationship between allergic rhinitis and *GSH-Px1 Pro198Leu* and *Mn-SOD Ala16Val* gene polymorphisms. Based on our results, Pro/Leu and Leu/Leu genotypes of *GSH-Px 1 Pro198Leu* polymorphism increased the susceptibility to allergic rhinitis by 0.9948 and 12,914 times, respectively, but this increase was not statistically significant. Val/Ala and Ala/Ala genotypes of Mn-SOD Ala16Val polymorphism increased the susceptibility to allergic rhinitis by 3.1048 and 2.9707 times, respectively, and this increase was statistically significant.

Study Limitations

The most important limitation of the study was that the patient group was small and none of the patients had a previous history of asthma or other allergic diseases.

Conclusion

The accumulation of FORs plays an effective role in the etiopathogenesis of many diseases. The number of studies on the etiopathogenesis of allergic rhinitis are limited in the literature. Therefore, to shed more light on the etiopathogenesis of allergic rhinitis, we examined gene polymorphisms in *GSH-Px, CAT* and MnSOD enzymes, which are known to act as antioxidants in our body. Based on the results of this study, although there was a statistically significant difference between allele frequencies and genoty-pes of *CAT-262 C/T* and *Mn-SOD Ala16Val* polymorphisms with respect to allergic rhinitis, no statistically significant difference was found between *GSH-Px 1 Pro198Leu* gene polymorphisms. In this study, we provided new treatment options by further illuminating the etiopathogenesis of allergic rhinitis. The results of this study indicate that antioxidant therapy may also be an option for treating allergic rhinitis.

Ethics Committee Approval: The study was approved by the Ethics Committee of Van Yüzüncü Yıl University Clinical Research Ethics Committee (approval number: 10, date: 27.10.2015).

Informed Consent: Informed consent wasn't obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - P.K.; Concept - P.K., N.B.; Design - P.K., N.B., M.B.; Data Collection or Processing - P.K.; Analysis or Interpretation - P.K., N.B., M.B., H.Ç.; Literature Search - P.K., H.Ç.; Writing - P.K.

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

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

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İstanbul Med J 2022; 23(3): 164-9
DOI: 10.4274/imj.galenos.2022.24478

Evaluation of Skin Cancers in a 7-year Plastic Surgery Archive (2014-2020)

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ABSTRACT

Introduction: Skin cancer is one of the most important health problems in our age. In this study, we determined the prevalence of skin cancer in the region by evaluating the reports of skin cancer patients diagnosed at our center.

Methods: This is a retrospective study of patients diagnosed with malignant skin cancer by biopsy between January 2014 and December 2020 for 7 years in a single center. The year, age, gender, tumor types, localization surgical treatments and recurrences of these reports were examined.

Results: A total of 852 patients were retrospectively analyzed. Four hundred and ninety-seven were men and 355 were women. The average age was 67.0 years (9 y - 98 y). Six hundred and twenty-seven were evaluated as basal cell carcinoma, 133 as squamous cell carcinoma, and 25 as melanoma. 84.8% (n=774) of the tumors were located in the head and neck region (most frequently in the nose region). All our patients were treated with appropriate surgical treatments (wide excision primary surgical repair, surgical repairs with flaps and grafts) and the patients were followed up for at least 1 year. Recurrence was observed in 71 of 852 patients after surgical excision and they were re-excised.

Conclusion: In our study; we have found that our region in general had a similar incidence of malignant skin tumors compared with the other studies. The localization, surgical treatment and follow-up results were also similar. This study will shed light on the characteristics, behaviors, preventive measures, diagnosis and treatment of skin cancers.

Keywords: Skin cancer, epidemiology, plastic surgery

Introduction

Today, skin cancer continues to be one of the important health problems, as it is with most cancers. Skin cancer is the most common cancer among all cancer types. In order of frequency of malignant tumors of the skin are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), malignant melanoma (MM) and skin appendage tumors (1-3).

BCC and SCC, which are non-melanocytic tumors, constitute 90% of skin malignant tumors. The mortality rate was 0.1%. BCC has a lower metastasis and mortality rate than SCC. Most are caused by ultraviolet B (UVB) and due to genetic anomalies. Apart from this, smoking, human papilloma virus, radiotherapy, chronic ulceration and burn, xeroderma pigmentosum, suppression of the immune system, chemicals such as arsenic - vinyl chloride, polycyclic aromatic hydrocarbons and alkylating agents are also carcinogens effective in their emergence (4,5). MM is the 7th most common type of cancer in the United States of America (USA). In the etiology of MM; UVB, genetic and environmental factors play a role. Tumors of the skin appendage include hair follicle tumors, sebaceous tumors, apocrine tumors, eccrine tumors and complex adnexal tumors. Although cases show autosomal dominant tumor suppressor gene mutation, generally no specific etiological agent has been found (6-8).

Basal and squamous cell cancers are easily diagnosed and often treated with excision when diagnosed early. The definitive diagnosis of malignant skin tumors is made by punch biopsy, incisional biopsy, or excisional biopsy. Tumor excision margins differ according to localization and pathological type. Defects formed after tumor excision are repaired using surgical techniques such as primary repair, graft repair, or flap repair. Lymph node dissection can be performed depending on the tumor location, pathological examination, the involvement of the lymph nodes and factors related to the patient. Postoperative radiotherapy can be applied in patients with perineural invasion, lymph node involvement, nodal extracapsular invasion, positive surgical margins in SCC, some patients with positive margins of BCC and some frequently recurring malignant skin tumors.

The abstract of this study was presented at the International Harran Health Sciences Meeting on 07.05.2021.

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Received: 03.04.2022
Accepted: 09.06.2022

Cite this article as: Toplu G, Altınel D, Serin M. Evaluation of Skin Cancers in a 7-year Plastic Surgery Archive (2014-2020). İstanbul Med J 2022; 23(3): 164-9.

The current article contributes to the epidemiology of skin cancers in our region, by determining the epidemiological characteristics, age, sex, location and histopathological types of patients with malignant skin tumors treated in our clinic, and by retrospectively evaluating the operation and recurrence rates.

Methods

Skin cancer patients over a 7-year period between January 2014 and December 2020 were included in this retrospective study. Patients diagnosed with malignant skin cancer by biopsy at our center were retrospectively analyzed. Patient consent was received from the patients. Before starting the study, the approval of the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee was obtained (approval number: 2959, date: 05.11.2021). Age, gender, tumor types, localization surgical treatments, and recurrences of these reports were analyzed. Data were entered using the Microsoft Office Excel program. Results are given as percentages and numbers.

Statistical Analysis

Descriptive analysis was performed using GraphPad Prism version 7.00 for Windows. (GraphPad Software, La Jolla California USA).

Results

A total of 852 patients were investigated retrospectively. Four hundred and ninety-seven were male and 355 were female. In total, 852 skin cancers were seen at an average age of 67.0 (9 y - 98 y) (Table 1). Eight hundred and five patients had a single lesion and 47 patients had multiple lesions, which were all BCC. There were total of 913 lesion (Table 2). Of 852 patients diagnosed with malignant skin cancer, In our study, the order of malignant tumors involving the skin was as follows; 627 of them were evaluated as BCC (most common subtype was nodular - n=370), 133 of them as SCC, 25 of them as melanoma (Table 3). 84.8% (774) of the tumors were located in the head and neck region (most commonly the nose) (Table 4). BCC was most frequently seen in nose (n=238), cheek (n=73) and periorbital (n=68) region (Table 5). SCC was most frequently seen upper extremity (n=18) and cheek (n=18) (Table 6). Melanoma was most frequently found in upper (n=6) and lower (n=8) extremities (Table 7). All of our patients were treated appropriately (wide excision, primary surgical repair, surgical repair with flap and graft), and the patients were followed up for at least 1 year (Table 8). Local anesthesia was used in majority of the cases (Table 9). One hundred and sixteen patients had positive surgical margin following the initial excision and were re-excised in the following 3 weeks (Table 10). Relapse was observed during the first postoperative year in 71 patients after surgical excision and they were re-excised (Table 11).

Table 1. Number of patients and sex distribution in each group

	Number (n)	Mean age	Range
Male	497	68	9-93
Female	355	65.5	12-98
Total	852	67	9-98

Table 2. Number of lesions in each patient

Number of lesions	Number of patients	Total number of lesions
1	805	805
2	36	72
3	9	27
4	1	4
5	1	5
	852	913

Discussion

Malignant skin tumors are mostly seen in Caucasians. It is one of the most common tumors and its mortality rates are low compared with other malignant tumors. The incidence is the same in developed and developing countries. However, the mortality rate is higher in developing countries since early diagnosis and treatment opportunities are more limited in these countries.

If there are no predisposing factors, mostly malignant skin cancers are seen after 40 years of age. It has been reported in the publication that skin cancers are more common in men. This is compatible with our findings. In our study, the order of malignant tumors involving the skin was as follows: 627 of them were evaluated as BCC (the most common subtype "Nodular" -n=370), 133 as SCC, 25 as melanoma. In most of the other studies, it was stated that the order of frequency was similar (2,9-11).

In our study, the most common location of malignant skin tumors was found to be the head and neck region, which is compatible with previous findings. In the head and neck region, the most common tumor localization was the nose. BCC is the most common type of

Table 3. Number of lesions according to histopathological subtype

Histological type	Number (n)	Percentage (%)
Basal cell carcinoma	627	73.6
Squamous cell carcinoma	133	15.6
In situ SCC	30	3.5
Melanoma	25	2.9
Basosquamous carcinoma	10	1.2
Lymphoma	8	0.9
Trichoblastic carcinoma*	4	0.5
Eccrine porocarcinoma*	4	0.5
Sebaceous carcinoma*	3	0.4
Dermatofibrosarcoma protuberans	3	0.4
Atypic spitz tumor	2	0.2
Verrucose carcinoma	2	0.2
Sebaceous carcinoma *in situ**	1	0.1
Spindle cell main mesenchymal tumors	1	0.1
Microcystic adnexal carcinoma*	1	0.1
Clear cell carcinoma metastasis	1	0.1
Carcinoma infiltration (lung metastasis)	1	0.1
Mycosis fungoides	1	0.1
Carcinosarcoma (metaplastic carcinoma)	1	0.1

SCC: Squamous cell carcinoma

Table 4. Number of lesions according to location

	Number (n)	Percentage (%)
Nose	253	27.7
Nose tip	19	2.1
Dorsum of my nose	212	23.2
Nose edge	9	1.0
Nose wing	13	1.4
Glabella	2	0.2
The forehead	50	5.5
Cheek	117	12.8
Lips	39	4.3
Upper lip	24	2.6
Lower lip	13	1.4
Commissary	2	0.2
Temporal	63	6.9
Scalp	54	5.9
Periorbital	4	0.4
Eyebrow	22	2.4
Eyelid	60	6.6
Jaw	14	1.5
Neck	22	2.4
Ear	61	6.7
Pre-auricular	14	1.5
Behind the ear	10	1.1
Auricular lobule	1	0.1
Nape	10	1.1
Nasolabial	3	0.3
Trunk	56	6.1
Supraclavicular	2	0.2
Body	12	1.3
Back	24	2.6
The sternum	5	0.5
Abdominal	8	0.9
Lumbar	2	0.2
Sacrum	2	0.2
Anal	1	0.1
Upper extremity	43	4.7
Axilla	2	0.2
Shoulder	8	0.9
Arm	6	0.7
Forearm	10	1.1
Hand	16	1.8
The elbow	1	0.1
Lower extremity	40	4.4
Inguinal	7	0.8
Hip	2	0.2
The thigh	8	0.9
Leg	11	1.2
The knee	2	0.2
Foot	10	1.1
Total	913	

Table 5. Number of BCC lesions according to location

	Number (n)	Percentage (%)
Nose	238	38.0
Glabella	2	0.3
The forehead	33	5.3
Cheek	73	11.6
Lips	19	3.0
Temporal	40	6.4
Scalp	31	4.9
Periorbital	68	10.8
Jaw	11	1.8
Neck	17	2.7
Ear	33	5.3
Nape	6	1.0
Trunk	24	3.8
Upper extremity	14	2.2
Lower extremity	8	1.3
Inguinal	10	1.6
Total	627	-

BCC: Basal cell carcinoma

Table 6. Number of SCC lesions according to location

	Number (n)	Percentage (%)
Nose	2	1.5
Glabella	0	0.0
The forehead	10	7.5
Cheek	18	13.5
Lips	16	12.0
Temporal	9	6.8
Scalp	11	8.3
Periorbital	6	4.5
Jaw	1	0.8
The neck	2	1.5
Ear	17	12.8
Nape	4	3.0
Trunk	9	6.8
Upper extremity	18	13.5
Lower extremity	9	6.8
Inguinal	1	0.8
Total	133	-

SCC: Squamous cell carcinoma

cancer in the Caucasian race and is the most common tumor of the skin. These cancers, which make up 50-75% of all skin cancers, are locally slow growing tumors that almost do not metastasize. In our study, BCC was the most common malignant skin tumor. It has been reported in the literature that the incidence of BCC increases age. In our study, the mean age of the patients diagnosed with BCC was 67 years. It has been reported that it is mostly located in the head and neck region. Shanoff et al. (12) reported that the distribution of BCCs in the body is

Table 7. Number of melanoma lesions according to localization

	Number (n)	Percentage (%)
Nose	0	0.0
Glabella	0	0.0
The forehead	1	4.0
Cheek	2	8.0
Lips	0	0.0
Temporal	1	4.0
Scalp	1	4.0
Periorbital	0	0.0
Jaw	1	4.0
Neck	0	0.0
Ear	0	0.0
Nape	0	0.0
Trunk	3	12.0
Upper extremity	6	24.0
Lower extremity	8	32.0
Inguinal	2	8.0
Total	25	-

Table 8. Types of surgical methods for defect closure

	Number (n)	Percentage (%)
Primary repair	673	79.0
Grafting	61	7.2
Local flap	69	8.1
Regional flap	49	5.8
Total	852	-

Table 9. The type of anesthesia implemented

	Number (n)	Percentage (%)
Local anesthesia	696	81.69
Local + sedation	81	9.51
General anesthesia	75	8.80
Total	852	-

93%, in the head and neck g most frequently in the nose 26%, then 18% malar, 8% eye, 8% ear, 5% infraorbital region, 5% upper lip, 5% forehead, percentage It was seen in 4 postauricular, 3% chin, 1% cheek regions.

SCC mostly develops on the damaged skin. For example, underlying sun damage, actinic keratoses, burn scars, chronic ulcers can be seen. In our study, we found that SCC was in the second place in terms of incidence following BCC, and of these 133 patients, 67% were male and 33% were female. The mean age of the patients was 72 years. Our findings are similar to the literature findings (1,2,10,11,13-16).

In the study by Freeman et al. (17), in which they investigated the localization of SCC, 79% of the lesions were seen in the head and neck region, 45% in the cheek and lip region, 13% in the nose region, and 12% in the ear region. In our study, the order of localization of SCC lesions was upper extremity, cheek, ear and lips. The most common localization of the lesions was in the head and neck region as stated in the literature (17).

Table 10. Number of positive surgical margin cases according to histological type

	Number (n)	Percentage (%)
BCC	78	67.2
SCC	15	12.9
Melanoma	7	6.0
Basosquamous carcinoma	6	5.2
Leiomyosarcoma	1	0.9
T-cell lymphoma	1	0.9
Sebaceous carcinoma	1	0.9
Dermatofibrosarcoma protuberans	1	0.9
Malignant mesenchymal tumor	1	0.9
Malign eccrine poroma	1	0.9
Trichoblastic carcinoma	1	0.9
Lentigo maligna	1	0.9
Carcinoma metastasis	1	0.9
Carcinosarcoma	1	0.9
Total	116	-

BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma

Table 11. Number of relapses according to the histological type

	Number (n)	Percentage (%)
BCC	40	46.5
SCC	10	11.6
Melanoma	10	11.6
Leiomyosarcoma	1	1.2
Basosquamous carcinoma	3	3.5
T-cell lymphoma	1	1.2
Sebaceous carcinoma	1	1.2
Dermatofibrosarcoma protuberans	2	2.3
Malignant mesenchymal tumor	1	1.2
Malign eccrine poroma	1	1.2
Trichoblastic carcinoma	1	1.2
Total	71	-

BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma

Among skin cancers, MM is the third most frequently reported type of skin cancer. In our study, MM was the third most common MM in 25 patients. Melanoma is common in the head and neck and extremities. It is more common on the trunk in men and on the lower extremities in women. In our study, patients diagnosed with MM were 48% female, 52% male, and the mean age was 54 years. We observed that the incidence was similar in men and women, and the most common localization was the extremities and cheek.

Skin appendage tumors are tumors with histopathological features resemble skin appendages. Most of them are benign and rarely seen in malignant character. In our study, the total number of malignant skin appendage tumors was 13. The mean age of the patients with a diagnosis of skin appendage tumor was 60.5 years. The patients were 27% female and 73% male. Skin appendage tumors are mostly located in the head and neck region, extremities. Similar to the literature, it was

observed that the lesions were located in the head and neck region in 75% of the patients in our study.

Koplin and Zarem (18) suggested an excision margin of 2 mm for lesions less than 1 cm in diameter and 3-4 mm for lesions larger than 1 cm in diameter. In our clinic, we performed excision by leaving a surgical margin of 2-5 mm in lesions smaller than 2 cm in diameter and 1 cm in lesions larger than 2 cm in diameter and recurrent lesions as the surgical margin in our patients diagnosed with BCC. We applied primary closure to 85% patients with BCC under local anesthesia, grafts and flaps were used in the remaining 15% of the patients. Because of the pathological examinations performed after the surgical treatment, it was observed that the surgical margin was positive in 12% patient diagnosed with BCC, and re-excision was applied to these lesions (18).

Brodland and Zitelli (19) argued that SCC lesions with a diameter of less than 2 cm should be excised with a surgical margin of 4 mm, and those with a diameter of more than 2 cm with a surgical margin of 6 mm. In our study, we left the surgical margin at 1 cm in our patients with SCC. We performed primary closure in 96 patients with SCC, 15 of them were grafted and 21 had repair with flaps. A 15 patients had positive surgical margins following surgery. Since SCCs can metastasize, their follow-up was performed regularly for 1 year, and preoperative and postoperative evaluations were made carefully in terms of LAP (19).

Tumor depth in MM is an important criterion for the prognosis of the disease and the type of treatment to be applied. Suggested surgical excision margins; 0.3-0.5 cm in lesions *in situ*, 1 cm in lesions less than 1 mm in tumor thickness, 2 cm in lesions between 1 and 2 mm, 2 cm in lesions between 2.01-4.0 mm, 2-3 cm in lesions larger than 4 mm has were determined. We performed excision in our patients in accordance with the surgical margins stated in the literature.

Even after the surgical treatment of malignant skin tumors, recurrence can still be seen. Relapse is the recurrence of a surgically removed lesion with the same histological features at the same location within 5 years. Factors such as the surgical treatment method, the experience of the practitioner, the histological features of the lesion, its location and size may affect the recurrence rates. The relationship between malignant lesion excision margin and recurrence is important. Pascal et al. (20) examined 143 cases of basal cell cancer in which the surgical margin was evaluated at the time of excision and followed the recurrence rate for 5 years. In this study, if the distance of tumor cells from normal tissue was above 0.5 mm, the recurrence rate was 1.2%; 12% if closer than 0.5 mm; reported as 33% if at least one surgical margin is persistent. In our study, however, the relationship between surgical margin positivity and recurrence was not evaluated. Because the patients with positive surgical margins are operated again and the intact surgical margin is reached. The entire group of patients we followed up consisted of patients who had intact surgical margins. Recurrence was observed at a rate of 8.3% of the 852 patients included in our study. The recurrence rate was determined as 6.3% for BCC and 7.5% for squamous cell cancers. All patients with non-melanoma skin cancer excised should be followed up for recurrence. There are two important points in the follow-up of patients with cutaneous MM. First; detection of local, regional, or distant recurrences, and the second is the early detection of MM. Patients with detailed anamnesis, physical examination, examination of all regional lymph nodes, and patients with a family history of MM or clinically dysplastic nevus should be offered lifelong follow-up. While early recurrences are seen in patients with large tumor thickness, recurrences can be detected in patients with low tumor thickness over a period of more than 10 years. In our study, MM was diagnosed in 25 patients. It was observed that recurrence developed in 40% of the patients. Additionally, the recurrence rate of patients diagnosed with skin attachment tumors included in our study was 25% (8,18,20).

In our study; it was concluded that the malignant skin tumor profile of our hospital and even our region and the frequency, localization, surgical treatment choices and follow-up principles seen throughout Turkey and even in the world are generally similar. Thus, this study will shed light on the characteristics, behaviors, preventive measures, and planning of additional diagnosis and treatment research of skin cancers.

Study Limitations

This study had several limitations. This was a retrospective study and the patient data were collected from a single-center. Patient standardization is imperfect and prospective randomized studies can overcome these limitations and support our findings.

Conclusion

Early diagnosis and treatment of malignant skin tumors are important both in terms of treatment efficiency and the surgical method to be used. Early diagnosis and treatment are critical for preventing the spread of lesions and reducing recurrence. The results of these studies suggest that keeping the excision margins narrow for various reasons, such as cosmetic and functional concerns, increases cancer recurrence. Regular follow-up of skin cancer patients is of great importance in the early diagnosis of new skin cancer formation and recurrence. Since UV damage is an important etiological agent in malignant tumors of the skin, particularly sun-exposed areas such as the head and neck region are at high risk of the development of these tumors. It causes functional and aesthetic deformation, sometimes mortal, especially in a psychologically and sociologically important location such as the head-neck region. Therefore, patients and healthcare professionals should be educated about skin tumors and awareness should be raised. Important steps can be taken in the early diagnosis and treatment of skin cancer by keeping national cancer registries regularly and raising awareness of people about skin cancer symptoms and treatment methods. Additionally, printed and visual images of society, especially on sun protection methods.

Ethics Committee Approval: The approval of the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee was obtained (approval number: 2959, date: 05.11.2021).

Informed Consent: Patient consent was received from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - G.T., D.A., M.S.; Concept - G.T., D.A.; Design - D.A., M.S.; Data Collection or Processing

- D.A., M.S.; Analysis or Interpretation - G.T., D.A., M.S.; Literature Search - G.T., D.A.; Writing - G.T., M.S.

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

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

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İstanbul Med J 2022; 23(3): 170–3
DOI: 10.4274/imj.galenos.2022.62362

Tumor/Nodule Size Ratio: A Possible Reason for False-Negative Thyroid Cytology

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ABSTRACT

Introduction: Fine-needle aspiration cytology is useful for the diagnosis and management of thyroid nodules. However, false negatives for malignancy may occur and affect treatment success. In this study, we investigated carcinoma size itself as another possible reason for false-negative results.

Methods: We retrospectively reviewed patient charts who had undergone total thyroidectomy and complementary thyroidectomy. A total of 613 cases were investigated. Patients who had a final histopathological diagnosis of thyroid carcinoma were included, and 138 cases were eligible for the study. Patients were categorized into three groups according to their fine-needle aspiration biopsy reports: Benign cytology and atypical cells of undetermined significance (group 1), cytology suspicious for a follicular/Hurthle cell neoplasm (group 2), and suspicious or positive for malignancy (group 3).

Results: Group 1 consisted of 55 patients with a mean tumor/nodule size ratio of 0.5236. Group 2 consisted of 21 patients with a mean tumor/nodule size ratio of 0.76. Group 3 consisted of 62 patients with a mean tumor/nodule size ratio of 0.848. There were no differences between the groups in terms of nodule size measured by ultrasonography ($p=0.209$), but the diameter of the carcinoma focus within the nodule was significantly smaller in false-negative cases ($p<0.001$). There were no statistically significant differences between the groups in terms of multicentricity ($p=0.197$).

Conclusion: The size of malignant tumors may be more important than nodule size in explaining false negativity.

Keywords: Fine-needle biopsy, thyroid nodule, thyroid carcinoma

Introduction

Thyroid nodules are a common condition encountered by physicians from different specialties. During the period when imaging methods were not as widespread, thyroid nodule incidence was reported to be 5 to 10% (1-3). As ultrasound, computed tomography, and magnetic resonance imaging became more available, the prevalence of thyroid nodules increased to a now-estimated range of 20 to 60% depending on age, gender, and geographical location (4,5).

Fine needle aspiration biopsy (FNAB) is the most respected method for the management of thyroid nodules. However, false-negative FNABs are a major problem, as they may result in a missed diagnosis of cancer. The sensitivity of FNAB for carcinomas in thyroid nodules is between 80% and 94%. False-negative rates are reported in a range from 3.6 to 21% in different studies (6-8).

In some studies, authors suggest that false-negative FNABs are more common in large nodules due to both sampling inaccuracy and altered pretest probabilities (9-14), but there is still no consensus because the data of others show that nodule size is not associated with false negative biopsy rates (15-19).

A obstacle in evaluating false-negative biopsy rates is that most of the benign cytology nodules do not need surgery. Therefore, most benign biopsies are not checked in the final histological diagnosis (15).

In this study, we reviewed patients who had undergone total thyroidectomy or complementary thyroidectomy. The general indications for surgery were suspicious or malignant biopsies, repeated inadequate biopsies, massive goiters causing compressive symptoms, significant retrosternal extension, and selected cases of hyperthyroidism.

The present study detect false-negative cytology performed in our clinic and to investigated possible reasons for false negativity.

Methods

Ethical approval was obtained from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethical Committee of the hospital (approval number 1557, date: 07.12.2018).

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Cite this article as: Boyoğlu NS, Yiğit Ö, Övünç O, Bilici S, Sünter AV. Tumor/Nodule Size Ratio: A Possible Reason for False-Negative Thyroid Cytology. İstanbul Med J 2022; 23(3): 170-3.

Received: 11.03.2022
Accepted: 21.06.2022

Patients who had undergone total thyroidectomy or complementary thyroidectomy in our clinic between 2010 and 2018 were reviewed. A total of 613 cases were analyzed, and those who had non-diagnostic cytologies, biopsies performed without ultrasonography, or biopsies performed at another hospital were excluded from the study. Of the remaining 400 patients, those who had a benign final histopathological diagnosis were also excluded. Patients with a carcinoma other than the biopsied nodule were noted. These cases of incidentally diagnosed carcinoma on histological examination of a non-biopsied nodule were classified on the basis of the FNAB report of the biopsied nodule. All FNAB are done under ultrasonography by a senior radiologist. All FNABs were performed using a 22-G needle on a 10 mL syringe with ultrasound guidance.

A total of 138 patients were included in the study. Each patient's carcinoma category, maximum carcinoma diameter, maximum biopsied nodule diameter, multicentricity, FNAB cytology diagnosis, age, and gender information were recorded.

Patients were categorized into three groups according to their FNAB reports: Benign cytology and atypical cells of undetermined significance (group 1), cytology that was suspicious for a follicular/Hurthle cell neoplasm (group 2), and cytology that was suspicious or positive for malignancy (group 3). FNAB results were considered false negative in all patients except those with suspicious or malignant cytology (group 3).

Statistical Analysis

Statistical analysis was conducted using SPSS version 24. To compare the three groups, Kruskal-Wallis test was performed for quantitative variables and chi-square test was performed for the categorical data. Statistical significance was concluded when $p<0.05$.

This study was presented as a written poster at AAO-HNSF 2019 Annual Meeting & OTO Experience.

Results

A total of 138 patients had a final histopathological diagnosis of thyroid carcinoma, of which 38 (27.5%) had a benign FNAB report, 21 (15.2%) had atypia of undetermined significance/follicular lesion of undetermined significance, 17 (12.3%) had a diagnosis of follicular neoplasia/suspicious for follicular neoplasia, 25 (18.1%) had a FNAB report of "suspicious for malignancy," and 37 (26.8%) had a malignant FNAB report.

The descriptive statistics of the patients are shown in Table 1. In group 1, there were 55 patients (10 male and 45 female) with a mean age of 48.82±11.65 years. The mean tumor size was 11.6 mm, the mean nodule size was 23.1 mm, and the mean tumor/nodule size ratio was 0.52. The cancer was multicentric in 22 patients (40%).

In group 2, there were 21 patients (10 male and 11 female) with a mean age of 46.38±12.38 years. The mean tumor size was 24.9 mm, the mean nodule size was 32.3 mm, and the mean tumor/nodule size ratio was 0.76. The cancer was multicentric in 11 patients (52%).

In group 3, there were 62 patients (22 male and 40 female) with a mean age of 51.44±14.00 years. The mean tumor size was 20.8 mm, the mean nodule size was 23.9 mm, and the mean tumor/nodule size ratio was 0.84. The cancer was multicentric in 35 patients (56%).

There were no differences between the groups in terms of nodule size measured by ultrasonography ($p=0.209$), but the diameter of the carcinoma focus within the nodule was significantly smaller in false-negative cases ($p<0.001$). There were no statistically significant differences between the groups in terms of multicentricity ($p=0.197$).

The distribution of carcinoma types is shown in Figure 1 and subtypes among groups are shown in Table 2.

According to the FNAB cytology results, the false-negative rate of the follicular variant subtype was significantly higher in all (micro + non-micro) papillary carcinoma cases ($p<0.001$).

We also noted that in 25 of the 66 patients with false-negative cytologies, FNAB was performed on the dominant nodule, but the carcinoma originated from another nodule.

Discussion

Many surgeons and clinicians are working to identify additional indicators to predict malignancy and hence to provide accurate recommendations for or against surgery. While nodule size is prioritized in many studies, nodule diameter and actual carcinoma diameter were treated together with similar importance in this study, each of them affects the ratio of the nodule size and tumor size.

Table 1. Descriptive statistics of the patients classified by groups

	Group 1 (n=55)	Group 2 (n=21)	Group 3 (n=62)	p-value
Male	10	10	22	0.023*
Female	45	11	40	
Age (year)	48.82±11.65 (17-71)	46.38±12.38 (24-67)	51.44±14.00 (18-83)	0.196**
Carcinoma size (mm)	11.60±14.15 (1-72)	24.90±21.14 (2-70)	20.85±18.69 (2-90)	<0.001**
Nodule size (mm)	23.10±15.22 (7-85)	32.30±21.54 (7-85)	23.90±18.41 (2-90)	0.204**
Tumor/nodule size ratio	0.52±0.35 (0.03-1.0)	0.76±0.34 (0.04-1.0)	0.84±0.21 (0.15-1.0)	<0.001**
Multicentricity	22 (40%)	11 (52%)	35 (56%)	0.197**

*: Chi-square test, **: Kruskal-Wallis test

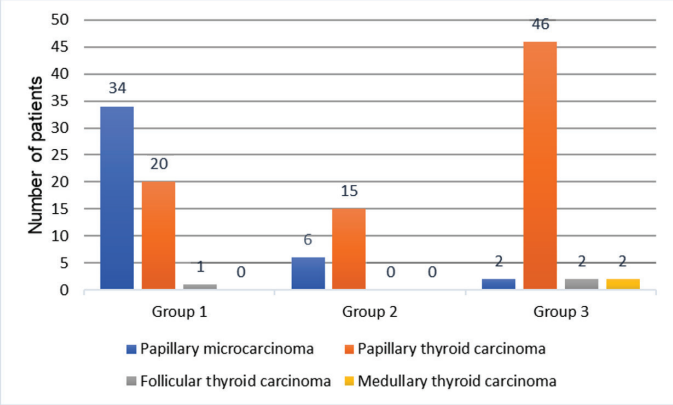

Figure 1. Carcinoma types among groups

Table 2. Carcinoma subtypes among groups

Carcinoma type	Group 1	Group 2	Group 3	Total
PMC-FV	18	3	2	23
PMC-CV	11	2	9	22
PMC-OV	5	1	1	7
PTC-FV	10	12	12	34
PTC-CV	6	2	26	34
PTC-OV	4	1	8	13
FTC	1	0	2	3
MTC	0	0	2	2

PMC-FV: Papillary microcarcinoma-follicular variant, PMC-CV: Papillary microcarcinoma-classical variant, PMC-OV: Papillary microcarcinoma-other variants, PTC-FV: Papillary thyroid carcinoma-follicular variant, PTC-CV: Papillary thyroid carcinoma-classical variant, PTC-OV: Papillary thyroid carcinoma-other variants, FTC: Follicular thyroid carcinoma, MDC: Medullary thyroid carcinoma

Although the nodule diameter has been investigated for malignancy predictor, data from several studies are conflicting. Mehanna et al. (15) found that in nodules larger than 3 cm, the false-negative rate was 10.9%, and in nodules smaller than 3 cm, it was 6.1%; however, this was not statistically significant.

In some studies, false-negative rates were reported to be 17 to 19.3% in thyroid nodules of size 3 to 4 cm with benign preoperative cytology (10,11). McCoy et al. (11) were the first to investigate false-negative FNAB cytology rates of larger nodules. They reported that false-negative rates were markedly higher in nodules of 4 cm or larger than in smaller nodules.

In contrast, Porterfield et al. (16) found a false-negative rate of 0.7% for nodules larger than 4 cm. Additionally, Albuja-Cruz et al. (20) did not find any significant false-negative results in nodules larger than 4 cm. In another study, Shrestha et al. (21) went further and found that the smaller the nodule size (<1 cm), the greater the probability of false negativity. Of note, large thyroid nodules are more common in many surgical series because they are more likely to be operated on than smaller nodules due to problems such as compression symptoms.

In fact, the most common cause of false-negative FNABs reported in some studies is micropapillary cancer, which cannot be needle-aspirated in the same nodule as an adenomatous goiter (6,17). Some studies do not even consider cases with microcarcinoma as false negative (6,10,18,22,23). However, one area of broad agreement is that the vast majority of false-negative FNABs are micropapillary carcinomas (24-26). Concordantly, in this study, we found that the false-negative ratio was higher in micropapillary carcinomas and especially in follicular variants of papillary thyroid carcinomas.

Because of the combination of benign and malignant regions in the dominant nodule, false-negative results may occur due to cells in the remaining parts showing benign cytological features. Taking multiple aspirations from various parts of the nodule could decrease false-negative results arising from this heterogeneity.

Ylagan et al. (27) noted a 4% false-negative rate in 255 patients, most of whom had micropapillary carcinoma. They also found that false negativity was due to interpretation errors in 14 (6%) of the 255 cases, which was explained by the emergence of overlapping cytological features in adenomatous nodules, follicular neoplasms, papillary thyroid carcinoma follicular variants, and Hashimoto thyroiditis (27). Mehanna et al. (15) also found that the probability of having a false-negative biopsy from a follicular variant papillary carcinoma was significantly higher than that of conventional or other papillary carcinoma variants, which is in line with the results of the study by Albuja-Cruz et al. (20) and our study (10).

In this study, we also noticed that 37.8% of the false-negative biopsies were biopsy taken from a different nodule other than the nodule the tumor was in. This supports the need to perform biopsies of all nodules and not only of the dominant nodule.

A obstacle in evaluating false-negative FNAB rates in thyroid nodules is that most nodules with benign cytology are not operated on. Therefore, the final histolopathological diagnosis is unknown, in most cases.

In this study, there was no difference between the groups in terms of nodule sizes, we found that the tumor diameter was much smaller in the false negative group. This resulted in a statistically significant difference in tumor size/nodule size ratio. We couldn't suggest a cut-off value because it seems that the reason for this ratio was the difference in the size of the cancer foci.

To date, researchers have focused mostly on nodule size because it is the most accurate data available to guide the decision-making process. However, our study is novel because it focuses on the tumor/nodule size ratio, which is a different perspective.

Study Limitations

This study has several limitations because we compared the results of FNAB with the postoperative pathology report. In other words, we only included patients who had undergone both thyroid nodule biopsy and surgery, and so patients who did not undergo surgery were excluded from the study. This was not a specificity-sensitivity study because we only evaluated carcinoma cases.

In this study, we could not suggest any information that could be useful in decision-making before thyroid surgery, but we have provided a different point-of-view regarding false-negative cytologies.

Conclusion

The size of malignant tumors may be more important than the nodule size in explaining the false negativity of FNAB. The smaller the tumor size, the higher the false negative rate.

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethical Committee of the hospital (approval number: 1557, date: 07.12.2018).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - N.S.B., Ö.Y., O.Ö., S.B., A.V.S.; Concept - N.S.B., Ö.Y., S.B., A.V.S.; Design - N.S.B., Ö.Y., O.Ö., S.B., A.V.S.; Data Collection or Processing - N.S.B., Ö.Y., O.Ö., S.B., A.V.S.; Analysis or Interpretation - N.S.B., Ö.Y., S.B., A.V.S.; Literature Search - N.S.B., Ö.Y., O.Ö., S.B., A.V.S.; Writing - N.S.B., Ö.Y., S.B., A.V.S.

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

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

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İstanbul Med J 2022; 23(3): 174-8
DOI: 10.4274/imj.galenos.2022.69077

Effects of Smoking Cessation on Peak Nasal Inspiratory Flow and Nasal Mucociliary Clearance

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ABSTRACT

Introduction: In this study, the aim was to investigate smoking cessation on peak nasal inspiratory flow (PNIF) and nasal mucociliary clearance (MCC).

Methods: Sixty-two (32 male and 30 female) smokers were included in this prospective study. Varenicline (Champix®, R-Pharm, Germany) was prescribed to all subjects who want to quit smoking as supportive therapy. Three-month treatment is planned. Day 0 (baseline), 3rd month (after smoking cessation), and 6 h (3 months after smoking cessation) PNIF values with decongestant (PNIFwD) and without decongestant (PNIFsD) and nasal mucociliary clearance time (MCT) with the saccharine test were determined. The acquired data were evaluated statistically.

Results: The mean age of the subjects was 36.77±9.63 (minimum: 18, maximum: 60) years. Significant differences were found between the PNIF and MCT values during different study periods ($p<0.05$). The medians of nasal MCT values for the 3rd and 6th months were significantly lower than the median of baseline values ($p<0.05$). Additionally, the median of nasal MCT values for the 6th month was significantly lower than the median of nasal MCT values for the 3rd month ($p=0.0003$, $p<0.05$). The medians of PNIFsD values for the 3rd and 6th months were significantly higher than the median of baseline values ($p<0.05$). Additionally, the median of the 6th-month values was significantly higher than the median of the 3rd-month values ($p=0.023$, $p<0.05$). There was no significant difference in terms of PNIFwD evaluations ($p=0.06$, $p>0.05$).

Conclusion: The results of this study showed that smoking cessation improves nasal MCC and airflow.

Keywords: Smoking, mucociliary clearance, nasal obstruction/diagnosis, nose/physiology, saccharin/pharmacokinetics

Introduction

Tobacco use is one of the major public health problem, killing more than 8 million people worldwide each year (1). The most commonly used tobacco product in the world is cigarettes (2). Smoking is a chronic disease and since the 20th century, this epidemic has been the most common reason for preventable deaths (2,3). More than 80% of all smokers worldwide live in low- and middle-income countries (3). Although smoking is one of the most common causes of mortality and morbidity in these countries, it has devastating effects on the economies of these countries (3). Turkey is a country with the most frequent smoking in the world, with 14.8 million smokers (4). Because of its effects on health and the economy, removing smoking is one of the most important common goals of the world. To achieve this goal, countries have their projects besides international projects. The World Health Organization Framework Convention on Tobacco Control, which has been accepted by 182 countries where more than 90% of the world's population lives, is one of these projects (2).

Smoking, which shortens the lifetime, is involved in the etiology of malignant neoplasms of many organs, especially the lung, and chronic diseases of many systems, especially the cardiovascular and respiratory systems (5). One of these organs is the nose, which is the entrance to the respiratory tract (6-10). Two of the main nasal functions are respiration and mucociliary clearance (MCC). The insufficiency of nasal respiration function is clinically manifested by nasal congestion characterized by raised nasal resistance and decreased airflow (11). MCC, which is the initial and key defense mechanism of the airways, can be described as the retention and removal of detrimental foreign particles in the inspired air by the mucosa (12). The impairment of this function of the nose may present with many chronic diseases, especially infectious diseases, in the clinic (12).

To give up smoking, which has all these harmful effects, both the addiction of the person should be treated and the behavior and habits of the person should be changed (3). Smoking cessation needs psychological and pharmacological supports. In smoking cessation, the main psychological support is behavioral counseling and the main pharmacological

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Cite this article as: Çakan D, Uşaklıoğlu S. Effects of Smoking Cessation on Peak Nasal Inspiratory Flow and Nasal Mucociliary Clearance. İstanbul Med J 2022; 23(3): 174-8.

Received: 06.05.2022
Accepted: 22.06.2022

treatments are nicotine replacement therapy, bupropion, varenicline, and cytisine (5). Varenicline is a selective partial agonist for one of the nicotine receptors, α4β2 nicotinic acetylcholine receptor (5). It alleviates withdrawal symptoms via this receptor (agonist activity), while reducing rewards by preventing nicotine binding (antagonist activity) (5,13). There are many studies showing the effectiveness of the use of varenicline, which helps quit smoking by preventing withdrawal symptoms, alone or along with other treatment methods (14).

Nasal damage can be alleviated or completely healed by smoking cessation like many damages caused by smoking (5,15). In this study, we purposed to examine the effect of smoking cessation in nasal respiratory and nasal MCC functions.

Methods

This prospective study was performed at Cerrahpaşa School of Medicine and Eyüpsultan State Hospital between April 2021 and January 2022 with the confirmation of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (approval number: E-83045809-604.01.02.74209, date: 14.04.2021).

Participants, Inclusion, and Exclusion Criteria

All subjects of the study consulted the Smoking Cessation Clinic of Eyüpsultan State Hospital. A detailed anamnesis was obtained from every subject and a full otorhinolaryngological examination was done. All participants signed an informed consent form. Subjects who had been smoking for at least one year were included in the study. A smoker was defined as a person who currently smokes daily and has smoked at least one-hundred cigarettes in her/his lifetime (16).

The subjects under 18 and over 60 years of age, with an abnormality (ontological or respiratory) in full-endoscopic examination, with anamnesis of cranial trauma, with a neurological disease and/or a chronic disease such as obstructive pulmonary disease, with anamnesis of airway infection in the last three months, with anamnesis of otologic or airway procedure, with anamnesis of regular drug use in the last six months, with an anamnesis of taste and/or odor disturbance were excluded from this study.

Subject Size

The minimal subject size was determined based on the article of Develioglu et al. (17). The minimal subject number with a 95% reliance gap and 5% bearable mistake assumptions was 62.

Data Collection

Study Design

Day 0 (baseline): Detailed medical histories of the subjects were recorded. Varenicline (Champix®, R-Pharm, Germany) was prescribed to all subjects as supportive therapy for smoking cessation. Three-month treatment is planned. The drug dose was planned to be 1x0.5 mg for the first 3 days, 2x0.5 mg between the 4th and 7th days, and 2x1 mg between the 8th and 90th days (5). The subjects were then referred to the otorhinolaryngology clinic. A complete endoscopic otorhinolaryngological examination was performed. Nasal MCT was determined by the saccharine test method, and Peak Nasal Inspiratory Flow (PNIF) was examined with a PNIFmeter.

Day 90 (3rd month): After the treatment, all subjects gave up smoking, and the tests were repeated.

Day 180 (6th month): At the end of the study, the measurements were repeated 3 months after smoking cessation.

All measurements of the patients were performed after a 30-minute relaxation at the same place at a temperature of 20-25 °C and 50-70% humidity. Evaluation of all subjects was done by the same internal medicine and the same otorhinolaryngology specialists. For success in varenicline therapy, smokers should stop smoking 1 to 2 weeks after starting treatment (5). All subjects stopped smoking on the 10th day of treatment.

Methods

Endoscopic Examination

The nose was first examined with a Hartmann nasal speculum (Karl Storz, Germany). Then, the nasal cavity, pharynx, and oral cavity were examined with a 3.5 mm flexible fiberscope (Karl Storz, Germany).

Saccharine Test Method

The smoker was positioned in a chair. The subjects were instructed to not move, to not sniff, not sneeze, to open her/his mouth, and to perform nasal and oral breathing. For the nasal MCC test, 5 mg saccharin (Sakarin-oro, Oro İlaç, Turkey) was placed on the medial surface 0.5 centimeters behind the anterior of the inferior turbinate with alligator forceps (Karl Storz, Germany). The subjects swallowed every 30 seconds. When the subject notices that she/he has tasted saccharin was determined as mucociliary clearance time (MCT; second, sec.) (17). The nasal MCT alters approximately 720-900 seconds in healthy people (17).

PNIFmeter

Twenty minutes later the saccharine test, PNIF values were calculated using a PNIFmeter (Clement Clarke International Limited, England). The smokers were placed in a chair and the PNIFmeter mask was positioned to cover the mount and nose, deep and rapid inspiration was performed then forced expiration with the mouth closed. The evaluation was repeated 10 min after nasal decongestant, with one puff to each nasal cavity, xylometazoline (Otrivine®, GlaxoSmithKline, UK) administration. To provide subject compliance, the measurement was repeated 3 times and the highest value of the PNIF with decongestant (PNIFwD) and the PNIF without decongestant (PNIFsD) were saved as liters/minute (L/min). The PNIF value of a healthy person is 138.4 L/min (18).

Statistical Analysis

The minimal subject number was estimated using the G*Power software 3.1 (19). Statistical analyses were performed with the SPSS 21 (SPSS Inc., USA) program. Normal distribution and homogeneity of data were analyzed with the Kolmogorov-Smirnov test and Levene's tests, respectively. Wilcoxon signed ranks test and Friedman test were used for analysis. The significance level was defined as $p<0.05$.

Results

A total of 62, 32 male and 30 female, smokers were included in this study. All subjects gave up smoking and completed the study. The mean

age of the individuals was 36.77±9.63 (minimum: 18, maximum: 60) years. No serious drug-related side effects were observed.

There was a significant difference according to the nasal mucociliary clearance time values of the smokers at different periods (p=0.00001, p<0.05). In the evaluation of nasal mucociliary clearance time, the median of the 6th-month values was significantly lower than the medians of baseline and the 3rd-month values (p=0.000001; p=0.000001, respectively, p<0.05). Additionally, the median of the 3rd-month values was significantly lower than the median of initial values (p=0.0003, p<0.05) (Table 1, 2) (Figure 1).

There was a significant difference according to the PNIFsD values of the smokers in different study periods (p=0.00001, p<0.05). In the evaluation of PNIFsD values, the median of the 6th-month values was significantly higher than the medians of baseline and the 3rd-month values (p=0.000001; p=0.000001, respectively, p<0.05). Additionally, the median of the 3rd-month values was significantly higher than the median of initial values (p=0.023, p<0.05) There was no significant difference in terms of PNIFwD evaluations (p=0.06, p>0.05) (Table 1, 2) (Figure 2).

Discussion

Smoking is involved in the pathophysiology of chronic diseases and malignancies of many organs, and systems, particularly the lung and the respiratory system (1-5). Because of these chronic diseases, smoking shortens the average life expectancy by 10 years (20). Many strategies are being implemented at the national and international levels against smoking, which is the most common cause of preventable deaths. Depending on these strategies, the number of people who quit smoking increases day by day. In parallel, many studies have been conducted examining the effects of smoking cessation (8,15,21). In this study, we investigated the effect of smoking cessation on nasal MCC and nasal respiratory functions. In this study, mucociliary clearance time values at the end of the smoking cessation period (3rd month) and 3 months after smoking cessation (6th month) were significantly lower than the initial values (p<0.05). Besides, the 3rd-month MCT values were significantly lower than the baseline values (p=0.0003). Additionally, 3rd-month and 6th-month PNIFsD values were significantly higher than the initial values (p<0.05), and the 3rd-month PNIFsD values were significantly lower than the initial values (p=0.023).

Respiration, airway defense, and olfaction are the basic physiological functions of the nose (11). Optimal nasal respiration is essential for the other basic functions (11,12). Nasal obstruction, which is the most common rhinological problem, leads to decreased nasal airflow and insufficiency in nasal respiratory function (11,12). Nasal endoscopy, computed tomography scan, and anterior active rhinomanometry (AARM) are some methods used in the diagnosis of nasal obstruction and evaluation of nasal respiration function (22,23). AARM is the most commonly used and most accurate method to determine nasal airflow and resistance (18,24). Measurement of PNIF with a PNIFmeter is an objective and cheap method, that is faster and easier to apply compared to AARM, to examine nasal airflow and indirectly nasal resistance with a high correlation with rhinomanometry in normal and pathological noses (18,24). A high PNIF value is compatible with low nasal resistance (24).

Nasal MCC is one of the major nasal functions and is described as the removal of pathogenic particles from the inspired air by the mucosa and their transport from anterior to posterior by epithelial ciliary movements (12). It is the first and basic defense mechanism of the upper airways (12). MCC can be studied *in vivo* tests using different materials such as dyes, radiopaque materials, and saccharin (12,17). The saccharine test is an inexpensive, safe, and easily applicable test that measures MCT, which is an indicator of MCC (12,17,25). With these features, it is the most commonly used MCC evaluation method in clinical studies (12,17,25).

The normal MCC depends on the volume and composition of airway surface liquid (mucus and underlying periciliary layer), the healthy ciliary epithelium and structure, appropriate beating frequency, and airway surface liquid-cilia interaction (10-12,25). The disturbances in the MCC mechanism can lead to obstructions in the respiratory tract, airway infections, structural changes in the respiratory tract, and damage to the respiratory organs (8,12). The MCC can be affected by various factors such as environmental heat, drugs, toxins, pH, fasting, chronic diseases, and rhinological surgeries (8,11,12,25). One of these factors is active or passive smoking (8,26).

Various studies have reported the damage caused by smoking on different tissue and organ functions (8,26). One of these organs is the nose. There are various studies examining the effects of smoking on different nasal functions (6,8,15,26). However, the number of studies on the reversibility of the damage caused by smoking on nasal functions is limited (8,15). We planned this study to examine the effects of smoking cessation on nasal defense (MCC) and respiratory functions. In this study, the factors affect MCC negatively, such as being over 60 years old, and having a history of nasal pathology or surgery were accepted as

Table 1. Evaluation of peak nasal inspiratory flow and nasal mucociliary clearance time measurements

Parameter	Baseline	3rd month	6th month	p*
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
MCT	809.68±196.9 (840)	711.45±205.51 (727.5)	694.03±196.18 (720)	**0.00001***
PNIF without decongestant	119.06±19.21 (120)	120.02±20.17 (125)	125.5±19.8 (130)	**0.00001***
PNIF with decongestant	135.64±20.95 (140)	136.16±22.53 (140)	136.97±21.85 (140)	0.06

*Friedman test, p<0.05. PNIF: Peak nasal inspiratory flow, MCT: Mucociliary clearance time, SD: Standard deviation

Table 2. Pairwise comparison of data according to study periods

	Compared values	MCT[a]	PNIFsD[b]
p	Baseline-3rd month	0.0003*	0.023*
	3rd-6th month	0.000001*	0.000001*
	Baseline-6th month	0.000001*	0.000001*

[a]Nasal mucociliary clearance time, [b]Peak nasal inspiratory flow without decongestant, *Wilcoxon signed ranks test, p<0.05, MCT: Mucociliary clearance time, PNIFsD: Peak nasal inspiratory flow without decongestant

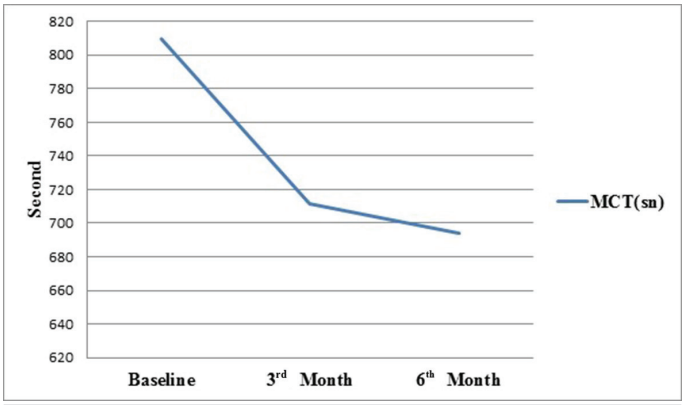

Figure 1. Nasal mucociliary clearance time values at baseline, 3rd month and 6th month
MCT: Mucociliary clearance time

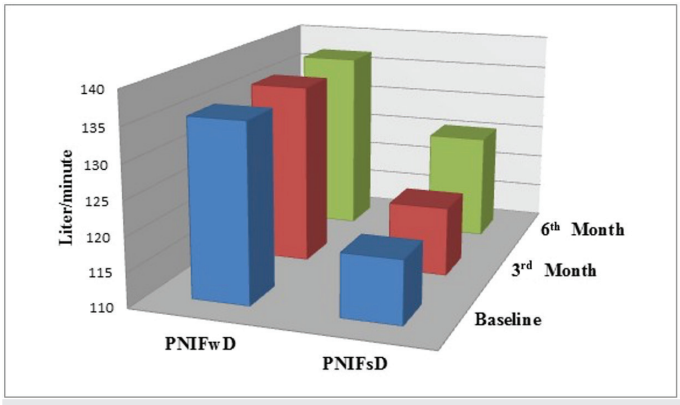

Figure 2. Peak nasal inspiratory flow with decongestant and peak nasal inspiratory flow without decongestant values at baseline, 3rd month, and 6th month
PNIFwD: Peak nasal inspiratory flow with decongestant, PNIFsD: Peak nasal inspiratory flow without decongestant

exclusion criteria from the study (25). All measurements were performed by the same person for standardization.

Various previous studies have examined the relationship between smoking and nasal MCC (7,8,10,26). Active or passive smoking increases inflammation and oxidative stress in the mucosa and induces changes in the epithelial structure and functions (8). Smoking causes produce high viscosity mucus by goblet cell hyperplasia and causes spread of an epithelium without ciliary functions in the airways by squamous metaplasia (8,27,28). Cigarette smoke impairs cilia structure and cilia regeneration (8,27,28). Additionally, cotinine, a metabolite of nicotine in cigarettes, decreases ciliary beat frequency (29). With all these effects, smoking, which impairs epithelial ciliary functions, leads to MCC deterioration and MCT elongation. This effect of smoking gradually returns with smoking cessation (8,28). Epithelial and ciliary regeneration increases after smoking cessation (8,28). Nasal MCC improve 1 month after smoking cessation, while nasal mucus properties return to normal 12 months after smoking cessation (8). In our study, the gradually decreasing MCT values in the first and 6th months in people who quit smoking support the existing information in the literature.

There are a limited number of studies in the English literature that objectively examine the effect of smoking on nasal respiratory functions (7-9,30). In these studies, increased nasal resistance and decreased airflow levels were found in smokers compared with normal individuals (7-9,30). The reasons for the deterioration in nasal respiratory function include increased nasal mucosal inflammation, increased mucosal edema, increased nasal mucosal congestion and decreased nasal decongestion capacity (7-9). In this study, we found a progressive increase in PNIFsD values after smoking cessation ($p<0.05$). Although the increase detected in the measurements made after the decongestion application (PNIFwD) was close to a significant level, it was not statistically significant ($p=0.06$). While there is a significant difference between PNIFsD values, the absence of a significant difference between PNIFwD values can be explained by the fact that smoking reduces the nasal decongestion capacity. Since the measurement value of the decongestion capacity is lost with the use of decongestion, the increase we obtained may not have been at a significant level.

Study Limitations

This study has several limitations. One of these limitations is the use of a PNIFmeter for nasal respiratory function. Although PNIFmeter show a high correlation with AARM in the evaluation of nasal respiratory function, they are not as valuable as AARM in clinical practice. Additionally, patient-related problems may occur during the PNIFmeter application. To limit the effect of this limitation on this study, we iterated the PNIFmeter tests 3 times and recorded the highest value we detected. Another limitation is the absence of a control group. In our opinion, there was no need for a control group because the effects of smoking on nasal functions are well known and our study aimed to investigate the effect of smoking cessation on nasal functions. Another parameter limiting the value of this study is that we did not determine the duration of smoking of the smokers included in this study. The pathologies related to smoking increase with the duration and amount of smoking (28).

Conclusion

In this study, we found that smoking cessation increases nasal MCC and airflow. This effect can be explained by the reversible nature of the changes caused by smoking in the nasal mucosa. More comprehensive studies, including biochemical, immunological, and histopathological examinations, are needed to reveal these effects more clearly.

Ethics Committee Approval: This prospective study was performed between April 2021 and January 2022 with the confirmation of İstanbul

University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (approval number: E-83045809-604.01.02.74209, date: 14.04.2021).

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Peer-review: Externally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - D.Ç.; Concept - D.Ç.; Design - D.Ç.; Data Collection or Processing - D.Ç.; Analysis or Interpretation - S.U.; Literature Search - S.U.; Writing - D.Ç., S.U.

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

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

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İstanbul Med J 2022; 23(3): 179-182
DOI: 10.4274/imj.galenos.2022.44788

Increased Serum Growth Differentiation Factor 15 Levels may be Associated with Diastolic Dysfunction in Acromegaly

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ABSTRACT

Introduction: Growth differentiation factor-15 (GDF-15) is a cytokine that is associated with various metabolic and cardiac changes. Acromegaly is a chronic multisystemic disease that causes cardiac dysfunction due to an increased levels of growth hormone. We evaluated the association of GDF-15 with diastolic dysfunction in acromegaly.

Methods: Fifty-four patients with acromegaly [active (n=24), inactive (n=30)] and 34 healthy controls were included in the study. The acromegaly group (AG) and control group (CG) were compared for their blood pressures, metabolic parameters, GDF-15 levels, and echocardiographic findings. The correlation analysis was performed between the GDF-15 and the parameters that may be associated with it in the AG.

Results: GDF-15 was significantly higher in AG than in the CG ($p<0.001$). GDF-15 was positively correlated with body mass index ($r=0.4$, $p=0.008$) and negatively correlated with fasting blood glucose ($r=-0.4$, $p=0.004$). GDF-15 was also positively correlated with diastolic blood pressure (DBP) ($r=0.4$, $p=0.002$). Among echocardiographic findings, end-diastolic volume (EDV), and stroke volume (SV) were negatively correlated with GDF-15 levels ($r=-0.4$, $p=0.003$, and $r=-0.4$, $p=0.03$, respectively).

Conclusion: GDF-15 was detected to be significantly increased in patients with acromegaly. This increment was associated with subtle changes in DBP, EDV, and SV. Therefore, GDF-15 may play a role in diastolic impairment at the cardiac involvement in acromegaly.

Keywords: Acromegaly, cardiac complications, diastolic dysfunction, growth differentiation factor 15

Introduction

Acromegaly is a multisystemic disease that also causes cardiac dysfunction (1). Both excessive insulin-like growth factor-1 (IGF-1) and growth hormone (GH) cause cardiac changes in acromegaly (2,3). Various underlying mechanisms involving changes in calcium influx, expression of muscle-specific genes, and the composition of myosin isoform are held responsible for the effects of IGF-1 and GH on the cardiovascular system (4-7). However, all the mechanisms have not yet been identified.

Growth differentiation factor-15 (GDF-15) is a novel cytokine that is a member of the transforming growth factor β superfamily released by activated macrophages (8,9). It is present in different tissues and cells, including the vessels and cardiomyocytes (10). Stress, ischemia, anoxia, and inflammatory cytokines are among the triggers of GDF-15 release (11,12). Increased GDF-15 is associated with metabolic and cardiac changes. GDF-15 predict increased cardiovascular risk and all-cause mortality (13,14). It also maintains vascular integrity and increases cardiomyocyte and endothelial cell viability (15). Whether GDF-15 takes a role in cardiac involvement in acromegaly is unknown. The We evaluated the association of GDF-15 with cardiac changes in acromegaly.

Methods

Fifty-four patients with acromegaly [active (n=24), inactive (n=30)] were involved in this prospective study. The healthy control group (CG) was composed of age, gender, and body mass index (BMI)-matched 34 subjects. Patients with acromegaly were monitored at our hospital endocrine clinic during this study. Patients with malignancy, rheumatologic disease, chronic kidney failure, and patients with older stents were excluded from the study based on these criteria. This study was approved by the Ethics Committee of Universtiy of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 2011-KAEK-50, date: 06.05.2022). A written informed consent form was obtained from all patients before the study.

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Cite this article as: Hacıoğlu Y, Pişkinpaşa ME, Kılıçkaya P, Niyazoğlu M, Hacıoğlu B, Hatipoğlu E. Increased Serum Growth Differentiation Factor 15 Levels may be Associated with Diastolic Dysfunction in Acromegaly. İstanbul Med J 2022; 23(3): 179-182.

Received: 26.04.2022
Accepted: 29.06.2022

The presence of typical clinical symptoms with failure to suppress the GH levels to less than 1 ng/mL according to the oral glucose tolerance test and elevated IGF-1 levels led to the diagnosis of acromegaly. Acromegaly remission criteria were defined as normal IGF-1 levels according to the age-adjusted range and GH levels less than 1 ng/mL. Active disease was defined as elevated IGF-1 levels according to the age-adjusted range.

Bilateral diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured three times with 5 min intervals in all patients.

The mean of each SBP and DBP was used for statistical analysis. Serum fasting blood glucose (FBG), hemoglobin A1C, insulin, cholesterol levels were measured in all patients. Homeostatic model assessment for insulin resistance has been used to determine insulin resistance (16).

The quantitative sandwich enzyme-linked immunosorbent assay method was used to measure the GDF-15 (Human GDF-15 ELISA Kit, USA).

For each attendant, transthoracic echocardiography at rest was performed with Philips EPIQ 7 diagnostic ultrasound system equipment and 2.5 MHz transducers. All measurements were taken at the same time of day and by the same competent cardiologist to the recommendations from the American Society of Echocardiography (17). Left ventricular internal end-diastolic diameter, left ventricular posterior wall thickness during diastole, left ventricular mass (LVM), left atrium diameter, interventricular septum thickness (IVST), end-diastolic volume (EDV), end-systolic volume (ESV), and left ventricular ejection fraction (EF) were measured. The Devereux and Reishek Formula was used to determine LVM. The ratio of the LVM to the body surface area was used to calculate the LVM index (18). Stroke volume (SV) was calculated using measurements of ventricular volumes from an echocardiogram, i.e., subtracting ESV from EDV.

Acromegalic group (AG) and healthy CG participants were compared concerning their laboratory, clinical and echocardiographic findings. Correlation analysis was performed between the GDF-15 and the parameters that may be associated with it in the AG.

Statistical Analysis

Statistical analysis was assessed using the SPSS 22.0 package program. The chi-square test was used to evaluate the categorical variables. Kolmogorov-Smirnov test was used to evaluate the normality of the distribution of the quantitative variables. Normally distributed data were evaluated using the student's test, and non-normally distributed data were evaluated using the Mann-Whitney U test. Pearson's correlation analysis was used to evaluate the associations between variables. Statistical significance was set with $p<0.05$.

Results

The mean age in the AG was 46.9±13.4 years, and it was 44.8±12.3 years in the CG (p=0.7). Gender was not different between the two groups (37 female/17 male in the AG and 22 female/12 male in the CG, p=0.7).

The mean time since the initial diagnosis in patients with acromegaly was 40.8±11.8 months. Thirty patients (57%) with acromegaly had controlled disease. The clinical findings of the patients with acromegaly are given in Table 1.

BMI in the AG and CG was 30.7±6 kg/m^2 and 29.3±5.3 kg/m^2, respectively (p=0.3). The mean SBP and DBP in the AG were 123.1±18 and 82.2 ±11.9 mmHg, and in the CG were 118.4± 14.8 and 78.4±10.5 mmHg (for SBP p=0.2, for DBP p=0.1).

GDF-15, IGF-1, and GH were significantly higher in AG than in the patients in the CG ($p<0.001$, $p<0.001$, and p=0.002, respectively). Laboratory findings in the AG and CG are shown in Table 2.

In patients with acromegaly, GDF-15 was positively correlated with BMI (r=0.4, p=0.008) and negatively associated with FBG levels (r=-0.4, p=0.004). GDF-15 was not correlated with the mean SBP (r=0.1, p=0.7), whereas it was positively correlated with DBP (r=0.4, p=0.002). GH, IGF-1 levels, cholesterol levels, and the passed time since the initial diagnosis of acromegaly was not correlated with GDF-15 levels.

LVM, IVST, EDV, and ESV was significantly higher in AG than in the patients in the CG (p=0.009, p=0.002, p=0.03, and p=0.005, respectively). Comparisons of the echocardiographic parameters between the two groups are given in Table 3. Among echocardiographic findings, EDV and SV were negatively associated with GDF-15 levels in AG (r=-0.4, p=0.003, and r=-0.4, p=0.03, respectively) (Figure 1). Additionally, EF was positively correlated with GDF-15 (r=0.3, p=0.01).

Table 1. Clinical findings of the patients with acromegaly

	Acromegaly (n=54)
Time elapsed since diagnosis (months)	40.8±11.8
Operation (n, %)	47 (87)
GKN/CKN (n, %)	11 (20)
Somatostatin analog (n, %)	30 (56)
Cabergoline treatment (n, %)	10 (19)
Controlled (n, %)	30 (57%)

GKN: Gamma knife, CKN: Cyber knife

Table 2. Laboratory findings of the patients with acromegaly and control groups

	Acromegaly (n=54)	Control group (n=34)	p-value
Fasting blood glucose (mg/dL)	108.9±34.1	104.2±32.1	0.5
HOMA-IR	3.8 ± 3.5	3.6 ± 2.7	0.8
Total cholesterol (mg/dL)	194.3±41.1	213.249.9	0.06
LDL cholesterol (mg/dL)	109 ±36	125.2±44	0.08
Triglyceride (mg/dL)	141.7±77.4	154.4±68.9	0.4
HDL cholesterol (mg/dL)	55.9±14.7	59.2±14.6	0.3
GDF-15 (pg/mL)	1179.7±344.6	510.6±319.8	**<0.001**[a]
GH[b] (ng/mL)	1.7 (0.7-3.2)	0.7 (0.1-1.8)	**0.002**[a]
IGF-1 (ng/mL)	287.5±169	135±46.3	**<0.001**[a]

HOMA-IR: Homeostasis model of assessment-insulin resistance, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, GDF-15: Growth differentiation factor, GH: Growth hormone, IGF-1: Insulin like growth factor-1, [a]Statistically significant p values, [b]Data were expressed as median and interquartile range

Table 3. Comparison of the echocardiographic parameters between the groups

	Acromegaly (n=54)	Control group (n=34)	p-value
EF (%)	59.8±3.3	63.5±3.9	**<0.001**[a]
LAd (mm)	35.9±4.5	34.4±5.6	0.2
LVID (mm)	4.7±0.4	4.5±0.8	0.2
LVPWd[b] (mm)	0.9 (0.9-1)	0.9 (0.9-1)	0.1
LVM (g)	170.8±42.1	145.9±40.4	**0.009**[a]
LVMI (g/m^2)	46.5±11.2	42.8±14.4	0.2
IVST (mm)	0.99±0.13	0.88±0.18	**0.002**[a]
EDV (mL)	17.9±4.6	15.1±2.5	**0.03**[a]
ESV[b] (mL)	4.8 (4.2-5.4)	4.3 (3.7-4.6)	**0.005**[a]
SV (mL)	12.1±7.6	10.9±2.4	0.6

EF: Ejection fraction, LAd: Left atrium diameter, LVID: Left ventricular internal end-diastolic diameter, LVPWd: Left ventricular posterior wall thickness during diastole, LVM: Left ventricular mass, LVMI: Left ventricular mass index, IVST: Interventricular septum thickness, EDV: End-diastolic volume, ESV: End-systolic volume, SV: Stroke volume, [a]Statistically significant p-values, bData were expressed as median and interquartile ranges

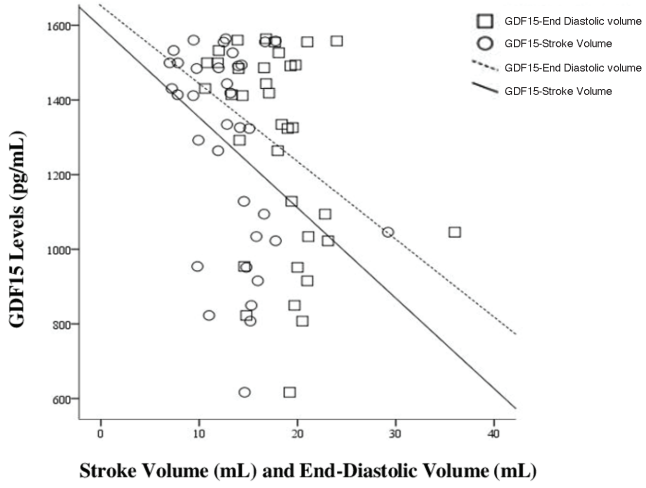

Figure 1. Correlation of GDF-15 with stroke volume and end-diastolic volume
GDF-15: Growth differentiation factor 15

Discussion

GDF-15 was found significantly higher in patients with acromegaly compared to healthy subjects in our study. Additionally, EF value was lower in patients with acromegaly, and EF value was positively associated with GDF-15 values. GDF-15 was also found to be negatively correlated with EDV and SV in acromegaly. Patients with acromegaly also had a higher LVM and a thicker interventricular septum.

GDF-15 is a novel anti-inflammatory cytokine released from activated macrophages (8,9). Although cytokines are mostly controlled via Nuclear Factor-κb transcription factors, GDF-15 is upregulated by p53, which is a tumor suppressor protein (12,19). Under physiological conditions, it is present at low levels, and it is increases because of an injury such as inflammation (20). Additionally, GDF-15 increases with cardiovascular events, including atherosclerosis, myocardial infarction, and heart failure (21-23). It is controversial whether GDF-15 is responsible for cardiac damage or whether it is secreted to protect against cardiac damage (11,21). GDF-15 is also depicted as a heart-derived hormone that blocks GH signaling (24). Changes in GDF-15 levels have not been previously investigated in acromegalic patients.

GDF-15 levels in patients with acromegaly were significantly higher than those in healthy subjects in this study. In a study that evaluated the between GDF-15 and obesity, GDF-15 was associated with BMI (25). This study determined that GDF-15 in obese individuals was an independent marker of impaired glucose control in obese individuals (25). Consistent with this study, we found that BMI was positively correlated with GDF-15 acromegaly. These results suggest that there is a link between GDF-15 and obesity. Interestingly, FBG was negatively associated with GDF-15 levels in our patients with acromegaly. These findings are in contrast to previous studies that showed glucose tolerance impairing effects of GDF-15 (25-27).

EF values were statistically lower in the acromegaly group. Additionally, EF was positively correlated with GDF-15 in acromegaly. However, lower EF values did not indicate systolic heart failure. As also previously known, EF may be normal due to remodeling and reduced ventricular cavity volume in hypertrophic cardiomyopathy and diastolic dysfunction (28,29). The chronic effect of an increased level of GH and IGF-I secretion in acromegaly causes biventricular concentric hypertrophy (3). Consistent with this, LVM and IVST was also significantly higher in the acromegaly group in our study. Also, patients with diastolic dysfunction are unable to increase SV by increasing their left ventricular EDV (30). Since GDF-15 was negatively related to EDV and SV, it may also have a role in diastolic impairment at the early stages of cardiac involvement in acromegaly. Moreover, GDF-15 was positively correlated with DBP. Therefore, GDF-15 may also have a role in hypertension observed in the patient with acromegaly.

It is well established that complications of acromegaly are linked to the increased levels of IGF-1 and GH levels (1-4). Neither GH nor IGF-1 levels were associated with GDF-15 levels in acromegaly. The time passed since the initial diagnosis of acromegaly was also uncorrelated with GDF-15. Therefore, the increment of GDF-15 in acromegaly may be an irreversible change, and it may be independent of the disease activity and IGF-1 levels.

Study Limitations

The main limitation of this study was that it is a single-center study with few cases, so further studies with a more significant number of patients are needed to confirm the associations between GDF-15 and cardiac changes in acromegaly.

Conclusion

GDF-15 was significantly increased in acromegaly. This increment was associated with subtle changes in cardiac functions, namely, diastolic dysfunction. An effective prediction model of GDF-15 needs to be explored in patients with acromegaly. Further prospective studies with a larger number of cases are needed to confirm our results.

Ethics Committee Approval: This study was approved by the Ethics Committee of Universtiy of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 2011-KAEK-50, date: 06.05.2022).

Informed Consent: A written informed consent form was obtained from all patients before the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.H., M.E.P., E.H.; Design: Y.H., M.E.P., B.H.; Data Collection or Processing: Y.H., P.K.; Analysis or Interpretation: B.H., E.H., M.N.; Writing: M.E.P., P.K., E.H.

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

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

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İstanbul Med J 2022; 23(3): 183-8
DOI: 10.4274/imj.galenos.2022.54077

Effects of Chitosan on Cisplatin-Induced Hepatorenal Toxicity in an Animal Model

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ABSTRACT

Introduction: We examined the effects of chitosan (CTS) for reducing cisplatin (CIS)-induced hepatorenal toxicity in a rat model.

Methods: A hepatorenal toxicity model was established by administering a single dose of 7 mg/kg CIS intraperitoneally (i.p) to Wistar albino rats. After seven days of once-daily normal saline or CTS (200 mg/kg; po) treatment, liver and kidney tissue samples were obtained from rats and Bcl-2/Bax ratio and caspase-3 expression levels were evaluated by Western blotting method. Histologically, structural damage was measured under a light microscope, and the degree of damage to organs was evaluated by a scoring system.

Results: CIS-induced hepatorenal damage was reversed with CTS treatment in the renal and hepatic tissues ($p<0.05$). Overall, CIS-induced increases in apoptotic parameters, Bcl-2/Bax ratio and caspase-3 expression, were reversed by CTS treatment ($p<0.05$).

Conclusion: It was determined that CTS, a biopolymer, may have a protective effect by preventing hepatorenal damage caused by CIS.

Keywords: Chitosan, cisplatin, apoptosis, hepatorenal toxicity

Introduction

Although cancer survival rates have increased from 50% to 70% recently, the need to produce new solutions to protect the quality of life of individuals both during and after chemotherapy continues (1,2). This situation led to the acceleration of studies aimed at mitigating the side effects of drugs that are in clinical use, apart from the studies in which new drugs and combination therapy of cisplatin (CIS) with other cancer drugs that can be used in clinical use have been developed (3). Today, CIS is widely used for treating many types of cancer cases. Although it varies depending on the dose and duration of the treatment, hepatorenal toxicity is one of the most common side effects seen in individuals with cancer during CIS-treatment (4). Hepatorenal injuries with a high risk of mortality may develop in approximately one-third of patients after CIS-treatment. The literature findings emphasize that CIS-treatment is also effective in oxidative stress-induced hepatotoxicity, as well as causing damage to liver and kidney tissue due to apoptosis (5).

Chitosan (CTS), a degradable and non-toxic biopolymer obtained by deacetylation of chitin, in which the basic monomer unit is glucosamine, has many applications in the pharmaceutical field due to its antibacterial, antimicrobial, hemostatic, antioxidant activity and antitumor properties (6-8). The main advantages of CTS are its non-toxicity and free radical scavenging properties have been reported in previous studies (9). CTS is widely used for various different medical applications such as wound healing materials and drug carriers (10). Because of these advantageous properties of CTS, we planned to investigate the protective effect of CTS on CIS-induced hepatorenal injury in a rat model and performed in vitro biochemical tests, including Bax/Bcl-2 ratio, when lower indicating resistance to apoptosis, and caspase (casp)-3 protein expression, when lower indicating resistance to apoptosis, in liver and kidney tissue as well as light microscopic assessments of hepatorenal histological damage indicating experimental tissue toxicity.

Methods

Drugs and Chemicals

CIS (50 mg/100 mL) (Koçak, İstanbul) was used for animal studies. All antibodies were purcashed from Santa Cruz Biotechnology (USA) and other compounds and chemicals were obtained from the Sigma-Aldrich (USA).

Animal Experiments

Experimental procedures were conducted in accordance with the international ethical guidelines for investigations in laboratory animals

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Cite this article as: Aykaç A, Şah H, Kükner A, Sayıner S, Şehirli AÖ. Effects of Chitosan on Cisplatin-Induced Hepatorenal Toxicity in an Animal Model. İstanbul Med J 2022; 23(3): 183-8.

Received: 28.01.2022
Accepted: 29.06.2022

and were approved by the Animal Research Ethics Committee of Near East University (approval number: 2019/101). The animals used in the study were obtained from the Near East University Animal Experiments Unit were fed with standard rat chow without any food and water intake restrictions. Wistar albino rats (male, 250-300 g and 8-10 weeks) were housed with three rats per cage under the conditions at 22±2 °C temperature, 50±10% humidity, and 12:12 h dark/light cycle.

Rats were divided into 4 study groups (n=8): normal saline (NS) group, received NS (0.9% NaCl) 0.01 mL/kg; CTS group, received CTS 200 mg/kg orally for 7 days; CIS group, received a single dose of CIS 7 mg/kg intraperitoneally on day 1; and CIS/CTS group, received a single dose of CIS 7 mg/kg intraperitoneally on day 1 and CTS 200 mg/kg orally for 7 days (11,12).

At the end of the experiment, all rats were sacrificed and dissected to obtain their liver and kidney tissue. Half of the liver and kidney tissue were transferred into 10% buffered formaldehyde for histopathological examinations, while the remaining tissues were stored at -80 °C for western blotting.

Histopathologic Assays

The fixation of the tissues was provided, tissue tracing procedures (Leica TP1020, Leica Biosystems Nussloch GmbH, Germany) were completed, and then were embedded in paraffin (Leica EG1150 H, Leica Biosystems Nussloch GmbH, Germany). Sections of 5 µm thickness were taken with a microtome (Leica RM2255, Leica Biosystems Nussloch GmbH, Germany) and hematoxylin and eosin staining was performed (Bancroft and Gamble 2008). Histomorphology examination of the sections was performed using a Leica DM500 light microscope combined with the Leica Microsystem Framework integrated digital imaging analysis system (Leica Application Suit ver. 3.0 series 38132019, Leica ICC50 HD, Leica Biosystems Nussloch GmbH, Germany).

Liver tissue was evaluated in terms of eosinophilic cytoplasm, necrosis, congestion and mononuclear cell infiltration criteria, and kidney tissue was evaluated in terms of tubular damage, glomerular damage and mononuclear cell infiltration criteria. For each tissue section, these criteria were evaluated in 10 different fields (13,14).

Immunoblotting Assays

Bax, Bcl-2, and casp-3 expression levels in the kidney and liver tissue were determined using western blot analyses. To determine the protein content of tissues, the Lowry method was used after the dissected tissues were centrifuged (15). Samples containing 100 µg protein were prepared and carried out in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins in the obtained gels were transferred to the membranes (Schleicher and Schuell, 0.45 µm, Germany) (16). Membranes were incubated with polyclonal primary antibodies for 12 h at +4 °C at a dilution of 1:200. Densitometric analysis of membranes was performed using Bio Rad Molecular Analyst software (www.totallab.com).

Statistical Analysis

Data are presented as mean with standard error of mean. At first, normality test in groups was performed using the Shapiro-Wilk test. Histopathological and molecular data were analysed using GraphPad Prism ver. 5.03 (GraphPad, San Diego, CA, USA) by Kruskal-Wallis ANOVA test followed by post-hoc Dunn's test. A p-value of 0.05 or less was required to confirm statistical significance.

Results

CIS-Induced Hepatorenal Damage was Reversed with CTS Treatment

The CIS treatment significantly increased the scores of tubular damages in kidney tissue compared with the NS and CTS treatments ($p<0.05$). The CTS treatment decreased tubular damage induced by the CIS treatment ($p<0.05$). The CIS treatment significantly increased both glomerular damage and mononuclear cell inflation in kidney tissue compared with the NS and CTS treatments ($p<0.05$). The CTS treatment significantly reduced both glomerular injury and mononuclear cell inflammation induced by the CIS treatment ($p<0.05$) (Table 1).

Microscopic examination revealed that the renal cortex and medullary structures were normal in the NS group (Figure 1a, b). In the CIS group, tubule structures were disrupted and Bowman's spaces in the glomeruli were very narrowed, as well as enlargements in the cortex and tubules (Figure 1c, d). In the CIS group, degeneration was also detected in the straight parts of the proximal and distal tubules in the outer medulla. The presence enlargement of the tubules was seen in the CIS/CTS group, which was less than the enlargement seen in the CIS group. In the CTS group, neither degeneration of the tubules in the the cortex and outer medulla nor narrowing of Bowman's spaces was detected (Figure 1e, f).

When the hepatocytes with eosinophilic cytoplasm and necrosis in the liver tissue were evaluated, most of the damage was observed in the CIS group. The number of hepatocytes with eosinophilic cytoplasm

Table 1. Histomorphological measurement scores of liver and kidney tissues

		NS (n=8)	CTS (n=8)	CIS (n=8)	CIS/CTS (n=8)
Liver	Eosinophilic cytoplasm	0.01±0.01	0.03±0.02^{+}	0.35±0.09*	0.1±0.05^{+}
	Necrose	0.02±0.01	0.02±0.02^{+}	0.42±0.06*	0.18±0.04$^{*,+}$
	Congestion	0.02±0.02	0.03±0.02^{+}	0.32±0.04*	0.22±0.07*
	Mononuclear cell infiltration	0.02±0.02	0.05±0.02^{+}	0.35±0.02*	0.22±0.04$^{*,+}$
Kidney	Tubular damage	0.05±0.02	0.03±0.02^{+}	0.82±0.1*	0.43±0.1^{+}
	Glomerular damage	0.01±0.01	0.05±0.02^{+}	0.62±0.1*	0.18±0.08^{+}
	Mononuclear cell infiltration	0.02±0.02	0.03±0.02^{+}	0.4±0.04*	0.2±0.09^{+}

NS: Physiological saline solution, CIS: Cisplatin, CTS: Chitosan, CIS/CTS: Cisplatin and chitosan.
Data were analysed using Kruskal-Wallis ANOVA test followed by post-hoc Dunn's test. $^{*}p<0.05$ vs. NS; $^{+}p<0.05$ vs CIS

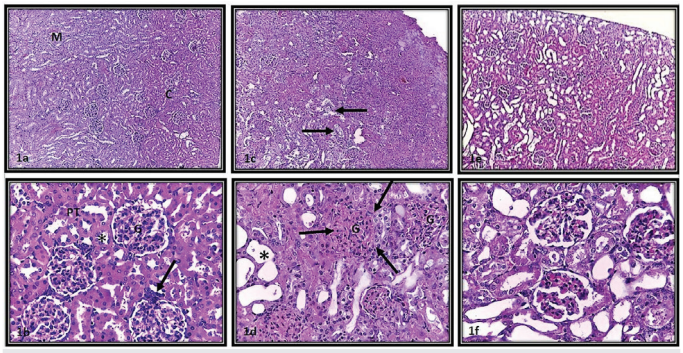


Figure 1. After normal saline (NS) exposure, a) the cortex (C) and medulla (M) (x10), b) glomeruli (G), proximal tubule (PT), distal tubule (*) and macula densa (→) structures of the kidney tissue were normal (x40). After cisplatin (CIS) exposure, c) glomerular structures could not be distinguished, and degeneration (→) was seen in tubules in the outer medulla (x10). d) Moreover, dilatation in tubular structures (*), closure of Bowman's space in glomerular structures, and tubule structures cannot be distinguished (x40). After CIS exposure followed by chitosan (CTS) treatment, e) tubular dilatation in the kidney tissue was reduced compared to the cisplatin exposure, and the degeneration in the tubules in the outer medulla region was very minimal (x10). f) The statuses of tubular degenerations were similar between the NS and CTS exposures (x40)

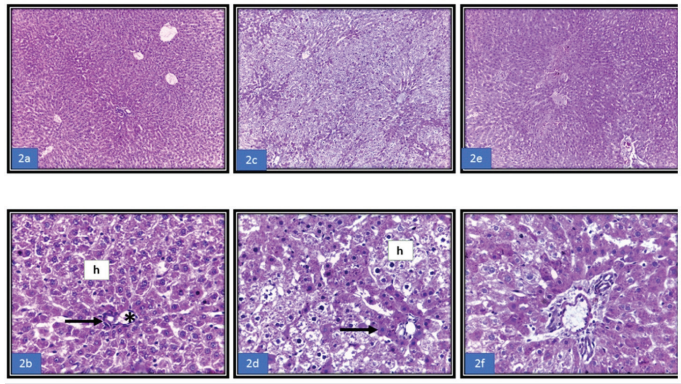


Figure 2. In the liver tissue of the normal saline group, a) hepatocyte cell cords and sinusoids (x10); b) bile duct, v. Porta branch (*) and hepatocytes (h) were seen to be in normal structure in the portal area (→) (x40). c) In the cisplatin (CIS)-exposed liver tissue, areas where hepatocytes dark and light stained cytoplasm were found (x10), d) hepatocytes with heterochromatic nuclei and light stained cytoplasm h), hepatocytes with normal structure (arrows) are seen around the portal area (x40). In the hepatic tissue that is chitosan-treated after CIS exposure, compared to the only CIS exposure, e) the light-stained areas were significantly reduced (x10), and f) hepatocytes with normal structures around the portal area were more common (x40)

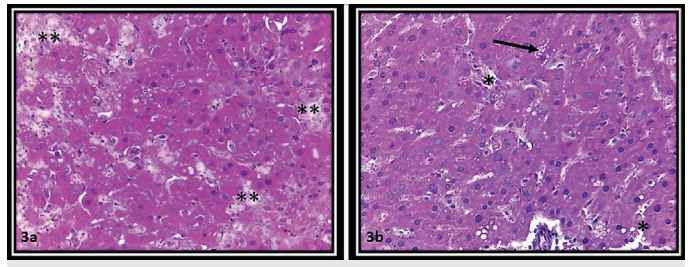


Figure 3. (a) Focal necrotic areas (**), (b) enlargement of sinusoids (*) and vacuolization of hepatocytes (→) (x40) were observed in the cisplatin-exposed liver tissue with H&E staining

and necrosis occurring in the CIS group was significantly higher than those of the NS and CTS groups ($p<0.05$). The damage to hepatocytes with eosinophilic cytoplasm in liver tissue due to CIS treatment was significantly reduced by CTS treatment ($p<0.05$). Moreover, the necrosis caused by CIS in the liver tissue decreased with the addition of CTS treatment ($p<0.05$). The congestion and mononuclear cell infiltration in the CIS group significantly increased compared to those of the NS and CTS groups ($p<0.05$). The CTS treatment applied to the CIS-induced hepatorenal toxicity group reduced mononuclear cell infiltration and congestion in the liver tissue induced by CIS ($p<0.05$) (Table 1).

By H&E staining of the cytoplasm of hepatocytes from the NS group, bile duct in the portal area, vessels and sinusoidal structures in the liver tissue was determined to be normal (Figure 2a, b). In the CIS group, the cytoplasm of hepatocytes around the v. centralis stained very lightly and their nuclei were heterochromatic and small, while the cytoplasm of hepatocytes around the portal area was stained darker (Figure 2c, d). The number of dark-stained hepatocytes around the v. centralis and portal area was increased in the CTS group compared in the CIS group (Figure 2e, f). While enlargement of the sinusoids, focal necrotic areas, and vacuolization in hepatocytes were determined in the CIS group (Figure 3a, b), necrotic areas and vacuolization were significantly reduced in the CIS/CTS group.

CIS-Induced Increase in Apoptotic Parameters Was Reversed by CTS-Treatment

The Bax/Bcl-2 ratios of liver and kidney tissue increased in the CIS group compared to the NS group ($p<0.05$) (Figure 4a, b). In the CIS/CTS group, Bax/Bcl-2 ratio significantly decreased in liver and kidney tissue compared to the CIS group ($p<0.05$). The casp-3 expression levels of liver and kidney tissue increased in the CIS group compared to the NS groups ($p<0.05$) (Figure 4c, d). The casp-3 expression level in the liver tissue increased in the CIS group compared to the CST group ($p<0.05$, Figure 4c). CTS administered after CIS treatment decreased the casp-3 expression level in the liver tissue compared to the CIS ($p<0.05$). However, CTS administered after CIS treatment did not cause any change in the casp-3 expression level in the kidney tissue. Representative images of the membranes obtained from western blotting are given in Figure 5.

Discussion

CIS is used to treat solid tumors; however, its clinical use is limited due to dose-related side effects such as nephrotoxicity, hepatotoxicity (17,18). Due to its many properties, such as anti-apoptotic and anti-inflammatory, CTS is widely used in different biomedical applications from tissue engineering to obesity treatment (19,20). Therefore, we evaluated the toxic effect of CIS on rats' liver and kidney tissue and the effectiveness of CTS treatment in alleviating CIS-induced toxicity.

The administration of CTS decreased the tubular damage induced by administrating CIS. The administration of CTS significantly reduced both glomerular injury and mononuclear cell inflammation induced by administrating CIS. On CIS treatment with microscopic examination, tubule structures were found to be disrupted and degeneration was detected in the straight parts of the proximal and distal tubules in the outer medulla. No degeneration of tubules in the cortex and outer medulla and narrowing of Bowman's spaces were detected with CTS treatment.

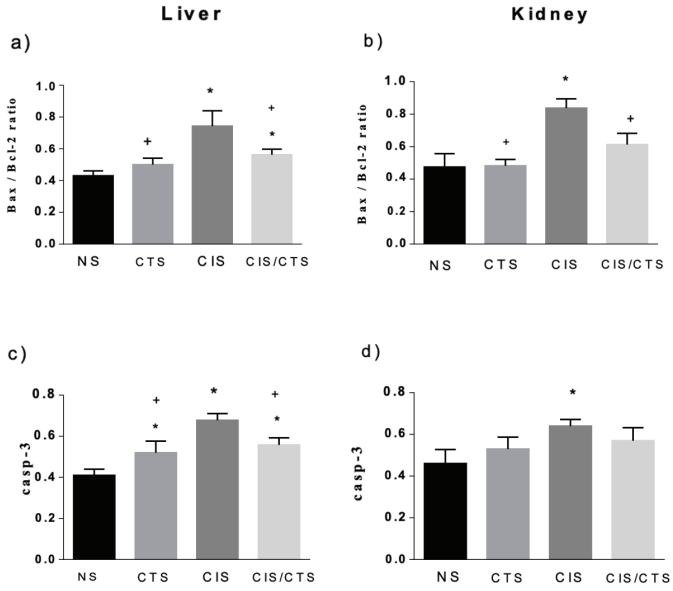


Figure 4. Bax/Bcl-2 ratio and casp-3 expression in CIS-induced hepatorenal toxicity
β-actin expression was used for the normalizations of all Bax, Bcl-2 and casp-3 expressions (n=4 for each group). *p<0.05 vs. NS; +p<0.05 vs. CIS. NS: normal saline; CIS: cisplatin, CTS: chitosan, CIS/CTS: cisplatin and chitosan, Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma, casp-3: caspase-3

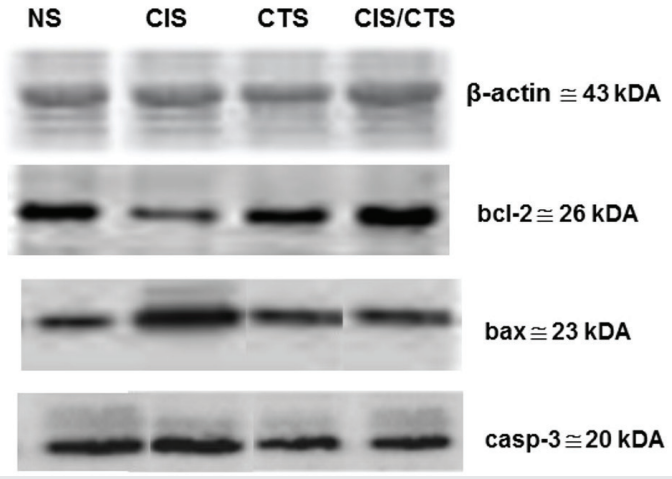
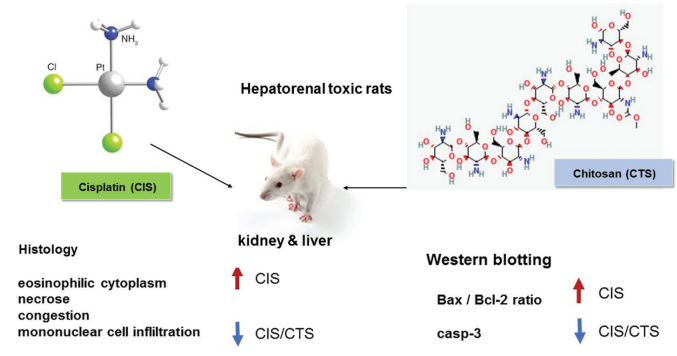


Figure 5. Representative images of expressions of studied proteins measured with western blot analysis
NS: Normal saline, CTS: Chitosan, CIS: Cisplatin and CIS/CTS: Cisplatin and chitosan

The damage to hepatocytes with eosinophilic cytoplasm in liver tissue due to CIS treatment was significantly reduced by CTS treatment. CIS-induced necrosis of liver tissue was reduced in the CIS/CTS group. The number of dark-stained hepatocytes around the v. centralis and portal area was increased in with CTS treatment compared to the CIS treatment. While enlargement of the sinusoids, focal necrotic areas, and vacuolization in hepatocytes were determined in the CIS treatment, necrotic areas and vacuolization were significantly reduced in the CIS/CTS group.

In the CIS/CTS group, Bax/Bcl-2 levels were significantly reduced in liver and kidney tissue compared with CIS treatment. It was determined that



Graph 1. Hepatorenal toxic rats
CIS: Cisplatin, CTS: Chitosan

the CIS/CTS group increased the casp-3 expression level in the liver tissue compared to with CIS treatment. However, CIS/CTS group did not cause any change in the casp-3 expression level in the kidney tissue. Apoptosis is a normal cell death process controlled by physiological stimuli. Anti-apoptotic Bcl-2 and pro-apoptotic Bax, members of the Bcl-2 protein family, control many important steps in the apoptosis process (21). Casp-3, one of the apoptosis markers, are responsible for the activation of the casp cascade. The effect of CIS treatment on apoptotic proteins was evaluated in this study. Consistent with the literature, in our study results, high Bax and casp-3 levels and low Bcl-2 levels were observed in the liver and kidney tissue of rats with CIS-induced hepatorenal toxicity. Faubel et al. (22) showed that CIS treatment to mice increased casp-3 activity in the kidney tissue, and Fatima et al. (23) showed that CIS increased casp-3 activation in the liver tissue. Similarly, other studies have shown that the Bax/Bcl-2 ratio increased in both liver and kidney tissue with CIS treatment. In our study, it was determined that Bax/Bcl-2 ratio decreased and casp-3 activation decreased in liver and kidney tissue with CTS treatment. It has been reported that CTS causes apoptosis in cancer cells (8,24,25), but increases the Bax/Bcl-2 ratio by increasing casp-3 activation in damaged inflamed cells. Therefore, it is suggested that CTS has a protective effect owing to its anti-apoptotic property. This suggests that CTS plays an active role in cancer treatment, especially due to its different effects on apoptosis in cancer and inflamed cells. In addition to highlighting once again the well-known profound effects of CIS on the regulation Bax and casp-3 levels and downregulating Bcl-2 expression, our study determined that CTS treatment modifies these adverse effects: Bax and casp-3 were down-regulated and Bcl-2 expression was up-regulated.

Molecular changes in liver and kidney tissue were accompanied by histological changes in CIS-treated rats. As reported in previous studies, portal inflammation, sinusoidal dilatation and granuloma formation in the liver; tubular atrophy, vacuolization and apoptosis were observed in the kidney (26-29). In the study by Işeri et al. (27) they determined severe degeneration in the proximal and distal tubules and glomeruli because of histological evaluation of the kidney of rats treated with CIS. Histological examinations of liver tissue in the same study revealed that CIS treatment caused acute activation of Kupffer cells, degenerated hepatocytes, and moderate enlargement of sinusoids (27). In their study, Palipoch and Punsawad emphasized that CIS administration is effective in pathologies such as the separation of tubular cells from the basement

membrane and tubular necrosis (30). CIS treatment both altered the overall tissue structure and significantly increased the histology score compared with normal rats in this study. Changes were reduced with CTS, as seen in other *in vivo* studies of histology score, CIS-induced liver and kidney injury. The anticancer effect of CIS is manifested by inducing apoptosis, which is programmed cell death. The cytotoxic effects of CIS have been demonstrated in many in vitro and fewer *in vivo* studies using different cell types. Both our histological and molecular findings emphasized the role of apoptotic mechanisms in hepatorenal damage caused by CIS, and it was determined that CTS therapy used against damage caused by CIS can be a protective agent, as reported in other studies.

Study Limitations

The most important limitation of this study was that the entire apoptotic pathway was not evaluated. In this context, the inability to evaluate the effective parameters in the apoptotic pathway by western blot or immunohistochemistry analyses is among the limitations of our study.

Conclusions

The preference of CTS usage for several purposes in experimental studies is related to its biodegradable polymer structure having no toxic effect. The findings of the current study support the that CTS has a potential for use to reduce the CIS-induced hepatorenal toxicity in a rat model. This hepatorenal protective ability of CTS may be linked with its anti-apoptotic and anti-inflammatory abilities.

Ethics Committee Approval: The Near East University Local Animal Experiments Ethics Committee granted approval for this study (approval number: 2019/101), date: 21.11.2019).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept - A.A., A.Ö.Ş.; Design - A.A., A.Ö.Ş.; Data Collection or Processing - A.A., H.Ş., A.K., S.S., A.Ö.Ş.; Analysis or Interpretation - A.A., H.Ş., A.K., S.S., A.Ö.Ş.; Literature Search - A.A., H.Ş., A.K., S.S., A.Ö.Ş.; Writing - A.A., H.Ş., A.K., S.S., A.Ö.Ş.

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

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

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İstanbul Med J 2022; 23(3): 189-93
DOI: 10.4274/imj.galenos.2022.85453

Risk Factors Associated with Femoral Neck Fracture Outcomes in Adults: Retrospective Clinical Study

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ABSTRACT

Introduction: The aim of this study was to evaluate the factors affecting the clinical and radiological results of the patients who underwent internal fixation due to femoral neck.

Methods: Sixty-nine patients are included in this study. Follow-up periods were determined as a minimum of six months. Fractures were classified with Garden and Pauwel's classifications. 12 hours was the cut-off limit between early and delayed fixation. The standard treatment was cannulated screw fixation. Trauma mechanism, reduction quality and patient dependent factors such as smoking and diabetes were evaluated. Non-union and avascular necrosis (AVN) was diagnosed with X-ray or computerized tomography if needed. Harris Hip score (HHS) was used as a functional scoring modality.

Results: Twenty-five (36.2%) patients were female and 44 (63.8%) were male. Twenty-six patients (37.7%) were under 40 years old and 43 (62.3%) were older. Forty-two (60.9%) of these fractures were classified as Garden 1-2 (non-displaced) and 27 (39.1%) were classified as Garden 3-4 (displaced). While 39 patients (56.5%) were operated on before the 12-hour cut-off point; 30 patients (43.5%) were operated on later. Fourteen patients (20.3%) were diagnosed with non-union and 12 (17.4%) with AVN. Mean HHS was 88.7±14.2 (47-100). Displaced group (Garden 3-4) showed a higher incidence of non-union (40.7% vs.7.1%) and AVN (25.9% vs. 11.9%) compared to the non-displaced group (Garden 1-2). The timing of surgery did not have a statistically significant impact on outcome in terms of non-union and AVN. The complication rates were higher in patients with poor reduction.

Conclusion: Our complication rates were similar to the literature. Age, gender, fracture side, smoking, and time to fixation were found as irrelevant with non-union and AVN rates. Non-union was significantly higher in the displaced group and no significant difference was found in AVN rates. The reduction quality was significantly associated with complications.

Keywords: Femoral neck fractures, internal fixation, time to surgery

Introduction

Femoral neck fractures are an important cause of mortality and morbidity with an increasing frequency. Although it was calculated as 1.3 million fractures per year in the 90s, it is expected to increase to 4.5 million in the 2050s as the average life expectancy increases (1). Femoral neck fractures constitute 3% of all hip fractures in the young population. Despite its low incidence, it maintains its importance due of high complication rates (2). The treatment of femoral neck fractures has always been controversial and no definitive algorithm has been formed. The main goal in young and active patients is to obtain a functional hip by preserving the femoral head. For this purpose, the primary choice has been internal fixation methods. In elderly patients with comorbidities, low expectation of union, and hip arthrosis, the first choice is usually arthroplasty.

According to meta-analysis studies, 11 to 19% osteonecrosis and 23 to 37% non-union are seen despite all these treatment protocols (3). Fracture type, time to surgery, reduction quality, stability, trauma mechanism, and comorbidities and their impact on outcomes are still debated. In this study, we evaluated the factors affecting the clinical and radiological results of the patients who underwent internal fixation due to the femoral neck.

Methods

Sixty-nine patients who underwent internal fixation for femoral neck fractures in our clinic between 2011 and 2018 were retrospectively evaluated. Verbal informed consent was obtained from all patients in the study. Pathological fractures, stress fractures, concomitant acetabular fractures, fracture-dislocations, and patients with a history of steroid use were not excluded. After a comprehensive trauma evaluation in

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Cite this article as: Acar B, Eren M, Demirci Z, Demirulus K, Öztürkmen Y. Risk Factors Associated with Femoral Neck Fracture Outcomes in Adults: Retrospective Clinic Study. İstanbul Med J 2022; 23(3): 189-93.

Received: 21.04.2022
Accepted: 07.07.2022

the emergency room, patients were prepared for surgery. Surgery was performed as soon as possible after anesthesia consultation. Relevant radiographs were taken and classified according to Garden and Pauwel's fracture classification. All patients were operated on in the supine position on the traction table. After closed reduction, all patients were fixed in the triangle configuration using three 6.5 mm cannulated screws. On the first postoperative day, patients were mobilized in a non-weight bearing regime. Postoperative X-rays were evaluated according to the Garden alignment index (4). Follow-up time was a minimum of six months with decreasing frequency. Patients' age, gender, past medical history, smoking, drug history, and complaints were recorded. The time to surgery cut-off was determined as 12 h. Those who were operated on before 12 h were determined as early and those who were operated on after 12 h were determined as delayed. Postoperative examinations included gait, range of motion, and limb length discrepancy evaluations. Hip anteroposterior and lateral radiographs and computerized tomography scans were performed when a non-union was suspected. Complications such as shortness, deformity, infection, non-union, and avascular necrosis (AVN) were recorded (Figure 1). AVN was classified according to the Ficat-Arlet classification using radiography. Harris Hip score (HHS) and visual analog scale (VAS) were used to determine the functional outcomes. This research was approved by the Institutional Review Boards of the authors' affiliated institutions.The study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 1070, date: 18.08.2017).

Statistical Analysis

SPSS 22.00 was used for data analysis. In the descriptive statistics of the data, mean, standard deviation, median lowest and highest, frequency and ratio were used. Kolmogorov-Simirnov test was performed for distributing variables. The chi-square test or Fisher test was used for the analysis of qualitative independent data. The statistical significance level was determined as $p<0.05$.

Results

Study Characteristics

Twenty-five of 69 patients (26.3%) were female and 44 patients (73.7%) were male. There were 26 patients (37.6%) under the age of 40 and 43 (62.4%) between the ages of 40 and 60. Our average follow-up period was 16.4±10.1 (6-84 months). Twenty-one patients (30.4%) presented with a simple fall, 30 (43.4%) patients with a fall from a height, and 18 (26.2%) patients with a traffic accident. Forty-two patients (60.9%) were evaluated as Garden type 1-2, 27 (39.1%) patients as Garden type 3-4 fractures on initial X-rays. According to Pauwel's classification, 26 patients (37.6%) were type 1, 36 patients (52.1%) were type 2, and 7 patients (10.3%) were type 3. While 39 patients (56.5%) were operated on before the 12-hour cut-off point; 30 patients (43.5%) were operated on later. Fixation was achieved with cannulated screws after closed reduction in all patients. Twenty-nine patients had a history of smoking, and 11 patients had a history of diabetes mellitus (DM). Sixteen patients underwent a second surgery due to complications. Eight of these were converted to total hip arthroplasty, 3 patients' implants were removed due to local irritation, and 5 patients needed re-osteosynthesis.

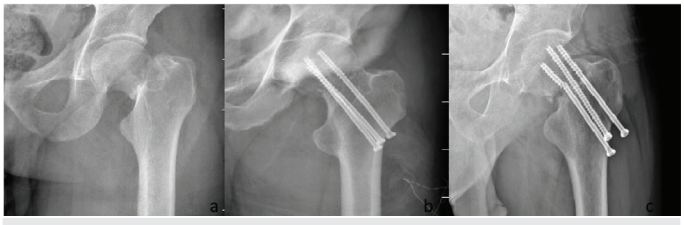

Figure 1. a) Displaced femoral neck fracture, b) Femoral neck fracture fixed with cannulated screw, c) Non-union and implant failure was seen at 3rd month control X-ray

Functional Outcomes

Non-union was detected in 20.2% (14/69) patients and AVN in 17.3% (12/69). According to the Ficat-Arlet classification, two patients were stage 2A, five patients were stage 2B, three patients were stage 3, and two patients were stage 4. Mean HHS was 88.7±14.2. HHS was excellent in 46 patients, good in 6 patients, fair in 8 patients, and poor in 9 patients. VAS score was 1.7±2.3 (Table 1).

Complications

Non-union and osteonecrosis rates were similar among patients who were younger and older than 40 years. In the younger group (n=40), 19.2% (n=5) non-union and 26.9% (n=7) osteonecrosis were observed. In the older group (n=43), non-union and osteonecrosis rates were 20.9% (n=9) and 11.6% (n=5) respectively, and showed no significant difference with respect to AVN and non-union ($p>0.05$) (Table 2).

Complication rates were significantly higher in patients with high-energy trauma. In the patients with low energy trauma (n=21), only 1 patient was diagnosed with non-union and 3 patients had radiographic signs of AVN, while in 48 patients who presented with high-energy trauma 13 (27.1%) were diagnosed with non-union and 9 (18.7%) with AVN ($p<0.05$) (Table 2).

In the non-displaced group (n=42), 3 patients (7.1%) were diagnosed with non-union and 5 patients (11.9%) showed radiographic evidence of AVN. The displaced group showed a higher incidence of both nonunion [40.7% (11/27)] and AVN [25.9% (7/27)]. Non-union was significantly higher in the displaced group ($p<0.05$). Fracture displacement didn't have a significant impact on osteonecrosis. ($p>0.05$) Non-union rate was 7.6% (2/26) with Pauwels' type 1, 22.2% (8/36) with type 2, and 57.1% (4/7) with type 3. The AVN rate was 7.6% (2/26), 22.2% (8/36) and 28.5% (2/7) respectively. Pauwels' classification showed no significant impact on the outcome in terms of non-union and osteonecrosis ($p>0.05$) (Table 2).

A good reduction was achieved in 81.1% (56/69) patients, a moderate reduction in 11.5% (8/69), and a poor reduction in 7,4% (5/69). Complications developed in 62.5% (5/8) of patients with moderate reduction and 80% (4/5) of patients with poor reduction. There was a significant relationship between reduction quality and complications ($p<0.05$) (Table 3).

The non-union rate was 30% (9/30) and the AVN rate was 16.6% (5/30) among smokers. The non-union and AVN rates were 12.8%(5/39) and 17.9% (7/39) respectively, with non-smokers. There was no significant difference between the smoking and non-smoking groups in terms of complications ($p>0.05$) (Table 2).

Discussion

Femoral neck fractures make up 3% of all hip fractures in the young adult population. Although the rate seems to be low, femoral neck fractures remain important due to the high complication rate (5,6). In our study, similar to the literature, we found 20.2% non-union and 17.3% AVN.

Despite all treatment protocols, the high complication and revision rates despite proper treatment require evaluation of each factor that could impact the results.

Table 1. Statistical comparison of demographic characteristics

		Min.-max.	Median	Mean ± SD/n-%
Age		19-60	48.0	46.2±11.8
Age	<40	-	-	26 (37.7%)
	≥40	-	-	43 (62.3%)
Gender	Female	-	-	25 (36.2%)
	Male	-	-	44 (63.8%)
Smoking	(-)	-	-	39 (56.5%)
	(+)	-	-	30 (43.5%)
DM	(-)	-	-	58 (84.1%)
	(+)	-	-	11 (15.9%)
Side	Right	-	-	28 (40.6%)
	Left	-	-	41 (59.4%)
Following time (month)		6-96	36.0	41.2±27.8
Trauma mechanism				
Simple		-	-	21 (30.4%)
Falling from high		-	-	30 (43.5%)
Traffic accident		-	-	18 (26.1%)
Garden	I-II	-	-	42 (60.9%)
	III-IV	-	-	27 (39.1%)
Pauwel	I	-	-	26 (37.7%)
	II	-	-	36 (52.2%)
	III	-	-	7 (10.1%)
Time to surgery	<12 H	-	-	39 (56.5%)
	≥12 H	-	-	30 (43.5%)
VAS		0-8	1.0	1.7±2.3
HHS		47.0-100.0	95.8	88.7±14.2
HHS	Poor	-	-	9 (13.0%)
	Fair	-	-	8 (11.6%)
	Good	-	-	6 (8.7%)
	Excellent	-	-	46 (66.7%)
AVN	(-)	-	-	57 (82.6%)
	(+)	-	-	12 (17.4%)
Union	(+)	-	-	55 (79.7%)
	(-)	-	-	14 (20.3%)
Revision	(-)	-	-	53 (76.8%)
	(+)	-	-	16 (23.2%)

DM: Diabetes mellitus, VAS: Visual analog scale, HHS: Harris Hip score, AVN: Avascular necrosis, min.: Minimum, max.: Maximum, SD: Standard deviation

The role of the patient's age in the results is one of the most discussed factors. Loizou and Parker (6) with 1,023 patients, a 20.6% complication rate was found for patients under 60 years of age, and 12.5% for patients over 60 years of age, and reported that complication rates at younger ages were higher. Schweitzer et al. (7) in a study they conducted with 29 patients, reported that the AVN rate increased with age but non-union did not change. In our study, we did not find a significant difference between groups over and under 40 years of age in terms of complications. We believe that more comprehensive prospective studies should be conducted in terms of the effect of the age on the results.

We found a significant difference in the impact on the outcome between high-energy trauma such as falls from height and traffic accidents compared to low-energy trauma such as a simple fall. While femoral neck fractures are usually caused by low-energy trauma in the elderly population, they usually occur after high-energy trauma in young adults. Schweitzer et al. (7) reported that low or high-energy trauma did not impact the rate of non-union and AVN. Zhou et al. (8) investigated 42 patients with different trauma mechanisms, such as a fall from height, traffic accident, or beating; similarly found no difference between groups. Slobogean et al. (5) reported that the displacement and complication rates increased because of high-energy trauma. We think that high-energy trauma affects the results because it causes displaced fractures.

Table 2. Statistical comparison of risk factors associated with AVN and non-union

		Non-union (n)	p	AVN (n)	p
Age	<40 years	19.2	0.865	26.9	0.104
	>40 years	20.9		11.6	
Sex	Female	8.0	0.056	20.0	0.667
	Male	20.2		15.9	
Smoking	(-)	12.8	0.079	17.9	0.889
	(+)	30.0		16.6	
Diabetes mellitus	(-)	18.9	0.530	15.5	0.390
	(+)	27.2		27.2	
Garden	1-2 (non-displaced)	7.1	0.001	11.9	0.134
	3-4 (displaced)	40.7		25.9	
Pauwel	I	7.6	0.063	7.6	0.116
	II	22.2		22.2	
	III	28.4		28.5	
Time to surgery	<12 H	25.6	0.208	20.5	0.435
	>12 H	13.3		13.3	

AVN: Avascular necrosis

Table 3. Complication rates associated with reduction quality

	Complication	(+)	(-)	p
Reduction quality (Garden index)	Poor	80.0%	20.0%	-
	Fair	62.5%	37.5%	0.001^{x2}
	Good	21.4%	78.6%	-

The time to surgery is the most discussed topic regarding the results of femoral neck fractures. Loizou and Parker (6) also reported no difference between the groups with a preoperative time of fewer than 12 hours and more than 48 hours in their study with 1,023 patients. Wang et al. (9) reported 14.4% AVN in 146 patients with femoral neck fractures who underwent internal fixation and found it unrelated to the time to surgery. Kang et al. (10) reported 10.7% AVN and 7.1% non-union in their study with 84 patients and found these complications unrelated to the preoperative delay. Despite many studies arguing that the time to surgery does not affect the rate of AVN, there is still controversy in the literature (11). Yeranosian et al. (12) in a systematic study examined 935 patients and reported that the risk of AVN increased in the group with time to surgery of more than 24 hours. Papakostidis et al. (13) in their meta-analysis of 7 studies, reported that the rate of non-union increased after 12 hours, but AVN incidence was not impacted. When all these studies are evaluated, it is seen that there is no consensus on the impact of the preoperative delay on the results. In our study, we did not find a significant relationship between preoperative time and complication rates.

One of the important factors affecting the outcome is the initial displacement of the fracture. The initial displacement can also be an indicator of the energy level of trauma. There is a consensus that the complication rates are high in patients with a high degree of displacement (14-17). In displaced fractures (Garden 3-4), 40.7% (11/27) non-union and 25.9% (7/27) AVN were observed. While the fracture displacement was associated with non-union ($p<0.05$), the difference in AVN rates was not statistically different ($p>0.05$). Although fracture displacement didn't seem to affect AVN rates, we believe that it should be re-evaluated with long-term follows up since AVN rates may increase in the future.

Another factor that may affect the results of femoral neck fractures in the method of fixation. Samsami et al. (17) found that a dynamic hip screw is superior to an anatomic plate or a cannulated screw construction in a vertical fracture in their study on cadavers. Kostic et al. (18) compared between 2 and 3 screw constructions; found similarly good results and argued 2 parallel screw construction is simpler and not inferior to 3 screw construction. Bhandari et al. (19) researched the optimal treatment method in their meta-analysis and found no significant difference between DHS and cannulated screws in all fracture types. In our study, we used 3 screw construction as the preferred method and we have found a similar rate of the union to the literature.

Femoral neck fracture fixation is generally a fairly simple procedure with close reduction and percutaneous fixation. Although the most important and challenging step of surgery is generally achieving a good reduction. The reduction quality is generally evaluated with the Garden alignment index in the literature (20,21). Wang et al. (9) found a correlation between reduction quality and osteonecrosis in their study. Chang et al. (22) detected complications in 21 of 28 patients with poor reduction quality and found the quality of reduction significantly associated with the results. Kang et al. (10) also argued poor reduction leads to early non-union and progressive loss of reduction. Garden et al. (23) in their original paper have found no cases of AVN in 57 patients with good reduction but 65% (53/81) osteonecrosis in patients with poor reduction. In our study, we have found lower complication rates in patients with good reduction (Garden alignment index) when compared to moderate and poor reduction.

The high risk of AVN in the femoral head is due to its limited vascularization. Factors such as smoking and DM can risk this fragile balance for the side of osteonecrosis (25-27). In our study, we found no statistically significant difference between the smoking and non-smoking groups and we couldn't evaluate the impact of diabetes on the outcome due to the limited number of patients.

Study Limitations

Our study has several limitations. This was a retrospective study with a limited number of patients. Due to limited follow-up time, some cases of AVN may not be detectable on an X-ray. The type of instrument and reduction was constant between groups and we couldn't compare different modes of fixation and reduction.

Conclusion

Femoral neck fractures in young adults maintain their importance because of high complication rates. Fixation with cannulated screws after closed reduction is a reliable treatment method. Although we found no difference in impact between demographic characteristics such as age, gender, and comorbidity, further studies with a greater number of patients can help identify patient-dependent risk factors. High-energy trauma results in a higher amount of displacement and poor reduction results in worse outcomes and a higher incidence of complications. Although we have found no relationship between time and surgery and complication rate; the timing of surgery is still debatable.

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 1070, date: 18.08.2017).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

Conflict of Interest: There is no conflict of interest in this study.

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

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İstanbul Med J 2022; 23(3): 194–8
DOI: 10.4274/imj.galenos.2022.92845

Evaluation of Hemoglobin and Hematocrit Values by the CO-Oximetry and Cyanide-Free Sodium Lauryl Sulphate Methods: A Retrospective Study

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ABSTRACT

Introduction: Blood gas analyzers (BGA) have recently been widely used as a rapid testing devices for the determination of hemoglobin (Hb) in the intensive care units and emergency services of hospitals. We compared the Hb and calculated-hematocrit (Hct) values by the CO-oximetry and cyanide-free sodium lauryl sulphate (SLS) methods.

Methods: Between January and June 2019, 12,049 patients who applied to the emergency department of İstanbul Training and research Hospital, for whole blood count and venous blood gas analysis were included. Samples were analyzed using SLS- Hb and CO-oximetry methods. Bland-Altman plot and Passing-Bablok regression analysis were performed to evaluate the accordance of the methods.

Results: The correlation coefficients of the methods for Hb and Hct were 0.89 and 0.87, respectively ($p<0.0001$). Passing-Bablok regression analysis showed a significant deviation from linearity ($p<0.01$). Bland-Altman plot showed insufficient agreement between of the two methods for each variable. Bias % calculated as 2.5% for Hb, and 1.1% for Hct. Total error calculated as 4.08% for Hb. Total error of CO-oximetric Hb value was within the limits of allowable total error.

Conclusion: Although each test shows a significant deviation from linearity, BGA's could be used for Hb measurements since the bias and total error were still acceptable.

Keywords: Blood gas analyzers, CO-oxymetry, hemoglobin, sodium lauryl sulphate, method comparison

Introduction

Hemoglobin (Hb) is a complex protein responsible for the transport of oxygen from the lungs through the arteries to the tissues. A lower level of Hb in the erythrocytes red blood cells (RBC) is one of the important factors that shows reduced tissue oxygenation (1). Hematocrit (Hct) is the ratio of the volume of RBC to the whole blood volume. Simultaneous measurements of Hb and Hct concentrations are used to evaluate conditions such as anemia, bleeding or hemorrhage (2).

Hb and Hct can be measured by different methodologies such as hematology analyzers, Hct centrifuge, cyanmethemoglobin method, gravimetric copper sulfate method and color code Hb estimation (3). The cyanmethemoglobin method is accepted as the gold standard for measuring Hb concentration by the International Council for Standardization in Hematology (ICSH). The principle of this method is to measure the absorbance of the final product at 540 nm wavelength by converting Hb to cyanmethemoglobin by adding potassium cyanide and ferricyanide. However, this method is not suitable for automated auto analyzers due to a low Hb conversion rate, and multiple sample processing is required. Furthermore, since cyanide, which has toxic effects on the environment and human health, is wasted in large volumes, laboratories have turned to alternative methods. So non-cyanide measurement methods have become the clinical standard (4,5).

In 1981, Oshiro et al. (6) developed a cyanide-free Hb assay method based on the Lambert-Beer Spectrophotometry principle using a non-toxic compound, sodium lauryl sulfate (SLS). CO-oximeter is another Hb based spectrophotometric method depending on Lambert-Beer laws. CO-oximeters are multi-wavelength photometers that determine the total amount of Hb and Hb derivatives such as oxyhemoglobin, reduced Hb, carboxyhemoglobin, and methemoglobin (7).

Blood gas analyzers (BGA) are used in intensive care units and emergency departments for assessing and monitoring oxygenation status, ventilation, and acid-base status of critically ill patients. Modern BGA have integrated CO-oximeter modules to estimate total Hb and Hb derivatives (8). The use of BGA has increased significantly over the years because of their ease of use and accessibility. The reliability of the results obtained from these devices should be tested using appropriate statistical tools.

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Cite this article as: Oğuz O, Serin H, Hocaoğlu FS. Evaluation of Hemoglobin and Hematocrit Values by the CO-Oximetry and Cyanide-Free Sodium Lauryl Sulphate Methods: A Retrospective Study. İstanbul Med J 2022; 23(3): 194-8.

Received: 21.03.2022
Accepted: 14.07.2022

In this study, we assessed the concordance of CO-oximetry and SLS- Hb methods in the terms of measuring Hb and calculating Hct values.

Methods

Samples and Data Collection

This is a retrospective study, conducted in the emergency laboratory of İstanbul Training and Research Hospital. We scanned the records of patients from the hospital information system between January and June 2019. Blood samples were collected in tubes containing lithium heparin (Ayset 2 mL sterile single-use syringe) for blood gas analysis and tubes with K2EDTA (BD Vacutainer™ Plastic Blood Collection Tubes with K2 EDTA) for complete blood count. The study was approved by the institutional ethical committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 1929, date: 09.08.2019).

Exclusion criteria were applied to the patients who underwent an emergency procedure, postoperative cases, and patients with hemoglobinopathy, and severe infection or sepsis. Inclusion criteria were determined as the simultaneous testing for both venous blood gas analysis and complete blood count at the same period. Following the establishment of the exclusion and inclusion criteria, the analytical results of 12,049 subjects were evaluated.

Analytical Measurements

The calibration and control of blood gas and complete blood count analyzers were performed according to manufacturers' instructions. The emergency laboratory participates in the RIQAS Blood Gas and the RIQAS Haematology External Quality Assessment programs, monthly.

Blood gas analyses were performed on RAPIDlab 1,265 BGA (Siemens Healthcare Diagnostics, Eschborn, Germany). Hb values were obtained from the CO-oximetry module using multiple-wavelength in the range of 500-680 nm. Hct values were calculated from the obtained Hb values using the formula.

Complete blood count analyses were performed on Sysmex XN1000™ (Sysmex, Norderstedt, Germany). The SLS Hb method was used for measuring Hb. The principle of this method is the binding of SLS to the heme group after the conversion of Hb to methemoglobin by oxidation of heme groups. The SLS-Heme complex is analyzed using a photometer.

Statistical Analysis

We used Excel 2013 (Microsoft, WA, USA), Excel XLSTAT 2019 (Addinsoft, New York, USA), SPSS 18 (IBM, New York, USA) for the statistical analyses. The normality of the distribution of the variables was tested using the Kolmogorov-Smirnov test. Since the data did not pass the normality test, a non-parametric Wilcoxon matched-pairs signed-rank test was performed to evaluate the significance of the difference between each set of matched pairs, and a comparison of each sample against the median values was performed. Passing-Bablok and Bland-Altman analyses were performed to evaluate the concordance of the analytical methods. A p-value less than 0.05 was set as the level of significance.

We calculated Bias % and total error for both tests by using the following formulas, respectively;

$$BIAS = ((C_1 - C_2) / C_2)) \times 100$$

$$TE = BIAS + 2\%CV$$

C_1 represents the mean concentrations obtained from Siemens Rapidlab-1,265, whereas C_2 stands for the mean concentration of the Sysmex XN1000 results. TE represents the total error and CV represents the coefficient of variation (9).

Results

The study included 12,049 patients (7,129 F, 4,920 M) with a mean age of 42.56±24.67 years (Table 1).

The mean and median values and interquartile range for each test using the two different methods are shown in Table 2. The difference between the two methods for each variable was statistically significant ($p<0.0001$). Correlation coefficients between both methods were found to be as $r=0.89$ and $r=0.87$ for Hb and Hct, respectively.

$y=-0.03412+0.9647x$ (intersection confidence interval: -0.08438-0.01569, slope confidence interval: 0.9608-0.9688) equation was obtained from Passing-Bablok regression analysis for Hb. For Hct, $y=3.3000+0.8850x$ (intersection confidence interval: 3.0818-3.5167, slope confidence interval: 0.8792-0.8909) equation was obtained. A deviation from linearity was observed for both variables between the methods ($p<0.01$) (Figure 1).

The Bland-Altman method plot created for the determination of agreeability between the methods, depending on the bias and mean values is shown in Figure 2.

Table 1. The characteristics of the patients for the comparison studies (n=12,049).

Variables	Mean ± SD	Min.-max.
Age (years)	42.56± 24.67	18-65
Gender		
Female	7,129 (59.2%)	
Male	4,920 (40.8%)	
Patient characteristics		
Outpatient clinics	6,921 (57.4%)	
Internalized patients	5,128 (42.6%)	

SD: Standard deviation, min.: Minimum, max.: Maximum

Table 2. Median, p and r-values and interquartile ranges for hemoglobin and hematocrit for two instrument analyses (n=12,049).

	Hemoglobin (g/dL)		Hematocrit (%)	
Instruments	Sysmex XN1000	RAPIDlab 1,265	Sysmex XN1000	RAPIDlab 1,265
Median value	13.1	13.45	40.2	40.9
Interquartile range	31.00-202.00		10.50-60.60	
p-value	<0.0001		<0.0001	
Correlation coefficient (r)	0.89		0.87	

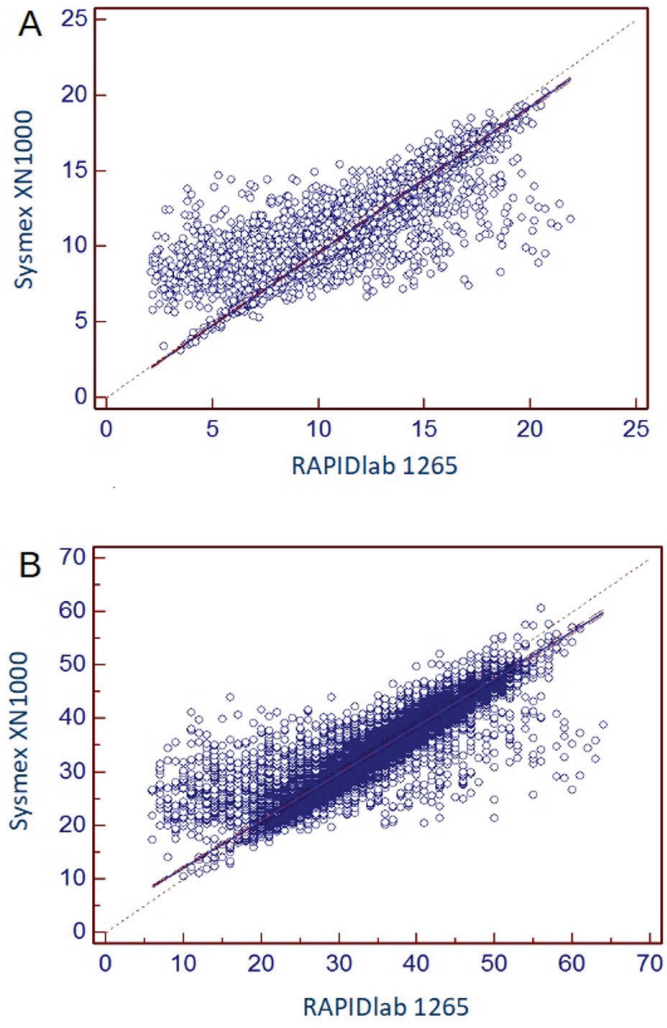

Figure 1. Passing Bablok regression analysis between the two measurement methods for hemoglobin A) and hematocrit B) (R^2=0.79 for hemoglobin and R^2=0.76 for hematocrit)

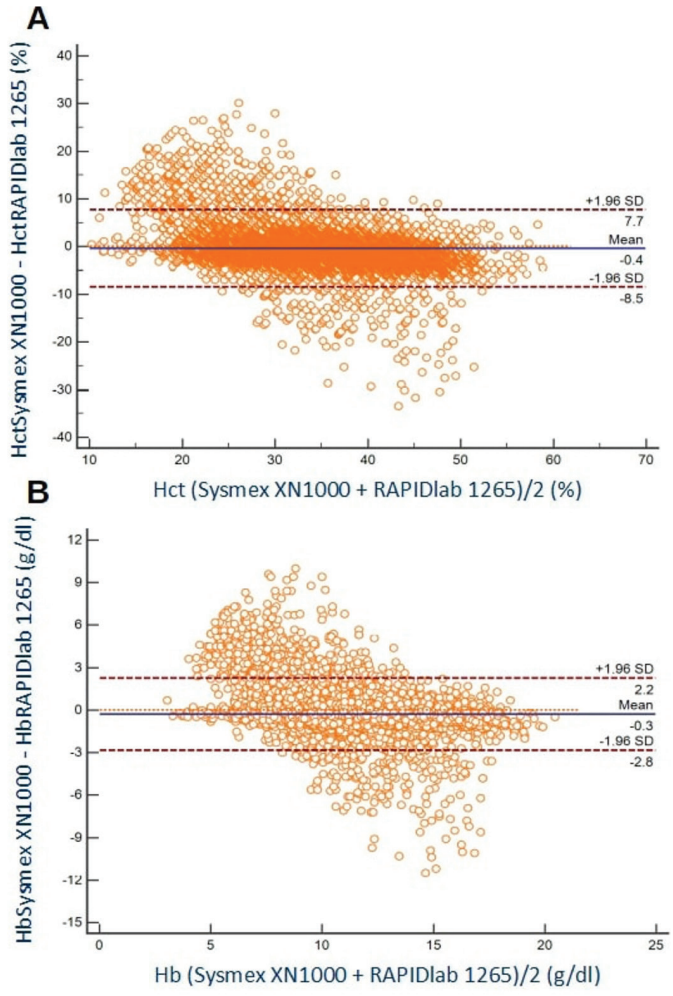

Figure 2. Bland-Altman analysis plots for the agreement of hematocrit A) and hemoglobin B) concentration with CO-oximetry (blood gas analyzer) and SLS-hemoglobin in 12,049 patients. The dashed lines represent the limits of agreement within which 95% of differences between measurements. Y-axes present the bias; X-axes present the average of the measurement results
SLS: Sodium lauryl sulphate

The formula calculation for the agreement of Hb results yielded a 2.5% bias and 4.08% TE. Bias for Hct measurement was calculated as 1.1% and the total error was calculated as 3.3%

The interassay coefficient of variations (CVs) for Hb was 0.9% and 1.6% for Sysmex XN1000 and RAPIDlab 1265 devices, respectively. The interassay CVs for Hct were 0.7% and 1.1% for the Sysmex XN1000 and RAPIDlab 1265 devices, respectively.

Discussion

In our study, we compared the Hb and Hct values simultaneously obtained from the BGA and the complete blood count autoanalyzer.

Hb levels might be measured by various methods such as the Sahli method, cyanmethemoglobin method, oxyhemoglobin method, and SLS- Hb method. The method recommended by Davis et al. (10) for measuring hemoglobin concentration is cyanmethemoglobin. SLS-Hb method is the method that we accept as the reference method and compared the BGA to evaluate the analytical performance. Although the SLS-Hb method is not considered a reference method, studies are indicating that the results correlate with the cyanmethemoglobin method (11,12). The main advantage of this method is its non-toxic nature and lower interference by lipemia and hemolysis (11).

According to the results of a study comparing the same BGA in our study, the mean Hb-value measured was found to be higher than the SLS- Hb method (135.10 g/L vs 130.60 g/L). Similarly, in our study, Hb values measured by the BGA were found to be higher than those the SLS- Hb method (119.60 g/L vs 116.60 g/L). However, the estimated correlation coefficient in this study was higher than that of our estimations (r=0.96, p<0.001). Contrary to our study, more than 95% of their samples remained within the limits according to the Bland-Altman analysis and were compatible with each other (13). This might be a result of several differences between the two study set-ups. Firstly, their data were normally distributed compared with our findings, with up to 5-fold more samples analyzed (2,548 vs 12,049). Furthermore, the variations between the populations and hospital management systems might result in minor analytical differences despite the use of similar devices. However, their study design was retrospective and based on an emergency laboratory setting.

Studies comparing the analysis results of BGA using venous blood for Hb measurement found higher mean Hb concentrations than the automated hematology analyzers (14). However, reports suggest that noninvasive measurement methods of Hb were more acceptable with the reference method than the measurement of Hb using a blood gas analyzer. However, both methods had significant variations from the reference method that can affect the clinical decision-making processes in the pediatric population (15).

In a multicenter cohort study on adult trauma patients, the initial Hb measurement on the trauma scene by point of care BGA predicted the presence of a significant hemorrhage with an area under the curve value of 0.72. Since early identification of excessive bleeding is crucial for adequate treatment, usage of point of care testing analyzers for measuring these variables in "outside the hospital" setting has the utmost importance to increase survival and decrease the possible morbidity (16).

Study Limitations

A limitation of our study was the lack of strict control of pre-analytical phase due to its retrospective design. Aruga et al. (17) reported that icterus, lipemia, cell free Hb and turbidity could interrupt the measurement of Hb. Although pre-analytical factors were controlled in routine laboratory applications, we believe that well-designed prospective studies with different patient groups such as pediatrics, suprageriatric (>85 years old) patients and individuals with hemoglobinopathies and anemia would be beneficial. Another limitation of our study was that broad clinical exclusion criteria were not applied. Among the 12,049 patient samples we compared, some patients applied to the emergency department and who were hospitalized and had chronic diseases. Due to these limitations, we consider our study not as a method comparison but as an evaluation of the method agreement study. The strong sides of our study are the higher subject number and the distribution of measured Hb values in a broad range. We also participated in an external proficiency testing program that revealed satisfactory performance for the analytes of interest.

Conclusion

Although each variable did not provide a satisfactory result in the Bland-Altman analysis when comparing the two methods, the calculated total errors for each analytes was lower than that reported by Westgard (4.20 % for Hb, 3.97 % for Hct) (9). Thus, the point of care BGA could be used for Hb measurements since the calculated bias remains acceptable. However, it is necessary to emphasize that the user and cost-related factors should also be evaluated within the scope of the laboratory pre-preanalytic phase related to the physician who will request the test. Since the blood count is performed using automatic systems, we must provide efficiency in terms of costs and qualified laboratory staff and manage faster access to test results, especially in healthcare institutions with many patients, in terms of laboratory practice.

Ethics Committee Approval: The study was approved by the Institutional Ethical Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 1929, date: 09.08.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: O.O., F.S.H., H.S.; Design : O.O., F.S.H., H.S.; Data Collection or Processing: O.O., F.S.H., H.S.; Analysis or Interpretation: O.O., F.S.H., H.S.; Literature Search: O.O., F.S.H., H.S.; Writing: O.O., F.S.H., H.S.

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

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

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İstanbul Med J 2022; 23(3): 199-204
DOI: 10.4274/imj.galenos.2022.03367

The Role of Hemogram Parameters in Predicting the Severity of Pulmonary Embolism

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ABSTRACT

Introduction: Acute pulmonary embolism (PE) is a disease with serious mortality and morbidity. Therefore, early diagnosis and treatment are important. A PE is a process accompanied by inflammation. Therefore, our study was designed to examine the relationship between hemogram parameters, which are easily accessible and indicative of inflammation, and late mortality from PE.

Methods: Two hundred and two patients who were hospitalized in our hospital between January 1, 2017 and July 1, 2020 and who were diagnosed with pulmonary angio computed tomography were included. Demographic and clinical data, laboratory, radiology and echocardiography results of the patients were analyzed retrospectively from the hospital information system.

Results: Seventy-eight of 202 patients included in the study were male (38.6%), 124 females (61.4%), mean age was 58.27±16.26 years. According to the results of univariate Cox regression analysis, age [hazard ratio (HR): 1,058, p=0.001], D-dimer (HR: 1,057, p=0.015), presence of malignancy (HR: 6,274, p=0.001), trauma history (HR: 2,931, p=0.039), long travel history (HR: 0.163, p=0.003), C-reactive protein (HR: 1,004, p=0.021), PE severity index (HR: 1,033, p=0.001), EF (HR: 0.944 , p=0.001), red-cell distribution width (HR: 1,125, p=0.001), lymphocyte (HR: 0.999, p=0.001), platelet-lymphocyte ratio (PLR) (HR: 1.013, p=0.001) and neutrophil-lymphocyte ratio (NLR) (HR: 1,017, p=0.001) significantly and it was associated with mortality.

Conclusion: Because of our study, we determined that NLR and PLR can be used as 12-month prognostic factors in patients with acute PE.

Keywords: Pulmonary embolism, mortality, PLR, NLR

Introduction

Acute pulmonary embolism (PE) is a cardiopulmonary disease with an incidence of 70 per 100,000 people, with significant mortality and morbidity. Therefore, early diagnosis and treatment are vital. It is usually observed after deep vein thrombosis (1-3). Biochemical markers such as troponin, brain natriuretic peptide (BNP), N-terminal proBrain natriuretic peptide (proBNP), heart type fatty acid binding protein, myoglobin and white blood cells (WBC) count as prognostic indicators in acute PE is used (4).

It has been shown that the progression of thrombosis in pulmonary arteries is associated with inflammation. Therefore, circulating inflammation-related markers are used as promising prognostic factors in thrombosis-related diseases. Among these biomarkers, it has been suggested that the neutrophil-lymphocyte ratio (NLR) and the platelet-lymphocyte ratio (PLR) are useful in determining the prognosis of patients with PE (5,6). Recent studies have found that NLR and PLR are better indicators of inflammation than WBC counts (5-12). In another study, it was suggested that NLR and PLR values are useful in determining the prognosis in patients with PE (13).

These studies included the groups in which the studies were conducted and inconsistencies due to the severity of the diseases and accompanying comorbidities. For this reason, we planned to evaluate the relationship between NLR and PLR, which are both cheap, easily accessible and can be evaluated from a routine hemogram, and prognosis in the service patients we follow-up. Unlike other studies, pulmonary embolism severity index (PESI), PE risk determination score (WELLS), computed tomography (CT) findings and cardiac findings were also evaluated.

Methods

Patients and Study Design

The study, which was planned as a retrospective cohort, was followed up with the diagnosis code ICD-10 "I26" in our service between January 1, 2017 and July 1, 2020, the data could be accessed from the electronic patient information system, the diagnosis of PE was confirmed by

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Cite this article as: Kara K, Sökücü SN, Tural Önür S, Özdemir C, Tokgöz Akyil F, Kahya Ö. The Role of Hemogram Parameters in Predicting the Severity of Pulmonary Embolism. İstanbul Med J 2022; 23(3): 199-204.

Received: 18.03.2022
Accepted: 14.07.2022

pulmonary CT angiography, 202 patients in whom no massive PE was detected; in whom there was no need for thrombolytic; not requiring intensive care follow-up were included.

Our study was conducted with University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital Ethics Committee cognitive consent (approval number: 2020-08, date: 06.08.2020). Informed consent was not obtained from the patients as it was a retrospective cross-sectional study.

Age, gender, comorbidities of the patients (diabetes mellitus, hypertension, Cerebrovascular disease, congestive heart failure, chronic renal failure, ischemic heart disease, chronic obstructive pulmonary disease, asthma, hyperlipidemia, malignancy, etc.), vital signs (blood pressure arterial, pulse and fingertip saturation), thoracic CT findings, echocardiography (ECHO) findings [ejection fraction (EF), right ventricular overload finding, pulmonary artery pressure (PAP), tricuspid regurgitation, mitral insufficiency, left ventricular hypertrophy, left ventricular diastolic dysfunction, left atrium dilatation, biatrial dilatation, pericardial effusion, mitral stenosis, left ventricular systolic dysfunction], hemogram and biochemical parameters (NLR, PLR), thrombocyte, neutrophil, lymphocyte, D-dimer, C-reactive protein (CRP), procalcitonin, proBNP, troponin level risk factors (immobilization or surgery history in the last 4 weeks, malignancy history, oral contraceptive use, trauma, thrombophilia), bilateral venous lower extremity doppler findings were obtained from the electronic patient information system and the 12th month mortality was questioned from the "Death Notification System".

Hemogram, D-dimer, troponin 1, proBNP, CRP values were obtained at the time of admission and before treatment. In our hospital, peripheral blood samples are taken into calcium-EDTA tubes and blood counts and differentials are analyzed using an autoanalyzer. PLR was calculated as the ratio of platelets (PLTs) to lymphocytes, and NLR was calculated as the ratio of neutrophils to lymphocytes in peripheral blood. ProBNP and troponin 1 levels and their quantitative analysis were studied in the ADVIA Centaur Analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). All D-dimer levels in plasma samples taken from patients [1 part sodium citrate (3.2%) 9 parts venous blood] were evaluated using the BCS XP coagulation analyzer. The PESI of the patients was evaluated using the data obtained from the electronic patient information system. ECHO findings and bilateral lower extremity Doppler ultrasonography results performed within the first 48 h were obtained from the hospital's electronic system. Philips Affiniti 30 ECHO devices were used for the ECHO evaluations.

Statistical Analysis

All statistical analysis were conducted using the SPSS 21.0 (IBM Statistical Product and Service Solutions version 21 Inc., Chicago, USA) program. Descriptive statistics were reported in the study, including mean (standard deviation), median (interquartile range), and percentage. Whether continuous variables showed a normal distribution was examined by Kolmogorov-Smirnov test. The difference in continuous variables between the living and death groups was evaluated by the two-sample t-test and the Mann-Whitney U test. Pearson's chi-square and Fisher's exact tests were used to the difference between categorical variables. Univariate Cox regression analysis was used to investigate the univariate predictors of total mortality throughout the study period. Receiver operating characteristic (ROC) analysis was used to determine the cut-off value of the NLR.

Results

Two hundred and two cases hospitalized in our clinic with the diagnosis of PE between January 1, 2017 and July 1, 2020 were included in the study. Seventy-eight of the patients were male (38.6%), 124 of them were female (61.4%), their mean age was 58.27±16.26 years. While 128 of the patients (63.4%) had a history of smoking, 74 patients (36.6%) did not smoking. Table 1 shows the comparison of demographic data of the patients according to the groups who died and survived. When the living and deceased populations were compared, a statistically significant difference was found in terms of age and body mass index (BMI) ($p<0.05$). While the age was higher in the deceased group (56.41±16.31 vs 69.86 ±10.05; $p=0.001$), it was found to be higher in the group with BMI (28.77±6.26 vs 23.81±10.01; $p=0.006$).

PESI, saturation, EF, PAP of the patients were statistically significant ($p<0.05$). PESI and PAP was higher in the deceased group (78.12±27.45 vs 108.04±18.95, 27.27±16.22 vs 32.75±20.41; $p=0.001$, $p=0.04$, respectively), saturation and EF was higher in the living group (95.09±3.94 vs 92.56±4.57, 58.31±5.96 vs 53.0±11.36; $p=0.004$ and $p=0.006$, respectively). In the statistical evaluation of 145 patients in terms of thoracic CT findings, WELLS score, and ECHO findings, there was no statistically significant difference between the two groups.

When laboratory parameters were compared, a statistically significant difference was found between the CRP, RDW, NLR, PLR and lymphocyte values between the surviving and deceased groups ($p<0.05$). CRP, RDW, PLR and NLR were higher in the deceased group (60.85±72.3 vs 95.41±62.51, 43.23±4.69 vs 46.71±6.49, 163.63±102.75 vs 255.21±256.74, 4.62±4.18 vs 8.16±9.25; $p=0.001$, $p=0.009$, $p=0.016$ and $p=0.003$ respectively), and lymphocyte was higher in the living group 2037.99±979.31 vs 1410±637.94, $p=0.001$. No statistically significant difference was observed between D-dimer, troponin, ProBNP, procalcitonin, mean platelet volume, PLT and neutrophil. A comparison of laboratory parameters is given in Table 2.

Long-term mortality predictors were examined using Cox regression analysis. According to the results of univariate Cox regression analysis, age [hazard ratio (HR): 1,058, $p=0.001$)], D-dimer (HR: 1,057, $p=0.015$), presence of malignancy (HR: 6,274, $p=0.001$), trauma history (HR: 2,931, $p=0.039$), long travel history (HR: 0.163, $p=0.003$), CRP (HR: 1,004, $p=0.021$), PESI (HR: 1,033, $p=0.001$), EF (HR: 0.944 , $p=0.001$), RDW (HR: 1,125, $p=0.001$), lymphocyte (HR: 0.999, $p=0.001$), PLR (HR: 1,013, $p=0.001$) and NLR (HR: 1,017, $p=0.001$) significantly affect mortality. Age, D-dimer, malignancy, CRP, PESI, RDW, NLR and PLR have affect on mortality since the HR is greater than 1. According to the results of multivariate Cox regression analysis, malignancy (HR: 0.087, $p=0.013$), trauma history (HR: 7,985, $p=0.049$), long travel history (HR: 0.144, $p=0.032$), Procalsitonine (HR: 1,586, $p=0.017$) and EF (HR: 0.924, $p=0.017$) significantly affect mortality. Table 3 provides Univariate ve Multivariate Cox regresyon analysis of the possible predictors of total mortality in the study population.

Table 1. Comparison of demographic and clinical parameters and outcomes of the study population

Demographic and clinical characteristics	Total population (n=202)	Alive (n=174)	Dead (n=28)	p-value
Age (years-mean ± st. dev.)	58.27±16.26	56.41±16.31	69.86±10.05	0.001
BMI (kg/m² - mean ± st. dev.)	28.08±7.08	28.77±6.26	23.81±10.01	0.006
Female, n (%)	124 (61.4)	105 (60.3)	19 (67.9)	0.449
Smoking history, n (%)	128 (63.4)	111 (63.8)	17 (60.7)	0.754
Risk factor, n (%)	168 (83.2)	143 (82.2)	25 (89.3)	0.351
Immobilization, n (%)	158 (78.2)	134 (77)	24 (85.7)	0.301
Malignancy, n (%)	34 (16.8)	20 (11.5)	14 (50)	0.001
Surgical operation history, n (%)	30 (14.9)	28 (16.1)	2 (7.1)	0.216
Oral contraceptive, n (%)	3 (1.5)	3 (1.7)	0 (0)	0.484
Trauma, n (%)	9 (4.5)	6 (3.4)	3 (10.7)	0.113
Travel, n (%)	81 (40.1)	78 (44.8)	3 (10.7)	0.001
Deep vein thrombosis, n (%)	15 (7.4)	15 (8.6)	0 (0)	0.232
History of thrombophilia, n (%)	2 (1)	2 (1.1)	0 (0)	0.569
Segmental involvement in tomography, n (%)	135 (66.8)	117 (67.2)	18 (64.3)	0.758
ECHO findings, n (%)	94 (46.5)	83 (47.7)	11 (39.3)	0.121
Wells score	5.32±2.42	5.23±2.43	5.86±2.36	0.201
PESI	81.9±28.3	78.12±27.45	108.04±18.95	0.001
Oxygen saturation, %	94.77±4.1	95.09±3.94	92.56±4.57	0.004
Pulse, min	87.57±158.61	86.87±15.54	92.44±15.54	0.095
Ejection fraction	57.63±7.07	58.31±5.96	53.0±11.36	0.006
Follow-up period, day	576.5 (315.5)	644.0 (291.7)	90.5 (105.5)	0.001

BMI: Body mass index, PESL: Pulmonary embolism severity index, st. dev.: Standard deviation, ECHO: Echocardiography, PESI: Pulmonary embolism severity index

Table 2. Comparison of laboratory parameters in the study population

Laboratory parameters	Total population (n=202) (mean ± st.dev)	Alive (n=174) (mean ± st. dev.)	Dead (n=28) (mean ± st. dev.)	p-value
D-dimer (mg/L)	4.26±5.79	3.89±5.09	6.560±8.81	0.279
Troponin 1 (ng/mL)	54.56±491.72	58.6±529.42	29.44±57.59	0.084
CRP (mg/L)	65.64±71.89	60.85±72.3	95.41±62.51	0.001
ProBNP (pg/mL)	116.54±519.47	98.27±505.45	230.11±596.96	0.192
Procalsitonine (ng/mL)	0.36±0.95	0.33±0.84	0.6±1.45	0.372
PAP, mm/Hg	28.03±16.92	27.27±16.22	32.75±20.41	0.040
MPV (fL)	9.28±1.59	9.28±1.52	9.26±2.01	0.409
RDW (%)	43.71±5.11	43.23±4.69	46.71±6.49	0.009
LYM ($10e^3$/uL)	1,950.94±963.04	2037.99±979.31	1,410±637.94	0.001
PLT ($10e^3$/uL)	270153.47±104,390.68	271,632.18±105,742.86	260,964.29±96,844.08	0.836
NEU ($10e^3$/uL)	7,089.21±3,207.47	6,984.71±3,148.69	7,738.57±3,543.34	0.216
NEU/LYM ratio (NLR)	5.11±5.46	4.62±4.18	8.16±9.25	0.003
PLT/LYM ratio (PLR)	176.32±137.65	163.63±102.75	255.21±256.74	0.016
PLT/NEU ratio (PNR)	45.16±25.42	45.92±25.43	40.41±25.27	0.156

CRP: C-reactive protein, PAP: Pulmonary arterial pressure, PROBNP: ProBrain natriuretic peptide, MPV: Mean platelet volume, RDW: Red-cell distribution width, LYM: Lymphocyte, PLT: Platelet, NEU: Neutrophil, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, PNR: Platelet-to-neutrophil ratio

In the ROC analysis, cut off values, area under the curve, sensitivity and specificity for NLR and PLR were found to be 4.365 and 174.72; 0.675 (0.576-0.774) and 0.642 (0.535-0.748); 64.3% and 53.6%; 67.2% and 70.1% respectively (Figure 1).

Discussion

PE, as a disease with a high mortality incidence, challenges clinicians in patient management in emergency units and inpatient services. There are many studies on mortality predictive parameters, and researches are still ongoing. Early detection of this disease is important for predicting

Table 3. Univariate ve multivariate cox regression analysis

Variables	Univariate model		Multivariate model	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Age	1,058 (1,027-1,089)	0.001	-	-
Gender	0.758 (0.340-1,659)	0.479	-	-
BMI	1,469 (0.614-3,518)	0.388	-	-
Smoking history	0.876 (0.410-1,872)	0.733	-	-
D-dimer	1,057 (1,011-1,106)	0.015	-	-
Troponin 1	1,000 (0.998-1,001)	0.785	-	-
Risk factor	1,790 (0.540-5,930)	0.304	-	-
Immobilization	1,779 (0.517-5,127)	0.286	-	-
Malignancy	6,274 (2,982-13,204)	0.001	0.087 (0.013-0.603)	0.013
Surgical operation	0.432 (0.103-1,821)	0.253	-	-
Oral contraceptive	0.152 (0.021-4,754)	0.653	-	-
Trauma	2,931 (0.883-9,727)	0.039	7,985 (1,012-62,986)	0.049
Travel history	0.163 (0.049-0.540)	0.003	0.144 (0.025-0.844)	0.032
Deep vein thrombosis	0.152 (0.089-2,785)	0.310	-	-
History of thrombophilia	1,325 (0.627-3,589)	0.720	-	-
Segmental involvement in tomography	0.897 (0.414-1,942)	0.782	-	-
CRP	1,004 (1,001-1,008)	0.021	-	-
Procalsitonine	1,167 (0.913-1,493)	0.218	1,586 (1,086-2,317)	0.017
Wells score	1,122 (0.950-1,325)	0.176	-	-
PESI	1,033 (1,019-1,047)	0.001	-	-
Pulse, min	1,019 (0.997-1,0429	0.096	-	-
Ejection fraction	0.944 (0.913-0.976)	0.001	0.924 (0.866-0.986)	0.017
RDW	1,125 (1,054-1,200)	0.001	-	-
Lymphocyte	0.999 (0.999-1,000)	0.001	-	-
NEU/LYM ratio (NLR)	1,071 (1,030-1,114)	0.001	-	-
PLT/LYM ratio (PLR)	1,013 (1,004-1,035)	0.001	-	-

BMI: Body mass index, CRP: C-reactive protein, RDW: Red-cell distribution width, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, CI: Confidence interval

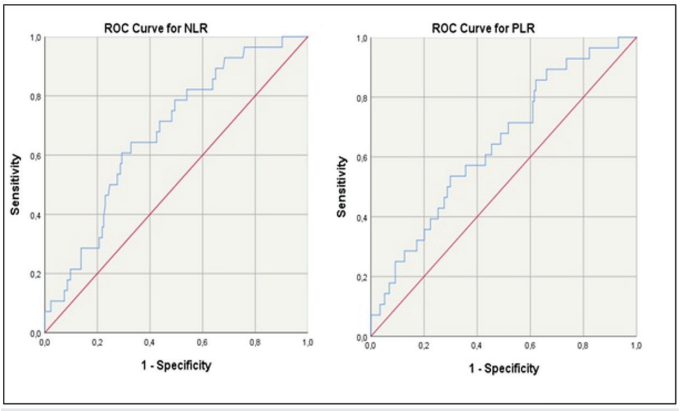

Figure 1. Receiver operating characteristic curve for NLR and PLR
ROC: Receiver operating characteristic NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio

mortality (1-3). Biochemical markers such as troponin, BNP, myoglobin and WBC count are used as prognostic indicators in acute PE (4). In our study, we predicted the mortality of the disease with the Complete Blood Count analysis found in each unit.

The incidence of PE increases with advanced age. Studies have shown that the average age is 50 years and above, and the risk of venous thromboembolism (VTE) increases with increasing age (12-16). In our study, 86 (42.57%) of the patients were under the age of 65 and 116 (57.43%) of them were over the age of 65, and the average age was 58 years, which was consistent with the literature. As in our study, a relationship between age and mortality was found in the study conducted by Karataş et al. (12).

It has been shown in the literature that the risk of VTE increased 6-22 times in the last 45-90 days after major surgical intervention (16). In our study, in accordance with the literature, 30 patients (14.9%) had a history of major surgical intervention, but it was not found to be associated with mortality. While PE was less mortal in patients with underlying long-travel history (41%) as a risk factor, mortality was higher in the group with a history of malignancy.

It has been shown in previous studies that NLR and PLR show increased inflammatory response in cardiovascular diseases and PE and have a prognostic value. Additionally, studies have shown that the increase in NLR and PLR is associated with 30-day mortality (17-19). In a study

performed in a group with massive PE and high PESI, the rate of NLR and PLR was found to be high (20). In the study conducted in 195 patients by Ozcan Cetin et al. (21), they found that NLR is a prognostic indicator of in-hospital adverse events and long-term mortality due to all causes. In another study, it was determined that NLR can be used as a 30-day mortality indicator depending on the increased inflammatory response (22). Additionally, in the study by Telo et al. (23), it was found that NLR and PLR increased in high-risk patients with PE, PLR could be a prognostic factor to predict 3-month mortality and showed that NLR can be used as a prognostic factor in-hospital, 3 months, and in total. Although most of the studies included patients with massive PE and in need of intensive care and thrombolytics, and short-term (1st and 3rd month) mortality was examined, we excluded this patient group in our study because we planned long-term mortality (12-month) study. Therefore, our mortality was found to be lower in our study. In our 12-month mortality study, consistent with the literature, NLR was associated with mortality according to both Cox regression analysis and hazard ratio. In another study, conducted with patients who were followed up after PE, NRL and PLR values at the time of diagnosis were found to be higher in the group who died during long-term follow-up, similar to our study (12). In the same study in which 203 patients were included in Turkey, NLR and PLR were associated with short- and long term mortality, and it was shown that NLR has a better prognostic value than PLR (12). In our study, it was found that NLR was more sensitive than PLR as a prognostic factor, while PLR was observed to be more specific.

In studies on RDW, RDW was found to be higher in patients with PE compared to the control group, and no statistically significant difference was found between those who lived or those who died (24). In contrast to other studies, we found RDW to be higher in the deceased group and was associated with mortality in univariate Cox regression analysis (25).

In studies examining the mean values of PESI in patients with PE, it was found that the mean values of PESI in patients who died were higher and the PESI score was associated with mortality (12,23,26). In our study, similar to the literature, the mean PESI value was found to be higher in the deceased group and it was observed that it was associated with mortality.

Additionally, we found that D-dimer, malignancy, and CRP are independent predictors of mortality in the 12-month period, in accordance with the literature (27-29).

Study Limitations

Our study was a single center retrospective study including a small sample group. The power of regression analysis can be increased with a larger sample group. We did not know the hemogram parameters of our patient before the acute PE. Therefore, it was impossible to compare the hemogram parameters before and after acute PE. Patients who were hospitalized in the ward were included in the study, and those who were followed up in an outpatient clinic or those who needed intensive care or thrombolytics were not included. Therefore, our mortality was found to be lower than that of other studies.

Conclusion

Our goal in this study was to determine the role of hemogram parameters in predicting the severity of PE. We found that NLR, PLR and RDW parameters were associated with long-term mortality. We think that hemogram parameters, which are easily accessible, can take their place in predicting the severity of PE and can be used as an indicator of mortality.

Ethics Committee Approval: Our study was conducted with University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital Ethics Committee cognitive consent (approval number: 2020-08, date: 06.08.2020).

Informed Consent: Informed consent was not obtained from the patients as it was a retrospective cross-sectional study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions: Concept - K.K., S.N.S., S.T.Ö.; Design - K.K., S.N.S., C.Ö.; Data Collection or Processing - S.N.S., S.T.Ö., C.Ö., F.T.A., Ö.K.; Analysis or Interpretation - K.K., S.N.S., S.T.Ö., C.Ö.; Literature Search - K.K., F.T.A., Ö.K.; Writing - K.K., S.N.S., S.T.Ö.

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

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

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İstanbul Med J 2022; 23(3): 205-9
DOI: 10.4274/imj.galenos.2022.84579

Predicting Length of Stay and Mortality in Acute Exacerbation of Chronic Obstructive Pulmonary Disease at the Intensive Care Unit

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ABSTRACT

Introduction: Chronic obstructive pulmonary disease (COPD) is a worldwide public health challenge because it affects more than 5% of the population. Early identification of the patients at risk of severe disease or death gives the clinician the chance to initiate rapid and aggressive treatment, and thereby save lives.

Methods: This was a single-center observational retrospective study. We included all patients aged ≥40 years admitted to the respiratory intensive care unit with a diagnosis of acute exacerbation of COPD (AECOPD) between January 2014 and December 2018.

Co-morbidities, hemogram and biochemistry values, and inflammatory markers were evaluated in both survivor and non-survivor groups. Results were evluated with SPSS.

Results: A total of 1,454 patients were assessed, 315 (21.6%) patients died during the hospital stay, and 1,139 (78.3%) patients were discharged. In the non-survivor group, mean white blood cell counts were higher than in survivors [14.1 (9.7-20.3), vs 11.8 (8.5-16.1), p<0.001]. However, the survivor group had significantly higher hemoglobin count [12.3 (10.6-14) vs 11.5 (9.8-13.2), p<0.001], lymphocyte % [6.9 (3.9-11.7) vs 5.2 (2.8-10.6), p=0.001], and eosinophil % [0.20 (0.00-0.90), vs 0.10 (0.00-0.60), p=0.001]. Additionally, C-reactive protein, and neutrophil to lymphocyte ratio were significantly lower in the survivor group on admission.

Conclusion: The findings of the current study may provide crucial information on several variables associated with in-hospital mortality for AECOPD patients.

Keywords: Chronic obstructive pulmonary disease, mortality, predictors, length of stay

Introduction

Chronic obstructive pulmonary disease (COPD) is a worldwide public health challenge due to it affecting more than 5% of the population (1,2). Approximately 10% of people aged 40 years or older have COPD which is which is expected to become the third major cause of death worldwide by the year 2030 (3).

An exacerbation of COPD, defined as a worsening of the patient's symptoms and the requirement of additional clinical treatment, is associated with accelerated lung function decline, quality of life impairment, and high hospital mortality (4-6). Early identification of the patients at risk of severe disease or death gives the clinician the chance to initiate rapid and aggressive treatment, and thereby save lives. Several identified factors, including congestive heart failure, older age, requirement of mechanical ventilation, nutritional status, and arterial oxygen and carbon dioxide partial pressure at entry have been independently associated with hospital mortality due to COPD exacerbations (7-10).

The decision to admit acute exacerbation of COPD (AECOPD) patients for the respiratory intensive care unit (RICU) were included according to the following GOLD guidelines: Hemodynamic instability, derangements in mental status, severe dyspnea that responds inadequately to initial therapy, worsening or impending respiratory acidosis and/or hypoxemia, the need for invasive mechanical ventilation (IVM) (4). Although, independent prognostic factors differ between clinical trials. To our knowledge, few studies have been conducted specifically to target patients admitted to the RICU. Thus, this study determined intensive care unit (ICU) mortality rate and factors affecting the prognosis of patients with AECOPD requiring RICU admission.

Methods

Study Design and Setting

This is a single-center observational retrospective study in our 16-bed RICU, which receives about 600 to 650 inpatients/year. The study protocol was approved by University of Health Sciences Turkey, İstanbul Training

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Cite this article as: Toptaş M, Kekeçoğlu A, Yurt S, Tural Onur S, Karapınar K, Akkoç İ, Haliloğlu M. Predicting Length of Stay and Mortality in Acute Exacerbation of Chronic Obstructive Pulmonary Disease at the Intensive Care Unit. İstanbul Med J 2022; 23(3): 205-9.

Received: 17.05.2022
Accepted: 20.07.2022

and Research Hospital Local Ethics Committee (approval number: 1857, date: 14.06.2019) by the Declaration of Helsinki. Because of the retrospective nature of the study design, informed consent was not obtained from patients regarding the use of medical data for publication. The identity information of all patients was strictly protected.

Study Population

We included all patients aged ≥40 years admitted to the RICU with a diagnosis of AECOPD from University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Anesthesiology, between January 2014 and December 2018. AECOPD is characterized by worsening the respiratory symptoms that is beyond normal variability, and changes therapy (4). COPD patients were excluded from the study if AECOPD was not the primary diagnosis, the patient had other acute events such as tuberculosis, lung cancer, interstitial pulmonary disease. Also, we excluded patients who needed early readmissions to the hospital occurring within ≤30 days of discharge.

Data Collection

All data from this study were obtained from retrospective querying of the institutional electronic system. The following variables were collected: 1) demographic characteristics including age, and gender; 2) characteristics of ICU stay, including the length of MV, length of ICU stay, 28-day mortality 3) blood tests, including red cell count and white blood cell count, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, C-reactive protein (CRP), creatinine, uric acid. All blood samples were collected within 24 h of admission.

Outcome Measures

The criteria for admission of AECOPD to the RICU did not change during the study period. The most common indications of AECOPD patients for RICU admission are worsening or impending respiratory failure and haemodynamic instability. The endpoint of this research was all-cause RICU mortality.

Statistical Analysis

All data were analyzed using SPSS 15.0 for the Windows program. Descriptive statistics; numbers and percentages for categorical variables, mean, standard deviation, median, and interquartile range for numeric variables. Since the numerical variables met the normal distribution condition, the comparisons of the two independent groups were made with the Mann-Whitney U test. Rates in the independent groups were compared with chi-square analysis. Relationships between numerical variables were made using Spearman correlation analysis since the parametric test condition was not met. The statistical alpha significance level was set as $p<0.05$.

Results

A total of 1,454 patients were assessed, 315 (21.6%) patients died during a hospital stay, and 1,139 (78.3%) patients were discharged.

The patients were divided into two groups based on in-hospital mortality: Survivors and non-survivors. Table 1 shows the demographics and comorbidities data of patients in the survival and non-survival groups. The majority were male in both groups, and survivors were younger than non-survivors [66 (58-75.2), vs. 69 (61-78), $p<0.001$]. The non-survivor group had significantly higher IVM requirements (51.1% vs 15.4%, $p<0.001$). The length of stay in the ICU was significantly longer in the non-survivor group. It was 7 (2-17) days in the non-survivor group and 4 (2-8) days in the survivor group ($p<0.001$). Comorbidities were similar in both groups and the most prevalent comorbidity was hypertension (<0.001).

Table 1. Clinical features and comorbidities

Variables	Survivors (n=1,139)	Non-survivors (n=315)	p-value
Male, n (%)	788 (69.2)	203 (64,4)	0.110
Age, years	66 (58-752)	69 (61-78)	<0.001
Requirement for IMV, n (%)	175 (15.4%)	161 (51.1%)	<0.001
Comorbidities, n (%)			
Hypertension	391 (34.3)	116 (36.8)	0.410
Diabetes	152 (13.3)	43 (13.7)	0.888
CHF	148 (13.0)	51 (16.2)	0.144
CKD	18 (1.6)	6 (1.9)	0.689
ICU length of stay	4 (2-8)	7 (2-17)	<0.001

Data presented as the median and interquartile range (25^{th}-75^{th} percentile) unless otherwise indicated. ICU: Intensive care unit, CHF: Chronic heart failure, CKD: Chronic kidney disease, IMV: Invasive mechanical ventilation

Table 2. Laboratory results of patients within 24 h after admission

Variables	Survivors (n=1,139)	Non-survivors (n=315)	p-value
Hemogram values			
WBC x10^9/L	11.8 (8.5-16.1)	14.1 (9.7-20.3)	<0.001
Neutrophil, (%)	83.7 (68.3-89.6)	85.1 (70.2-90.7)	0.125
Monocyte, (%)	4.7 (2.5-7.6)	4.3 (2.5-6.5)	0.059
Lymphocyte, (%)	6.9 (3.9-11.7)	5.2 (2.8-10.6)	0.001
Eosinophil, (%)	0.20 (0.00-0.90)	0.10 (0.00-0.60)	0.001
Basophil, (%)	0.20 (0.10-0.30)	0.10 (0.10-0.30)	0.078
Hemoglobin g/dL	12.3 (10.6-14)	11.5 (9.8-13.2)	<0.001
Hematocrit, (%)	38.9 (33.45-44.6)	35.9 (30.1-40.5)	<0.001
Mean platelet volume fL	87.9 (83-92.6)	88 (83-92.5)	0.712
Platelet count 10^9 L	235 (174-317)	228 (160-304)	0.062
Mean platelet volume fL	9.4 (8.4-10.3)	9.5 (8.3-10.4)	0.504
Biochemistry			
Blood glucose mg/dL	150 (119-203)	159.5 (127-195.75)	0.575
BUN mg/dL	41 (21.7-62)	55 (25.5-90.2)	<0.001
Serum creatinine mg/dL	0.71 (0.45-1.08)	0.88 (0.5-1.32)	<0.001
AST U/L	27 (19-43)	38.5 (24-74.75)	<0.001
ALT U/L	21 (14-37.25)	29 (16-55)	<0.001
Inflammatory markers			
CRP mg/dL	41.5 (9.2-121.7)	90.3 (16.5-195.1)	<0.001
NLR	11.7 (6.3-19.5)	15 (7.5-27.5)	0.002
PLT/MPV	26.5 (19.1-37.9)	25.9 (17.5-35.8)	0.079

Data presented as the median and interquartile range (25^{th}-75^{th} percentile) unless otherwise indicated. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CRP: C-reactive protein, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, PLT/MPV: Platelet to mean platelet volume, WBC: White blood cell count

The hemogram, biochemistry values, and inflammatory markers on admission are shown in Table 2. It was discovered in our study that white blood cell count, hemoglobin count, lymphocyte %, eosinophil %, was significantly different in both groups. In the non-survivor group, mean white blood cell counts were higher than in survivors [14.1 (9.7-20.3), vs 11.8 (8.5-16.1), p<0.001]. However, the survivor group had significantly higher hemoglobin count [12.3 (10.6-14) vs 11.5 (9.8-13.2), p<0.001], lymphocyte % [6.9 (3.9-11.7) vs 5.2 (2.8-10.6), p=0.001], and eosinophil % [0.20 (0.00-0.90), vs 0.10 (0.00-0.60), p=0.001]. Additionally, CRP, and neutrophil to lymphocyte ratio (NLR) were significantly lower in the survivor group on admission. No significant differences were in platelet-to-mean platelet volume ratio values were noted.

Discussion

Our study identified several risk factors for death in adult patients treated in the RICU for AECOPD. In particular, age, requirement of IMV, white blood cell count, lymphocyte %, eosinophil %, CRP, NLR, aspartate aminotransferase, alanine aminotransferase, serum creatinine (SCr), blood urea nitrogen was associated with mortality in the ICU.

Knowledge about the prognosis of the disease and factors that will cause poor outcome helps physicians plan treatment and advise patients about the expected natural course. Different risk factors predicting death from AECOPD have been identified in previous studies. For example, the relationship between CRP, NLR, PLR, D-dimers value, N-terminal proBrain natriuretic peptide, and in-hospital mortality in AECOPD patients has been reported (11-14).

Similar to the study by Ai-Ping et al. (15), gender was not related to mortality in our study. We noted that increasing age associated with in-hospital mortality, as a reason given for that is the patients' forced expiratory volume in the first second (FEV1) decreases at a more accelerated rate in elderly patients with COPD than in younger ones (16). In our study, the age was 69 (61-78) years among the nonsurvivors and 66 (58-75.2) years among the survivors (p<0.001).

Comorbidities were not correlated with mortality in our study, this finding is consistent with Connors et al. (17). In this study, several laboratory parameters were investigated for their potential prognostic properties, some of our observations were supported by past studies rising of SCr (18), eosinopenia (19), and anemia (20).

As far as we know, few studies based on lymphocytopenia to predict mortality have been conducted in AECOPD patients. In elderly patients with moderate-to-severe COPD, a relative lymphocyte count ≤20% was related to a higher risk of mortality (21). In another study, the lymphocyte counts of patients who died from AECOPD were lower than those who survived, but the lymphocyte count was not an independent risk factor for death (22). In this study, lymphocyte percentage was found to be a factor affecting in-hospital mortality in patients with AECOPD. Several factors should be considered for the mechanisms of lymphocytopenia predicting mortality in patients with AECOPD. In the elderly, lymphocyte count may be decreased (23), and the elderly are also a risk factor for death in hospitalized COPD patients (9).

This study suggests that CRP and NRL are useful laboratory biomarkers for prognosis in patients with AECOPD. Xiong et al. (22) and Sørensen et al. (24). Noted that elevated NLR may be related to death in patients with AECOPD. To the fact that CRP's relevance to mortality is inferior to that of NLR, it is still correlated with mortality (22).

When the need for respiratory support is established, candidates for noninvasive respitarory ventilation should be screened for possible contraindications. Generally, gastrointestinal hemorrhage, recurrent vomiting, and impairment swallowing are risk factors for vomiting, they are probably unsuitable (25). Also, patients, who are unable to protect their airway due to derangements in mental status, are poor canditates (25). Additionally, patients in AECOPD with cardiovascular instability are probably poor canditates (26). In spite of that, carbon dioxide narcosis in AECOPD should not be considered contraindication (27). We shown that the requirement for IMV was associated with in-hospital mortality. This finding is consistent with that of Brown et al. (9), and Ongel et al. (28); however, five other studies failed to find any association between IMV and in-hospital mortality (29-33). Several factors should be considered in this association; it is probably related to disease severity, and patients who require IMV rather than non-invasive ventilation are in a severe disease stage. Seneff et al. (32) demonstrated that IMV does not affect short- or long-term mortality when controlling for the severity of ilness.

We noted that the length of stay (LOS) in the ICU was related to in-hospital mortality. To our knowledge, predicting mortality based on ICU LOS in adult patients treated for AECOPD has been reported in only a few studies. Ai-Ping et al. (15) shown that LOS in the hospital was associated with mortality, while Hill et al. (34) reported that patients with COPD who died had significantly shorter LOS than those who survive. This is probably related to end-of-life practices, which differ significantly between ICUs.

Study Limitations

Our study also has some limitations. Due to retrospective design at a single center, our results may not be generalized. Additionally, data collection was limited to existing medical records, and not all study variables could be collected. However, the results of this study include many patients with COPD, who are valuable for this specific disease and deserve consideration.

Conclusion

The current study findings may provide crucial information on several variables associated with in-hospital mortality of AECOPD patients. The results may become important for medical decisions to decrease mortality and the LOS in patients with AECOPD requiring RICU admission.

Ethics Committee Approval: The study protocol was approved by University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 1857, date: 14.06.2019) by the Declaration of Helsinki.

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept - A.K., S.T.O., M.H.; Design - İ.A., M.H.; Data Collection or Processing - A.K., S.Y.; Analysis or Interpretation - M.T., M.H., K.K.; Literature Search - A.K., S.T.O.; Writing - M.T., M.H.

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

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

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İstanbul Med J 2022; 23(3): 210–5
DOI: 10.4274/imj.galenos.2022.62443

Prediction of Breast Cancer Distant Metastasis by Artificial Intelligence Methods from an Epidemiological Perspective

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ABSTRACT

Introduction: Despite significant advances in breast cancer (BC) management, the prognosis for most patients with distant metastasis remains poor. We predicted distant metastasis in BC patients with artificial intelligence (AI) methods based on genomic biomarkers.

Methods: The dataset used in the study included 97 patients with BC, of whom 46 (47%) developed distant metastases, and 51 (53%) did not develop distant metastases, and the expression level of 24,481 genes of these patients. An approach combining Boruta + LASSO methods was applied to identify biomarker genes associated with BC distant metastasis. Mann-Whitney U test was used to examine the difference between groups in terms of gene expression levels in statistical analyses, and Cohen d effect sizes and odds ratios were calculated. AdaBoost and XGBoost algorithms, which are tree-based methods, were used for BC distant metastasis prediction, and the results were compared by evaluating comprehensive performance criteria.

Results: After Boruta + LASSO methods, 14 biomarker candidate genes were identified. These predictive genes were *PIB5PA, SSX2, OR1F1, ALDH4A1, FGF18, WISP1, PRAME, CEGP1, AL080059, NMU, ATP5E, SMARCE1, FGD6,* and *SLC37A1*. In effect size results; in particular, show that the AL080059 (Cohen's D: 1.318) gene is clinically predictive of BC Metastasis. The accuracy, F1-score, positive predictive value, sensitivity, and area under the ROC Curve (AUC) values obtained with the AdaBoost algorithm for BC metastasis prediction was 95%, 96.3%, 100%, 92.6%, and 98.8%, respectively. The model created with the XGBoost algorithm, on the other hand, obtained 90%, 92.9%, 92.9%, 92.9%, 97.6% accuracy, F1-score, positive predictive value, sensitivity, and AUC values, respectively.

Conclusion: Identifying genes that successfully predict BC distant metastasis with AI methods in the study may be decisive for future therapeutic targets and help clinicians better adapt adjuvant chemotherapy to their patients. Additionally, the AdaBoost prediction model created can discriminate patients at risk of BC distant metastases.

Keywords: Breast cancer, distant metastasis, genetic risk factors, genomics, artificial intelligence

Introduction

Breast cancer (BC) is among the leading causes of mortality and morbidity among women. GLOBOCAN 2020 results reveal that BC is the most frequent cancer with an incidence of 11.7% and the fifth most frequent cause of death due to cancer with an incidence of 6.9% (1). Lifetime BC risk of a woman in a developed country is 12.5%, whereas the risk of mortality due to BC is 3.4% (2). A wide distrubition of incidence is also present between different ethnic groups and caucasian or afro-american populations of the same country.

BC is a significant public health problem for either developed or developing countries regarding financial and psychosocial issues. BC incidence is relatively higher in countries with higher income compared with BC incidence in middle and lower income countries. Epidemiological studies to analyse this difference revealed the impact of environmental factors, lifestyle, nutritional factors and sociocultural status as triggering factors of BC.

Predisposing factors for BC are considered in seven subgroups: demographic (age, female gender), reproductive (late age of menopause, pregnancy characteristics), hormonal (hormonal contraceptive methods, postmenopausal hormone therapy), breast-related factors (some benign breast disorders), lifestyle (obesity or overweight, alcohol consumption, smoking, diet), others (air pollution, night work, socioeconomic status, diabetes, radiation) and hereditary factors (genetic factors, positive family history of BC) (3,4). Genetic factors, which are among the hereditary risk factors, have been studied for many years. Mutations of either oncogenes or anti-oncogenes and abnormal amplification effects formation and progression (4). BC-associated genes revealed in previous studies are *BRCA1, BRCA2, c-erbB-2 (HER2), c-erbB-1 (HER1), TP53, PTEN, PALB2, STK11, CDH1, ATM, CHRK*, c-Myc ve Ras (4,5). However, there are many genes whose relationship with BC is still at the research level.

Despite the advances in BC treatment in the last 20-30 years, patients with metastatic disease still have a poor prognosis with a survival of five

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Cite this article as: Akbulut S, Yagin FH, Colak C. Prediction of Breast Cancer Distant Metastasis by Artificial Intelligence Methods from an Epidemiological Perspective. İstanbul Med J 2022; 23(3): 210-5.

Received: 07.06.2022
Accepted: 25.07.2022

to ten years (6,7). Recent studies determined locoregional recurrence and distant metastasis rates as 5-15% to 11-18.7% respectively (8-10). Hence, distant metastasis and recurrence have a negative impact on recurrence-free and overall survival.

The vast majority of studies for the molecular structure of BC are focused on primary cancers.

Gene expression profiles divide BC into different subgroups and clinical trial point out these transcriptional signatures to impact making therapeutic decisions (7,11). Recent large scaled genomic analyses ease revealing complicated mutational configurations. Despite the largely defined genomic configuration of BC, the same success is not actually present for genetic configurations of locoregional or distant-metastasis BC. Studies for metastatic disease up to date clonally determined relationship between metastases and primary tumor, presence of various common mutations and presence of typically additional mutations, which are not present in primary tumors (12,13).

Microarray technology, which provides simultaneous quantitative monitoring of expression levels of thousands of genes, is an important research topic in the early diagnosis of primary BC and its metastases (locoregional recurrence, distance metastasis) with artificial intelligence (AI)/machine learning (ML) methods. However, the predictive performance of AI/ML models may be adversely affected by many genes unrelated to the disease(s) and may not contribute to the classification. A technology that can be used to eliminate this problem is ML. ML is widely recognized as the choice approach in BC pattern classification and forecast modeling due to its unique benefits in detecting essential characteristics/genes from complicated BC datasets. Recently, ML approaches have played an essential role in the diagnosis and prognosis of BC by using classification techniques to identify persons with BC, differentiate benign from malignant tumors and predict prognosis. Accurate categorization can also help clinicians prescribe the best treatment regimen (14,15). Considering these data, this study intended to identify biomarker candidate genes in predicting BC recurrence with AI modeling.

Methods

Dataset

Gene expression and clinical data used in the study were obtained from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database. The dataset included 97 patients with lymph node-negative (pN0) BC, of which 46 (47%) had developed distant metastasis within 5 years and 51 (53%) had not developed distant metastasis. Clinical data included information on patients' age, pathological tumor size and grade, ER and V-ERB-B2 avian erythroblast leukemia viral oncogene homolog 2 (ERBB2) statutes, and follow-up results. In the gene expression data set, 97 patients had expression levels of 24,481 genes (16).

Data Preprocessing and Modeling

At baseline, there were 24,481 gene expression levels in the BC metastasis dataset. The Boruta + LASSO method was used to select candidate gene biomarkers associated with metastasis. Boruta is a method that iteratively removes from the dataset variables that have been statistically proven to be less relevant to the response (here, metastasis). LASSO for variable selection obtains a sparse regression model. For a given dataset (X,y), X is the explanatory variable and y is the variable to be explained. The LASSO method estimates the β parameters of the model. It then selects the important variables by applying a λ constraint to the predicted parameters. Variables with β parameters shrinking to zero are considered unimportant. Of the variable selected data set, 80% is randomly split to train the model and the remaining 20% to test the model. This split was repeated 100 times and average scores were calculated 100 times in the evaluation of the models. Two different models, AdaBoost and XGBoost, were created for BC metastasis prediction based on genomic biomarkers. The performance of the generated models was evaluated by accuracy, F1-score, positive predictive value, sensitivity, and the area under the ROC curve (AUC), and the results were compared.

Study Protocol and Ethics Committee Approval

This study, which was prepared using the NCBI GEO open-access dataset, involving human participants, was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was obtained from the İnönü University Institutional Review Board in Non-Interventional Clinical Research (approval number: 2022/3645, date: 07.06.2022). Strengthening the reporting of observational studies in epidemiology guideline was used to assess the likelihood of bias and overall quality of this study (17).

Statistical Analysis

Qualitative variables are summarized as numbers and percentages. Quantitative variables were digested with the median and inter-quantile range. Two groups were compared with the Mann-Whitney U test. Statistical tests with a p-value of less than 5% were considered significant. The Cohen's D effect size was calculated for variables with a significant p-value. For the Mann-Whitney U test, the effect size (Cohen's D) was interpreted as a small effect between 0.20-0.50, a medium impact between 0.50-0.80, and a large impact above 0.80 (18). Additionally, odds ratio estimates for quantitative biomarker genes with significant p-value were obtained by logistic regression analysis. All statistical analysis were performed in IBM SPSS Statistics for Windows version 26.0 (New York; USA) and Python 3.9.

Results

Descriptive statistics on patient information in the clinical dataset are given in Table 1. In the study, 74 (76%) patients were older than 40 years and the remaining 23 (24%) patients were younger than 40 years old. The tumor size was smaller than 20 mm in 44 (45%) patients and larger than 20 mm in 53 (55%) patients. 37 (38%) of the patients were stage I-II and 60 (62%) were stage III BC. While 72 (74%) had a positive ER status, 25 (26%) were negative. While 15 (16%) were HER2 positive, 82 (84%) were negative. Fourty-six (47%) of 97 patients had a recurrence of metastasis within 5 years, and 51 (53%) had no metastasis.

Descriptive statistics, effect sizes, and odds ratios (95% confidence interval) of selected genes after Boruta + LASSO feature selection methods are given in Table 2. There was a statistically significant difference between the metastasis and no-metastasis patient groups in terms of expression levels of all 14 genes selected as biomarker candidates that may be effective in the diagnosis and treatment of BC distant metastasis. Effect size results; in particular, show that the *AL080059* (Cohen's D: 1.318) gene is clinically predictive of BC Metastasis (Table 2). When the odds ratio estimations are examined; a one-unit decrease in the expression levels of the *PIB5PA, OR1F1, ALDH4A1, FGF18, WISP1, CEGP1,* and SMARCE1 genes increases the risk of metastasis by 10.75, 125, 166.66, 43.47, 100, 5.52, 83.33 times, respectively. In contrast, a one-unit increase in the expression levels of the *PRAME, AL080059, NMU,* and *ATP5E* genes increases the risk of metastasis by 4.454, 57.248, 35.396, and 728.461 times, respectively. Table 3 shows the results of the performance criteria of the AdaBoost and XGBoost models created for BC metastasis estimation. When Table 3 is examined, the accuracy, F1-score, positive predictive value, sensitivity, and AUC values obtained in the test data set for the AdaBoost algorithm for BC metastasis prediction are 95%, 96.3%, 100%, 92.6%, 98.8%, respectively. The model created with the XGBoost algorithm, on the other hand, obtained the accuracy, F1-score, positive predictive value, sensitivity, and AUC values of 90%, 92.9%, 92.9%, 92.9%, 97.6%, respectively, in the test data set.

Table 1. Descriptive statistics on clinical information in the breast cancer dataset

Patient's clinical information		(n=97)
Age	≥40 years	74 (76%)
	<40 years	23 (24%)
Tumor size	<20 mm	44 (45%)
	≥20 mm	53 (55%)
Grade	1-2	37 (38%)
	3	60 (62%)
ER status	Positive	72 (74%)
	Negative	25 (26%)
HER2 status	Positive	15 (16%)
	Negative	82 (84%)
Metastatic relapse within 5 years	Yes	46 (47%)
	No	51 (53%)

Discussion

Despite significant advances in BC treatment recently, the prognosis for most patients with distant metastasis remains poor. BC patients with the same disease stage may have markedly different treatment responses and overall outcomes. The strongest predictors of metastasis (eg, lymph node status and histological grade) cannot accurately classify BCs based on their clinical behavior. Additionally, an in-depth understanding of the molecular phenotype of distant metastasis is critical to pave the way for earlier detection of metastasis and more effective treatments. Therefore, in this study, we predicted distant metastases in patients BC using AI methods based on genomic biomarkers (18,19).

Microarray data of 24,481 genes of 97 patients with and without distant metastasis were used in the study. For AI models, the fact that microarray data contain thousands of gene information belonging to few patients both lead to computational inefficiency and reduces the performance of prediction models. Additionally, it may be useless to use information about thousands of genes in clinical practice, and there may be many genes unrelated to the disease of interest in these datasets containing many genes. From this perspective, the identification of a small subset of genes with AI methods not only facilitates transfer to clinics but also limits the identification of false-positive predictive genes. For this reason, in this study, a methodology combining Boruta + LASSO methods was applied to identify candidate biomarker genes that

Table 2. Statistical analysis results of selected genes as a result of Boruta + LASSO

Genes*	Breast cancer		p-value	ES	OR (95% CI)
	No-metastasis	Metastasis			
PIB5PA	-0.005 (0.367)	-0.337 (0.495)	<0.001	0.828 (large)	0.093 (0.022-0.326)
SSX2	0.092 (0.414)	-0.07 (0.298)	0.001	0.717 (medium)	1.032 (0.993-NA)
OR1F1	0.112 (0.193)	0.029 (0.074)	<0.001	0.81 (large)	0.008 (0-0.167)
ALDH4A1	0.126 (0.194)	-0.071 (0.262)	<0.001	0.974 (large)	0.006 (0-0.072)
FGF18	0.06 (0.416)	-0.242 (0.284)	<0.001	0.995 (large)	0.023 (0.003-0.127)
WISP1	0.064 (0.278)	-0.079 (0.208)	<0.001	0.816 (large)	0.01 (0.001-0.124)
PRAME	-0.77 (0.228)	0.054 (1.17)	<0.001	0.786 (medium)	4.454 (2.145-10.27)
CEGP1	0.065 (0.484)	-0.755 (0.734)	<0.001	1.032 (large)	0.181 (0.076-0.394)
AL080059	-0.391 (0.344)	0.076 (0.436)	<0.001	1.318 (large)	57.248 (12.013-364.325)
NMU	-0.302 (0.256)	-0.06 (0.392)	<0.001	1.091 (large)	35.396 (6.389-280.071)
ATP5E	-0.054 (0.128)	0.054 (0.166)	<0.001	0.981 (large)	728.461 (121.328-8541.318)
SMARCE1	-0.005 (0.288)	-0.18 (0.238)	<0.001	0.985 (large)	0.012 (0.001-0.106)
FGD6	0.029 (0.235)	-0.121 (0.162)	<0.001	0.86 (large)	0.143 (0.012-0.979)
SLC37A1	-0.064 (0.253)	0.069 (0.315)	0.002	0.655 (medium)	23.439 (2.749-245.161)

*: Gene expression levels are summarized as "median (IQR)", OR: Odds ratio, CI: Confidence interval, ES: Effect size

Table 3. Results of performance measures for models created to predict breast cancer

Models	Accuracy	F1-score	Positive predictive value	Sensitivity	AUC
AdaBoost	0.95	0.963	1.000	0.926	0.988
XGBoost	0.90	0.929	0.929	0.929	0.976

AUC: Area under the ROC curve

may be associated with distant metastasis. In this way, 14 genes that may be associated with BC metastasis were identified. These predictive genes were *PIB5PA, SSX2, OR1F1, ALDH4A1, FGF18, WISP1, PRAME, CEGP1, AL080059, NMU, ATP5E, SMARCE1, FGD6,* and *SLC37A1*. Then, two different models, AdaBoost and XGBoost, were created using 14 genes determined for distant metastasis prediction. The accuracy, F1-score, positive predictive value, sensitivity, and AUC values obtained with the AdaBoost algorithm for BC metastasis prediction were 95%, 96.3%, 100%, 92.6%, and 98.8%, respectively. The model created with the XGBoost algorithm, on the other hand, obtained the accuracy, F1-score, positive predictive value, sensitivity, and AUC values of 90%, 92.9%, 92.9%, 92.9%, and 97.6%, respectively. The results showed that AdaBoost outperformed XGBoost in BC distant metastasis prediction.

Our gene selection results were generally compatible with the literature. In a study in the literature, it was reported that high PIB5PA levels are associated with limited tumor progression and better prognosis in patients with BC (16). Greve et al. (20) investigated the phenotypic and molecular changes associated with SSX2 expression in human melanoma and BC cells and showed that the *SSX2* gene has oncogenic potential. Additionally, the study highlighted the potential of this gene as a therapeutic target (20).

ALDH1A1 is an essential element in the retinoic acid signaling pathway that regulates self-renewal and differentiation of normal stem cells and may play an important role in cancer progression. Liu et al. (21) emphasized that high expression of ALDH1A1 mRNA in tumor tissues may be an independent predictor of a positive triple-negative BC outcome. Marcato et al. (22) In another study, they showed that ALDH1A3 expression could predict metastasis in BC patients. Song et al. (23) showed that the *FGF18* gene promotes epithelial-mesenchymal transition and migration in BC cells and emphasized that FGF18 expression may be a potential prognostic therapeutic marker for BC.

WISP1 genetic polymorphisms were highlighted in a study in the literature to be associated with platinum-based chemotherapy toxicity and sensitivity to platinum-based chemotherapy responses in patients with lung cancer (24). It has been reported that WISP1 can also predict a patient's susceptibility to cervical cancer and hepatocellular carcinoma (25,26). Wang et al. (27) emphasized that WISP1 polymorphisms play a critical role in BC. In another study, Sokol et al. (28) emphasized that the expression of the *SMARCE1* gene in patients diagnosed with early-stage BC would be a strong indicator of recurrence and metastasis. Additionally, they reported that SMARCE1 expression identifies early-stage breast, ovarian, and lung cancers that are likely to progress and metastasis.

Epping et al. (29) emphasized that PRAME expression is a prognostic marker for the clinical outcome of BC. The results of the study showed that PRAME was an independent predictor of shortened metastasis-free interval in patients not receiving adjuvant chemotherapy. PRAME expression was associated with tumor grade and negative estrogen receptor status. Lu et al. (30) reported that CEGP1 expression is associated with locoregional tumor recurrence or distant metastasis in patients with BC.

In a recent study, it was emphasized that the *AL080059* gene is one of the ten prognostic marker genes that differ between normal and tumor tissues of patients with BC (31). Galber et al. (32) reported that ATP synthase contributes to cancer development or metastasis Amino acid changes in ATP synthase encoded by the *ATP6* gene have been detected in pancreatic cancer cells (33), thyroid (34), cervical, bladder, and head/neck cancers, as well as in leukemia (35) and acute myeloid leukemia (36) patients. In a study, it was observed that the *A6L* gene, which is derived from ATP8, is mutated in ovarian, breast, cervical, and thyroid cancers (35). Additionally, Grzybowska-Szatkowska et al. (37) found homoplasmic mutations in the *ATP6* and *ATP8* genes in patients with BC. However, there was no information in the literature that ATP5E, one of the 14 genes we selected, is directly related to BC. Future studies should examine whether ATP5E is a predictive biomarker for patients with BC.

Garczyk et al. (38) have identified NMU as a drug response biomarker candidate for patients with BC. Additionally, they reported that NMU may be a putative therapeutic target to reduce the metastatic spread of BC cells (38). Another study conducted on the *FGD6* gene, which was selected as a biomarker candidate in our study, reported that it is an independent prognostic risk factor for the survival of patients with gastric cancer (39). However, no study was found that reported the association of this gene with BC. In future studies, investigating whether the *FGD6* gene is associated with BC and metastasis may be important for future therapeutic targets.

In the literature, several different studies have been found that predict metastasis with the dataset we used in this study. For example, in a study using the same data set, a variable selection was made and distant metastasis was predicted with 88.55% accuracy (40). In another study using the same data, the Elastic net method was used and an accuracy rate of 59% was obtained in the prediction of metastasis (41). It can be said that the AdaBoost model created in the current study has a more successful performance in estimating distant metastasis in BC patients compared to the literature.

Study Limitations

As with all retrospective case-control studies, this study has some limitations. Most genomic analysis are usually conducted with few samples, as they require high budgets for each sample. For this reason, a limitation of this study is the sample size. Secondly, the open-access data set was used in this study, which means that some variables can be ignored since all possible factors cannot be accessed in such studies. In future studies, it should be aimed to present a model that can be created for predicting BC metastasis to users with a web-based interface.

Conclusion

To sum up, the identification of genes that successfully predict BC distant metastases with AI methods in the study may be decisive for future therapeutic targets and may help clinicians better adapt adjuvant chemotherapy to their patients. Additionally, the predictive model AdaBoost created can distinguish patients at risk of distant metastasis.

Ethics Committee Approval: Ethical approval was obtained from the Inonu University Institutional Review Board in Non-Interventional Clinical Research (approval number: 2022/3645, date: 07.06.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept - S.A.; Design - S.A., F.H.Y.; Data Collection or Processing - S.A., F.H.Y., C.C.; Analysis or Interpretation - F.H.Y., C.C.; Literature Search - S.A., F.H.Y.; Writing - S.A., F.H.Y.

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

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

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İstanbul Med J 2022; 23(3): 216-9
DOI: 10.4274/imj.galenos.2022.84748

Low Oocyte Maturity Rate and Asynchronous Follicle Development: Other Unnoticed Groups in the Bologna Criteria for Poor Responders?

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ABSTRACT

Introduction: This study aimed to evaluate the prognosis of patients with low rates of oocyte maturity and compare those who are aforethought poor responders with respect to the Bologna criteria.

Methods: All assisted reproductive technology (ART) cycles conducted from 2004 to 2018 in a tertiary center in İstanbul were analyzed retrospectively. Patients were grouped into three accordingly the count of total retrieved oocytes and metaphase-II [(M-II) -mature] oocytes after denudation (group 1: ≤3 oocytes and ≤3 M-II oocytes; group 2: >3 oocytes and ≤3 M-II oocytes; group 3: >3 oocytes and >3 M-II oocytes). A Low oocyte maturity rate was diagnosed when ≤50% of all harvested oocytes were in the M-II stage before the fertilization procedure.

Results: During the study period 14,899 intracytoplasmic sperm injection cycles were evaluated. The study group's mean age was 32.6±5.3. The mean counts of total and mature oocytes were 9.8±5.9 and 7.3±4.5, respectively. A mean count of 2.38 embryos was transferred in 10118 cycles. The group 3 patients had a considerably higher live birth ratio compared to the group 1 and 2.

Conclusion: We propose oocyte maturity rate and the count of M-II oocytes as two diagnostic criteria for the case definition of asynchronous follicle growth. Based on our findings, stimulation cycles ending with low oocyte maturity rate (≤50%) and ≤3 M-II oocytes would be considered asynchronous follicle development. Patients with low oocyte maturity rate and asynchronous follicle development should be counseled and informed regarding potential poor prognosis of the treatment.

Keywords: Oocytes, fertilization in vitro, ovarian reserve, ovarian stimulation, metaphase

Introduction

Asynchronous follicle growth is a frequent finding in women with poor ovarian reserve and usually ends with a limited count of mature oocytes available for intracytoplasmic sperm injection. The ESHRE Working Group on Poor Ovarian Response proposed Bologna Criteria using the total count of oocytes as the primary determinant to define a poor responder (1). However, this definition is prone to overlook patients who do not fulfill the criteria based on the total count of harvested oocytes despite a limited count of mature ones. Clinical experience denotes that the prognosis of women with asynchronous follicle growth and low oocyte maturity rate is as poor as the Bologna defined as poor responders (2-5). Therefore, we designed this study to evaluate the prognosis of patients with low oocyte maturity rates and compare those who were evaluated as poor responders in accordance with the Bologna criteria.

Methods

All assisted reproductive technology (ART) cycles conducted between 2004 and 2018 in the tertiary in vitro fertilization (IVF) center of İstanbul were retrospectively analyzed. Data collected from the electronic database included female age, previous failed cycles, stimulation protocol, count of oocytes collected, count of mature oocytes and clinical outcome. Patients, who had undergone one of two commonly used stimulation protocols, i.e., long luteal GnRH agonist and GnRH antagonist protocols and had at least one mature oocyte following follicle aspiration were included in the study. Patients who underwent in vitro maturation, natural cycle, or modified natural cycle treatment, preimplantation genetic screening/diagnosis, and who used testicular sperm for fertilization were excluded. Following the exclusions, the final dataset included 14,899 ICSI cycles.

Details of stimulation protocols and ART laboratory procedures employed in our unit are reported elsewhere (6). A low oocyte maturity rate was diagnosed when ≤50% of all harvested oocytes were in the metaphase-II (M-II) stage before the fertilization procedure.

The Koç University Faculty of Medicine Local Research Ethics Committee authorized the count of total and M-II oocytes (approval number: 2022.132.IRB1.048, date: 05.04.2022).

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Cite this article as: Ertaş S, Yakın K. Low Oocyte Maturity Rate and Asynchronous Follicle Development: Other Unnoticed Groups in the Bologna Criteria for Poor Responders?. İstanbul Med J 2022; 23(3): 216-9.

Received: 07.04.2022
Accepted: 26.07.2022

Statistical Analysis

Continuous variables were defined by mean (±2 standard deviation) and categorical variables were defined by number and percentage. Two-tailed Pearson correlation test and linear regression analysis were conducted to determine confounding variables that are associated with live birth. Cycles were categorized into four groups with respect to female age (≤30, 31-35, 36-40, ≥40). The groups were compared using analysis of variance test with Bonferroni correction. Receiver operator characteristics (ROC) curves were constructed to evaluate predictive values for live birth. In the context of two-way hypothesis evaluation, $p<0.05$ was considered statistically significant. The Statistical Package for the Social Sciences software (SPSS version 22) was used to analyze the data and create the figures.

Results

The mean age of the study group was 32.6±5.3. The mean counts of total and M-II oocytes were 9.8±5.9 and 7.3±4.5, respectively. A mean count of 2.38 embryos was transferred in 10118 cycles.

The overall oocyte maturity rate was 74%. The proportion of mature oocytes to the total count of retrieved oocytes was similar among the four age groups (73.4% for ≤30 years, 74.2% for 31-35 years, 74.8% for 36-40 years, and 75% for >40 years).

However, the low oocyte maturity rate was increased with advancing female age ($p=0.001$) linearly (4.6%, 5.9%, 6.0%, and 7.9%), showing the highest rate in >40-year-old women.

Patients were grouped into three according to the count of total retrieved oocytes and M-II oocytes after denudation (Table 1).

The association with live birth ratio and the count of entire harvested and M-II oocytes is given in Figure 1.

Women who had more than 3 M-II oocytes available for fertilization (group 3) had considerably higher live birth ratio compared to those who had ≤3 M-II oocytes (group 1 and 2). Nevertheless, the live birth ratio was similar for women with ≤3 M-II oocytes regardless of the whole count of retrieved oocytes (group 1 and 2, respectively; 11.9 and 16.7%).

Figure 2 elucidates the association between live birth and oocyte maturity rates. Women who had ≤50% oocyte maturity rate have a significantly lower live birth ratio.

The live birth ratios according to the count of total and M-II oocytes concerning oocyte maturity rate are given in Table 2.

Live birth ratios were directly proportional to the count of both mature and entire harvested oocytes, being significantly higher for women with >3 M-II oocytes (group 3) ($p<0.01$). However, for a given count of M-II oocytes, the live birth ratio did not significantly differ according to the entire count of harvested oocytes, despite being lower in women with low oocyte maturity rates ($p>0.05$). The count of M-II oocytes was more predictive of a live birth than the whole count of retrieved oocytes.

Figure 3 shows ROC curves of the count of mature oocytes and the total count of collected oocytes for discriminating cycles resulting in a

Table 1. Patient groups according to the count of total retrieved oocytes and M-II oocytes

	Group 1	Group 2	Group 3
Total oocyte number	≤3	>3	>3
Count of M-II oocytes	≤3	≤3	>3

M-II: Metaphase-II

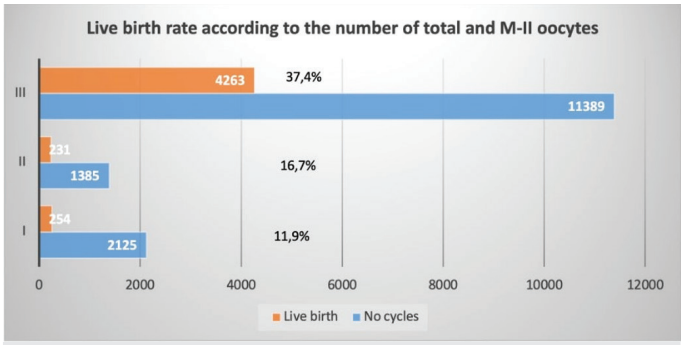

Figure 1. The live birth ratio according to the count of total harvested oocytes and M-II oocytes
M-II: Metaphase-II

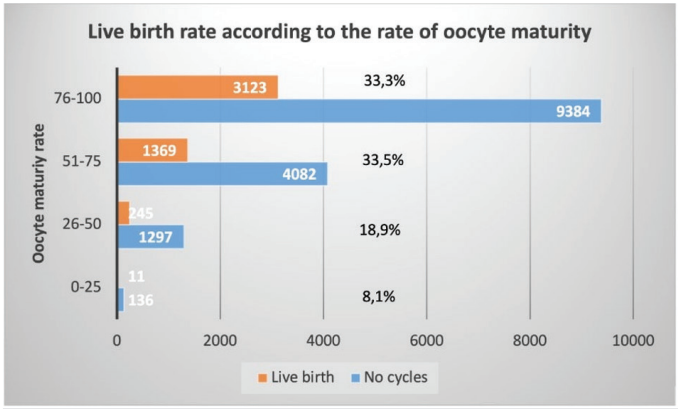

Figure 2. The live birth ratio according to the oocyte maturity rates

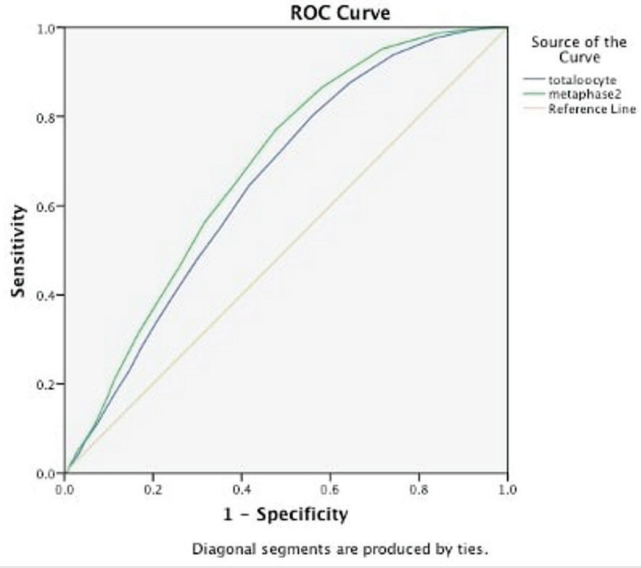

Figure 3. Receiver operating characteristic curves of the count of mature oocytes and the total count of collected oocytes for discriminating cycles resulting in live birth or non-live birth
ROC: Receiver operator characteristics

Table 2. Live birth ratios according to the count of M-II and total harvested oocytes

No M-II oocytes	Total harvested oocytes	Count of cycles	Embryo transfer cycles	Live birth (% per cycle)
1	≤3	957	589	52 (5.4%)
1	>3	90	42	4 (4.4%)
2	≤3	826	770	118 (14.3%)
2	≥4	376	309	38 (10%)
3	3	342	337	84 (24.6%)
3	4-5	651	639	138 (21.2)
3	≥6	268	247	51 (19.0%)
4	4-7	989	975	332 (33.6%)
4	≥8	110	106	30 (27.3%)
≥5	≥5	10,290	10,118	3,901 (37.9%)

M-II: Metaphase-II

live birth. In the prediction of live birth; the area under the ROC curve was 0.684 [95% confidence interval (CI): 0.671-0.697] for the count of mature oocytes and 0.653 (95% CI: 0.639-0.666) for the entire count of retrieved oocytes. These results showed that the count of M-II oocytes was better predictive of live birth than the entire count of harvested oocytes (p=0.0007).

Discussion

Marked discrepancies in follicular size during controlled ovarian stimulation would be damaging to pregnancy success since a considerable fraction of oocytes in the cohort will fail to complete the maturation process. In cases with low oocyte maturity; the count of M-II oocytes rather than the entire count of oocytes determines the patient's prognosis. Asynchronous follicle growth and subsequent low oocyte maturity are associated with a poor clinical outcome. The prognosis is similar to the Bologna criteria, defining poor responders if only ≤3 M-II oocytes are available within a pool of ≥3 retrieved oocytes. As the Bologna criteria fail to cover this group of patients, we suggest that this group of patients should be counseled accordingly.

Throughout controlled ovarian stimulation, the majority of the early antral follicles are expected to grow synchronously in reply to exogenous gonadotropins. However, 15-30% of recovered oocytes are reported to be immature (7-10). In our study group, 26% of the harvested oocytes were immature after denudation. Although these immature oocytes can be matured in vitro and fertilized, the derived embryos had a lower post-implantation developmental potential (11-13) and a higher incidence of cytogenetic abnormalities (14). In contrast to the physiological selection of the dominant follicle in a spontaneous cycle, ovarian hyperstimulation may lead to the rescue of follicles harboring intrinsically abnormal oocytes that would otherwise be destined to undergo atresia (15).

Immature oocytes may also stem from small antral follicles during oocyte retrieval or from large preovulatory follicles that do not respond adequately to hCG. A robust rate of oocyte immaturity was reported to be linked with ovarian stimulation protocols (8,9,16,17), inadequate timing, dose or activity of hCG (18), and early follicle aspiration (19). In contrast, we did not find a difference between stimulation protocols regarding oocyte maturity rate.

Asynchronous follicle growth and a higher rate of immaturity among retrieved oocytes are more frequently encountered in women with diminished ovarian reserve. A plausible explanation for this finding might be the earlier start of development of antral follicles due to the stimulatory effect of higher FSH levels during the late luteal phase of the previous cycle (20). We did not find a correlation between the overall rate of oocyte immaturity and female age. However, a low oocyte maturity rate was linearly correlated with advancing female age.

The Bologna criteria were proposed by the ESHRE Working Group on Poor Ovarian Response considering the entire count of oocytes retrieved as the major determinant to define a poor responder in addition to female age and ovarian reserve tests (1). According to these criteria, women who had ≤3 oocytes in one or two treatment episodes of ovarian stimulation (according to her age group) were poor responders. Several concerns have been raised about the design, reliability, applicability, and prognostic value of these criteria (21-25). Our findings will add further criticism to the Bologna criteria since the use of total count of oocytes as a criterion overlooks the group with asynchronous follicle growth. In cases with a limited count of growing follicles, the count of mature oocytes rather than the total count of oocytes will determine the prognosis of the patient.

Study Limitations

The retrospective design of our study was limitation. Although clinical heterogeneity within the dataset may be noted a drawback, such differences enhance the generalizability of our findings. Since female age was closely related to the chance of live birth, a post-hoc stratification according to different age groups was performed in the analysis phase. Since only ICSI cycles were included in the study, our findings cannot be translated into IVF cycles where oocyte maturity is not assessed before incubation with spermatozoa. However, indications for ICSI are becoming less stringent, and it is used more often, particularly in cycles with a low oocyte yield, exceeding 50% of all ART cycles in the U.S. (26).

Conclusion

Asynchronous follicle growth is a vaguely defined terminology. Much of the available literature includes a heterogeneous group of women, only some of whom have the condition of interest. Studies on risk

factors, diagnosis, management, and prognosis are hampered by a lack of uniform definition, either clinical or laboratory. We propose that oocyte maturity rate and the count of M-II oocytes as two diagnostic criteria for the case definition of asynchronous follicle growth. Based on our findings, stimulation cycles ending with low oocyte maturity rate (≤50%) and ≤3 M-II oocytes would be considered of asynchronous follicle development. Patients with low oocyte maturity rate and asynchronous follicle development should be counseled and informed regarding potential poor prognosis of the treatment.

Ethics Committee Approval: The Koç University Faculty of Medicine Local Research Ethics Committee authorized the count of total and M-II oocytes (approval number: 2022.132.IRB1.048, date: 05.04.2022).

Informed Consent: Our study design was retrospective; therefore, informed consent was not obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.E., K.Y.; Concept: K.Y.; Design: K.Y.; Data Collection or Processing: S.E.; Analysis or Interpretation: K.Y.; Literature Search: S.E.; Writing: S.E., K.Y.

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

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

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İstanbul Med J 2022; 23(3): 220-3
DOI: 10.4274/imj.galenos.2022.37029

Evaluation of Anxiety Symptoms in Patients with Chronic Obstructive Pulmonary Disease

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ABSTRACT

Introduction: Mood disorders are frequently seen in the patients with chronic obstructive pulmonary disease (COPD). The literature lacks the frequency and effects of the anxiety disorder, which affects daily life and success of the treatment. In the present study, we evaluated the anxiety levels of patients with COPD.

Methods: The relatives diagnosed with COPD of the patients who applied for psychiatric examination as out-patients to our clinic were included in the study between January 2018 and December 2021. Eighty patients were randomly selected for the COPD group. The fourty-eight volunteer relatives of the patients were included in the control group. The two Spielberger State and Trait Anxiety Inventory subscores-state (STAI-S) and trait (STAI-T)- were used to examine anxiety levels of the patients with COPD and control groups.

Results: There were 48 (60%) men and 32 (40%) women in the COPD group and 24 (50%) men, 24 (50%) women in the control group. The mean scores of Spielberger State Anxiety Inventory and Trait Anxiety Inventory were significantly higher in the COPD group when compared control group [STAI-S COPD group (n=80), mean score: 43.384, standard deviation (SD): 8.68 versus control group (n=48), mean score: 36,232, SD: 7.64; $p<0.05$] [STAI-T COPD group (n=80), mean score: 44,128, SD: 6.168 versus control group (n=48), mean score: 36,344, SD: 8.188; $p<0.05$, respectively].

Conclusion: The anxiety symptoms in the patients with COPD should be considered by physicians and should be appropriately assessed when diagnosed with COPD for not only the effects on treatment compliance but also on mortality, disability and quality of life. We believe that psychiatric aid is important in patients with COPD, particularly in handling anxiety, so that the anxiety-dyspnea vicious cycle will be easier to break with insensitive behavior therapies.

Keywords: Chronic obstructive pulmonary disease, anxiety, mood disorder

Introduction

Chronic obstructive pulmonary disease (COPD), manifested by coughing, shortness of breath, sputum production, wheezing, and reduced physical performance. COPD is one of the most frequently diagnosed and also most fatal lung diseases, approximately 400 million people suffering from this disorder (1). However, some symptoms are indirectly related to respiratory disorders in patients with COPD. The number of cases is systematically growing, and the World Health Organization predicts that by 2030 COPD may become the third leading cause of death worldwide.

COPD, as a chronic disease, it is common to worsen patients' quality of life, to interfere with the perception of other symptoms even it may be a cause of mortality (2-4). Women population has a higher number of deaths than men from COPD in the United States since 2000 (1). Between 1999 and 2014, age-adjusted mortality rates of men because of COPD has decreased; however, it was stable in women (1).

Patients with COPD show symptoms of depression and anxiety more frequently than the general population (2,3). The prevalence of anxiety and depression in patients with COPD is high. It is estimated that one in four people with COPD has symptoms of depression and anxiety. Depression and anxiety are frequent co-morbidities in patients with COPD, with an estimated prevalence of 8-80% and 2-96%, respectively (5,6). Many patients with COPD suffer from anxiety that affects daily life and it worsens the relationships with family members and working life. Besides the anxiety about dyspnea may affect sexual life. Anxiety, itself, causes hyperventilation, increases the feeling of dyspnea and as a result, it creates a vicious circle (7). Physical activity is reduced, the frequency of exacerbations increases and so does the use of health resources and causes high levels of psycho-social distress. Clinical anxiety has also been recognized as a significant problem in COPD, with an estimated prevalence of up to 40% (8,9).

Shortness of breath and fear of death in patients with COPD can lead to acute catastrophic anxiety that forces all the mental strength of the patient. Various behavioral changes such as anxiety, depression, discomfort and psychological defenses such as denial and avoidance, can be seen in these patients (10). It was suggested that symptoms of

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Cite this article as: Güzelhan Y. Evaluation of Anxiety Symptoms in Patients with Chronic Obstructive Pulmonary Disease. İstanbul Med J 2022; 23(3): 220-3.

Received: 31.01.2022
Accepted: 28.07.2022

dyspnea often lead to anxiety. Various risk factors have been identified for developing anxiety and depression. In addition to depression is associated with chronic stress, which leads to sustained activation of the sympathetic nervous system and an increase in the systemic inflammatory response. The most common psychological pathology in patients with COPD is anxiety and depression disorder (11). The patients avoid even the slightest physical activity because of fear of developing shortness of breath (10).

In the present study, it was aimed to evaluate the anxiety levels of patients suffering from COPD.

Methods

This study approval by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Ethics Committee (approval number: 167, date: 20.05.2022). Informed consent was obtained.

The relatives of patients who applied for psychiatric examination diagnosed with COPD of the patients by the lung disease clinics who as out-patients to our clinic were included in our study. The study was conducted between January 2018 and December 2021. Ninety patients were selected by random sampling and the study was completed with 80 patients. The fourty-eight volunteer relatives of the patients were included in the control group. The subtypes of COPD were not considered in our study.

The study was based on a questionnaire examination. Anxiety is a common mental health problem and is associated with physical and psychological discomfort. All the anxiety disorders share common symptoms, such as fear, anxiety, and avoidance. Other anxiety-related symptoms include fatigue, restlessness, irritability, sleep disturbances, reduced concentration and memory, and muscle tension. Among the anxiety disorders, the most common are specific or social phobias and generalized anxiety disorder. The participants completed the proprietary State-Trait Anxiety Inventory (STAI) which was designed to diagnose anxiety symptoms questionnaire.

Evaluation of Anxiety

The two STAI subscores- state (STAI-S) and trait (STAI-T)- were designed to diagnose generalized anxiety symptoms. Likert type answers are rated on a fourpoint scale of how well they describe the patient's current or typical mood, from "not at all" to "very much."

The STAI consists of two 20-item scales that measure "state" (current) and "trait" anxiety (general). The STAI tests diagnose the level of anxiety and distinguish it from depressive syndromes. The two STAI sub-scores ranged from 20 to 80, with higher scores indicating more severe symptoms and greater anxiety.

The Spielberger State and Trait Anxiety Inventory was used to examine anxiety levels in both the COPD patient group and control group. We accepted STAI scores <40 to indicate no or minimal symptoms and ≥40 to indicate moderate or severe symptoms. A range of demographic and clinical factors were collected as potential determinants of outcome.

Statistical Analysis

Statistical analysis was performed using the statistical software SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Data are expressed as mean standard deviation for continuous variables and as numbers with percentage. The evaluation of non-parametric data (discrete variables) was compared using the χ^2 test, and the evaluation of parametric data (continuous variables) were compared using a two-tailed t-test. A p-value of 0.05 or less was accepted to be significant.

Results

There were 48 (60%) men and 32 (40%) women in the COPD group and 24 (50%) men, 24 (50%) women in the control group. While 67 (84%) patients were smokers in the COPD group, it was 26 (53%) in the control group.

When the Spielberger State Anxiety Inventory was compared between COPD and control group; the mean score was found to be significantly higher in the COPD group [COPD group (n=80), mean score: 43.384, standard deviation (SD): 8.68; control group (n=48), mean score: 36,232, SD: 7.64; p<0.05] (Table 1).

When the Spielberger Trait Anxiety Inventory was compared between COPD and control groups; there was found a statistically significant difference between the mean scores of the two groups [COPD group (n=80), mean score: 44,128, SD: 6.168; control group (n=48), mean score: 36,344, SD: 8,188; p<0.05] (Table 1).

Table 1. Comparison of individuals with the COPD group and control group in terms of Spielberger State and Trait Anxiety Inventory scores

	COPD group (80 pts) (n, %)	Control group (48 pts) (n, %)	p
Age			
Mean (year)	52.3±8.3	57.4±6.1	ns
Gender			
Male	48 (60%)	24 (50%)	ns
Female	32 (40%)	24 (50%)	ns
Education level			
Mean (years)	8.125±2.2	7.850±2.9	ns
Primary school and lower	16 (20%)	9 (18.75%)	ns
Secondary school	40 (50%)	25 (52.08%)	ns
High school	16 (20%)	9 (18.75%)	ns
University and higher	8 (10%)	5 (10.42%)	ns
Smoking history			
Smoker	67 (84%)	26 (53%)	p<0.05
Anxiety (STAI)			
STAI-S	43.384±8.680	36.232±7.640	p<0.05
STAI-T	44.128±6.168	36.344±8.188	p<0.05

COPD: Chronic obstructive pulmonary disease, STAI: State-Trait Anxiety Inventory, STAI-S: State-Trait Anxiety Inventory "state" (current), STAI-T: State-Trait Anxiety Inventory "trait" (chronic)

Discussion

The anxiety in many patients with COPD is reported to have a negative impact on the patient's family and professional life, increasing the dyspnea as a vicious cycle (2). It is emphasized that most commonly anxiety disorders are seen in patients with COPD, and that the most important factor is the dyspnea itself and the fear of suffocation and death, and that fear of shortness of breath causes the patient to avoid social, professional and physical activities, thus affecting the patient's recovery negative (7,10).

The incidence of anxiety, depression and panic are more common in patients with COPD than in the normal population. These disorders may increase the mortality rate of patients and the risk of acute exacerbation worsen the quality of life and prognosis of patients (12).

In a study by Yohannes et al. (13), the efficacy of pulmonary rehabilitation and behavioral therapy used in patients with COPD and other co-modality activities were examined. Pulmonary rehabilitation and cognitive behavioral therapy have been reported to reduce both anxiety and dyspnea symptoms in patients with COPD (13). Janson et al. (14) reports that there was no statistical difference between patients with respiratory systems in terms of depression and anxiety, but there are often significant psychiatric problems in respiratory patients. Yellowlees (15) reported that the patients with ongoing airway blockages had psychiatric problems with 58% rate, and panic disorders and other anxiety disorders were the most common pathologies. In a review by Volpato et al. (16), depression is common in patients with COPD and has a negative effect on treatment. The review has focused on the relationship between anxiety, depression, and compliance in patients with COPD (16). patients with COPD are ten times more likely to experience panic disorder or panic attacks compared with general population samples (17). Moreover, depression and anxiety interfere with other risk factors, such as tobacco use, and, in general, they impair patients' quality of life (18,19). Patients with COPD need support for managing symptoms and medication also identifying anxiety and depression. Comorbid depression and anxiety in COPD are associated with a disproportionate increase in functional disability, work absence, healthcare usage rates and costs (20). These co-morbidities complicate the therapeutic approach and increases hospitalizations and health expenditures. The literature lacks about the frequency and effect of the anxiety on the patients with COPD.

In our study, we found both state and trait anxiety levels to be statistically higher in patients with COPD compared to the control group. The increased level of anxiety in these patients may cause psychophysiological function changes and cause the patient to experience their existing complaints more severely, while also causing complaints related to other systems. The hormonal system may be more affected, especially due to increased anxiety levels. Additionally, increased anxiety level may lead to depression secondary to anxiety after a while in these patients.

The potential impact of psychological problems in compliance with COPD treatment should be considered by physicians and should be appropriately assessed when diagnosed with COPD for not only the effects on treatment compliance but also on mortality, disability and quality of life. We also investigated the influence of psychological factors on cardiac surgical outcomes and to evaluated the hypothesis that symptoms of anxiety are associated with adverse clinical outcomes who undergoing elective open heart surgery (21,22).

We believe that psychiatric aid is important in the monitoring of patients with COPD, especially in handling anxiety, and that it is beneficial to confront the patient in a specific program with fear and dyspnea and to apply insensitive behavior therapies, so that the anxiety-dyspnea vicious cycle will be easier to break and the patient may be more motivated to treatment.

Conclusion

The patients with COPD are recommended to evaluate the anxiety symptoms using STAI scores clinically. More patients are needed to evaluate more precisely the long-term effects of anxiety in COPD.

Ethics Committee Approval: This study approval by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Ethics Committee (approval number: 167, date: 20.05.2022).

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

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

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İstanbul Med J 2022; 23(3): 224-8
DOI: 10.4274/imj.galenos.2022.01212

Septal Deviation in Newborns: A Prospective Study and Literature Review

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ABSTRACT

Introduction: In this study, our aim is to investigate the frequency of nasal septum deviation in newborns and the reasons for the emergence of these pathologies; then to follow and determine the results in the following year and review the literature.

Methods: Three hundred and seventy-two babies of the mothers between the ages of 14 and 45, including the mothers who gave birth in a hospital and migrated from Syria, were included in the study. The sex of all babies, birth weight, head circumference, presence of nasal septum deviation, as well as the age of all mothers, gestational period, delivery method (normal vaginal birth/cesarean birth), and the number of births was determined. Nasal septa of the infants with some nasal septal deviations were reposed, and follow-up results were reported.

Results: Among all 372 newborn babies, 210 of them (56.4%) gave birth with vaginal delivery, and 162 of them (43.6%) with cesarean delivery. The nasal septum deviation was detected in 45 of the 372 newborn babies (12%). A closed reduction was performed using a nasal septal elevator. Because of the 12-month follow-up case, it was observed that the deviation in the nasal septum showed improvement in 32 babies. In conclusion, no statistically significant difference was found between neonatal nasal septum deviation and maternal age, gender ($p>0.05$). However, a statistically significant difference was found between neonatal nasal septum deviation in the form of birth, the number of births, pregnancy duration, weight, head circumference ($p<0.05$).

Conclusion: It is necessary to have an early diagnosis and intervention for nasal septal deviations in newborn babies. To prevent nasal obstruction and permanent impairment, all newborn babies must be examined by an ENT specialist immediately after birth.

Keywords: Nasal septum, newborn, infant

Introduction

The causes of nasal deformities in infants may be congenital malformations, intrauterine pressure, and fetal malposition, of which, the most common cause is the minor traumas during childbirth. Most of the simple deformities are resolved spontaneously in a short time; however, some of them are not resolved in time and need to be corrected by closed reduction (1). There are two types of nasal deviations in newborns: The nasal septal dislocations that can be replaced replicated in the midline by manipulation and those that cannot be replicated (2).

The nasal septum deviation usually occurs because of mild or severe trauma in the face, but in some cases, it does not necessarily have any facial trauma in the face (3). Although trauma during child birth is an important factor, other intrauterine factors may lead to this deformity (4).

The diagnosis of nasal septal dislocation should be made as early as possible after delivery and should themselves. Various hypotheses have been claimed to explain the etiology of nasal septal dislocation in patients without a history of trauma; however, there are no definitive finding.

Intrauterine pressure, strain in newborns, strain at the first stage of birth in prim parous are the factors affecting nasal septal dislocations. When the nasal septum deviation is detected at birth, the nose should be immediately corrected to prevent nasal obstruction and permanent impairment. This is the main reason why it is so crucial to diagnose nasal septum dislocation at an early stage. The nasal deviation should be distinguished from the temporary flattening of the nose, which appears during childbirth and heals spontaneously in time. For definitive diagnosis, the nasal septum dislocation is felt with the porthole inserted along the base of the nose, and both nasal passages are examined with an otoscope (5).

It is recommended to use the Metzenbaum sign (asymmetry of the nostrils) and the test of Jeppesen and Windfeld (pressure at the tip of the nose) together with rhinoscopy. The diagnosis and treatment of nasal septum deviation are of great importance (6).

According to Stoksted and Schønsted-Madsen (7), there are 3 types of nasal deformities in newborns: 1) Self-healing-fixed deviations caused by trauma in the prenatal period, 2) repository deviations, 3) pressure deformities requiring treatment.

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Cite this article as: Erdoğdu S. Septal Deviation in Newborns: A Prospective Study and Literature Review. İstanbul Med J 2022; 23(3): 224-8.

Received: 22.04.2022
Accepted: 07.08.2022

According to Sooknundun et al. (8), communication between a pediatrician, an ENT, and an obstetricians is important for early detection and treatment of nasal septal deviation. The incidence of nasal septum deviation varies between 0.08% and 23% in newborns reported in the literature (9).

Methods

This was a prospective study, included the mothers who gave birth in the Beykoz State Hospital between October 2018 and March 2019 and the newborn, including the Syrian immigrant child-mothers under 18, and discussed the findings obtained after 12 months follow-up. Nasal examinations of newborn babies were performed with a Karl-Storz 0 degrees 2.7x110 mm optic otoscope. When moderate curvature was seen in the nasal septum, the Cottle elevator was used to direct the nasal septum toward the midline without anesthesia in the first three days after birth. The elevator was placed in the nose and the nasal septal cartilage was placed in the groove of the vomer. Sometimes a clicking sound is heard when replacing the nasal septum and the nasal septum position is felt to improve. Follow-up examinations were performed on the second day after the reduction, one month later and then twelve months later. The mothers of the newborn were informed about the possibility of unsuccessful repositioning and spontaneous flattening in the following months, and their written consent was obtained. The sex, birth weight, head circumference, presence of nasal septum deviation and deformation of all babies and the age, gestational period, delivery method (normal vaginal birth/cesarean birth), and the number of mothers were recorded.

The study was approved by the Beykoz State Hospital Ethics Committee (approval number: 45, date: 18.07.2018) and the written informed consent was obtained from the parents of all patients.

Statistical Analysis

Data were analyzed with SPSS 15.00 package program. Percentage, mean, standard deviation (SD), and chi-square tests were used in the analysis of the data. Statistical significance was set as a statistically significant $p<0.05$ in the study.

Results

In the hospital, the birth outcomes included mothers aged 14 to 45 (mean age: 28.59±5.53), including children who migrated from Syria, and the findings of 372 newborn babies were examined. It was determined that 210 (56.4%) of 372 newborn babies were born with vaginal delivery and 41 of these were difficult births. 162 (43.6%) of the remaining births were delivered through cesarean section. Fifty-two of them were emergency cesarean. The maximum number of deliveries by mothers was 8, and most of them being 2 deliveries. Births after 39-40 weeks of gestation were more common. The weight of the newborn babies ranged from 1735 to 4800 g. The babies born at a weight of 3000-3500 g made up the majority. There were 24 babies with a birth weight of more than 4000 g. The nasal septum deviation was detected in 45 (12%) babies in total, particularly in difficult and large births (Table 1).

When the findings were compared statistically: There was no statistically significant difference between the formation of neonatal septum deviation and maternal age, gender ($p>0.05$). In the study, the presence of deviation was found in 26 patients with difficult vaginal deliveries. The head circumference was found to be 34-36 cm in 25 newborns. The neonatal septum deviation seen in first births is more common. When the head circumference is large, neonatal septum deviation is more common. A statistically significant difference was found between the formation of newborn deviation and form of birth, number of births, pregnancy duration, weight, head circumference of the newborn ($p<0.05$) (Table 2).

In the study, 1 twin birth was detected in 371 pregnant women with age ranging from 14 to 45 years (mean: 28.59±5.53). The number of births ranged from 1 to 8 (1.98±0.98). While the gestational period is 33-42 weeks (38.96±1.28) the weight of newborn babies ranged from 1735 to 4800 gr (3337.43±434.77), and the head circumference from 28 to 39 cm (34.71±1.42) (Table 3).

The nasal septum deviation was observed in babies born with emergency and elective cesarean section. Because of the 12-month follow-up of these babies, 32 nasal septum deviations were improved. The remaining babies were considered to be operated at an advanced age and their follow-up would be kept in time.

Discussion

The development of the nose is completed in the 16th week of intrauterine life. After that time on, the nose is susceptible to all kinds of trauma. According to the general belief, treatment should be performed a few days after birth because babies tolerate interventions better in the early period than 1-2 weeks later. Anesthesia was not required. The nose i5s pulled upward by the gauze with the thumb and forefinger from the dorsum. The tip is inserted into the nasal passage with dislocation along the base. The elevator is lifted or rotated along its axis and seated in the nasal septum groove. During this time, a clicking sound is heard (5).

It is critical to detect nasal septal dislocation immediately after birth. It does not require treatment and should be distinguished from the temporary nasal flattening, which is caused during delivery and improves spontaneously. Although the external nasal deformity causes both cosmetic and psychological problems, internal deformity in the nasal cavity results in a significant discomfort in the respiratory system. It is observed that humidification deteriorates, nasal airflow decreases, scab and snoring increase, sinusitis occurs, a tendency to upper respiratory tract infection grows, and impaired tooth development occurs (4).

Deformities of the nose and nasal septum are divided into 3 groups: 1) Flattened nose that does not require treatment, 2) cartilage nasal septum subluxation, 3) combined deformities. They have reported that neonatology and otolaryngology specialists should have an early diagnosis and early treatment together (10).

While the frequency of neonatal nasal septum deviation in babies who have spontaneous births has a rate of 22%, Kawalski and Spiewak (11) found that the frequency of neonatal nasal septum deviation in babies by cesarean section at a rate of 3.8%.

Brain (12), said that nasal septum deviations occur in prenatal, newborn, and postnatal periods. Newborn deviations were first reported by

Table 1. Parameters of neonatal septum deviation

		No deviation		Deviation			
				Mild		Moderate	
		n	%	n	%	n	%
Maternal age (years)	14-19	11	2.96	3	0.81	1	0.27
	20-24	73	19.62	8	2.15	2	0.54
	25-29	104	27.96	6	1.61	3	0.81
	30-34	96	25.81	8	2.15	3	0.81
	35-39	32	8.60	4	1.08	6	1.61
	40-45	11	2.96	0	0.00	1	0.27
Form of birth	Vaginal normal	161	43.28	5	1.34	3	0.81
	Vaginal hard	15	4.03	18	4.84	8	2.15
	Cesarean emergency	43	11.56	4	1.08	5	1.34
	Cesarean elective	108	29.03	2	0.54	0	0.00
Number of births	1.00	102	27.42	13	3.49	10	2.69
	2.00	146	39.25	12	3.23	2	0.54
	3.00	59	15.86	3	0.81	4	1.08
	4.00>	20	5.38	1	0.27	0	0.00
Pregnancy duration	33-35 weeks	5	1.34	0	0.00	0	0.00
	36-38 weeks	116	31.18	6	1.61	2	0.54
	39-40 weeks	174	46.77	19	5.11	13	3.49
	41 weeks >	32	8.60	4	1.08	1	0.27
Gender	Female	163	43.82	17	4.57	9	2.42
	Male	164	44.09	12	3.23	7	1.88
Weight (gr)	2000<	2	0.54	0	0.00	0	0.00
	2001-2500	9	2.42	0	0.00	0	0.00
	2501-3000	62	16.67	2	0.54	2	0.54
	3001-3500	151	40.59	12	3.23	4	1.08
	3501-4000	91	24.46	9	2.42	4	1.08
	4001-4500	11	2.96	4	1.08	6	1.61
	4500>	1	0.27	2	0.54	0	0.00
Head circumference (cm)	28-30	2	0.54	0	0.00	0	0.00
	31-33	55	14.78	5	1.34	0	0.00
	34-36	245	65.86	14	3.76	11	2.96
	37-39	25	6.72	10	2.69	5	1.34
Nasal septal deviation	Normal	327	87.90	0	0.00	0	0.00
	Right	0	0.00	16	4.30	5	1.34
	Left	0	0.00	13	3.49	11	2.97

Metzenbaum in 1936. The reason for these deviations has been linked to developmental factors and a trauma that occur both at birth and during pregnancy. After all: 1) Nasal septum deviations occur at a rate of 3% in the newborn. 2) The two most common causes are developmental factors and birth trauma.

Na et al. (13) found that the rate of neonatal nasal septum deviation was 11.5% in 131 newborn babies. There was no statistically significant difference between these and pregnancy conditions. As a result, they said that nasal trauma is likely to occur in birth and pregnancy during nasal septal deviation. In the absence of postpartum trauma, the nasal septal deformity is thought to be caused by congenital etiology, or a trauma either during intrauterine life or during the passage through the birth canal. There is no difference in the frequency of nasal septal deformity between normal delivery and cesarean delivery (6).

Al-Amro (14) carried out a study on 130 newborn babies, of which 67 (51.5%) were unattended normal birth, and 63 (48.5%) were delivered by cesarean. No statistical significance was found between the nasal septal deviation and delivery method. He concluded that, in uncomplicated cases, the mode of delivery is not the cause of nasal septal deviation.

When Saim and Said (15) examined 674 newborn babies, they detected nasal septal deformity in 147 (21.8%) of the babies. In the prevalence of this, they found that the mode of delivery and the difficulty of delivery

Table 2. Evaluation of septum deviation in newborns

		Deviation		p
		Yes	No	
Maternal age (years)	≤24	14	84	0.109
	25-29	9	104	
	30-34	11	96	
	≥35	11	43	
Form of birth	Vaginal normal	8	161	0.000
	Vaginal hard	26	15	
	Cesarean emergency	9	43	
	Cesarean elective	2	108	
Number of births	1.00	23	102	0.008
	2.00↑	22	225	
Pregnancy duration	≤38 weeks	8	121	0.037
	39-40 weeks	32	174	
	≥41 weeks	5	32	
Gender	Female	26	163	0.318
	Male	19	164	
Weight (gr)	≤3000	4	73	0.000
	3001-3500	16	151	
	3501-4000	13	91	
	4001-4500	12	12	
Head circumference (cm)	≤33	5	57	0.000
	34-36	25	245	
	≥39	15	25	

Chi-square test, ↑: ≥2

Table 3. Evaluation of the parameters in the study

	Minimum	Maximum	Mean ± SD
Maternal age (year)	14.00	45.00	28.59±5.53
Number of births	1.00	8.00	1.98±0.98
Gestation period (week)	33.00	42.00	38.96±1.28
Newborn weight (gram)	1735.00	4800.00	3337.43±434.77
Head circumference (cm)	28.00	39.00	34.71±1.42

SD: Standard deviation

was statistically insignificant. They recommended routine screening to reduce the morbidity associated with this disease and to correct it early.

Alpini et al. (16) said that the etiology of the congenital deviations of newborn babies in the nasal septum is still controversial. They reported that the age was unimportant in the occurrence of neonatal nasal septum deviation, but the number of births and the duration of the tram was significant. The incidence of newborn according to birth weight: In the current series, it has been observed that the incidence of nasal septum deviation increases increasing birth weight. No statistically significant relationship was observed between the weight of the newborn and nasal deformities. Nasal septal deviation incidence according to the mother' sparity: In this series, it has been observed that the incidence of DNS is highest in the primipara (48%) and decreases as the parity increases (4).

The study has been conducted on 423 babies; 315 of them were natural births, and 108 were cesarean births, including 10 pyramid deformations and 12 nasal septal deviations. As a result, they reported that age was unimportant, but the number of births and the duration of the tram were significant (16). In this study, it was found that maternal age, gestational period, and number of births given was unimportant, but newborn weight and head circumference was significant.

Bhattacharjee et al. (17) prospectively found the incidence of SD to be 14.5% in a 1-year follow-up of 200 babies. The vaginal delivery rate was 55%, delivery with forceps was 24%, and cesarean delivery was 21%. They found that 55% of babies with SD had higher birth weights and 48% of them had more frequent first births. Besides, intrauterine malposition was found to occur at a rate of 45%, particularly breech, and prolonged labor was also involved in the SD formation.

Approximately 1/5 of the babies were found to have nasal septal deformities at birth, and the frequency of deformity was prolonged with younger babies and it was high in difficult deliveries (18).

The nasal septum dislocation may cause intrapartum due to nasal septal trauma intrauterine pressure, but more commonly occurs during vaginal delivery. Most of the specified nasal septal deviations are mild and spontaneously resolve within 1 month after birth. The nose should be conservatively corrected using immediate nasal septal manipulation (19).

In their study on 273 newborns, Kawalski and Spiewak (11) found the nasal septum deviation in 22.2% of infants who were delivered spontaneously and this rate to was 3.9% in cesarean babies.

Kent et al. (20) detected nasal septal deformity in 29 (2.9%) of 1,000 babies. 44% of these babies were not excised, but after 5 months, the nasal septum recovered spontaneously. Korantzis et al. (21) detected the nasal septum dislocation at a rate of 17% of 447 newborn babies' research. 5% of them constitute birth by cesarean.

Patel and Carr (22) in his 34-case series, found a nasal septal deviation in 2 (6%) babies in which neonates investigated nasal obstructions, and nasal septal deviation would improve rapidly with the presence of maternal estrogens in newborns. The nasal septum is composed entirely of cartilage during the neonatal period. At the end of the 1st year, the ossification of the nasal septum begins and is completed at the end of the adolescent age (23).

Spiewak and Kawalski (24) included 254 normal births and 52 cesarean births and their newborn in their study. They detected deformations in the cartilage part of the nasal septum in 26 babies and the bone part in 6 babies. These babies were automatically reposed in 19 (73%) of 26 deformations in the following 3-4 weeks after birth. They said that all bone deformities remained the same.

When Collo (25) examined 1,030 newborn babies, they detected traumatic nasal septal deviations in 19 of them and corrected them without surgery.

Emami et al. (26) reported that closed manipulation of the nasal septum on the first l-2 days of a baby's life was performed by many

otolaryngologists with good results. However, the use of this technique is generally limited to subluxations of the anterior cartilage, which are diagnosed immediately after birth or within a very short time.

Pentz et al. (2) detected a nasal deviation in 110 (3.23%) of 3425 newborn babies. Eighty-one (2.37%) of these 110 babies were reclassified with closed repositioning. It was impossible to repose in the remaining 29 (0.86%).

Soboczyński et al. (27) researched 410 newborn babies, of which 297 were born with natural birth and 113 of them with a cesarean. 14.3% of the nasal septum deviation was detected. It was followed for 4-6 months; most of them were observed deviation.

Study Limitations

First, the short follow-up time limited this study. It will be more comprehensive if the nasal examination findings of childhood are included in the study. Second, there is a need for studies with larger study samples.

Conclusion

The nasal examination of newborns should be performed carefully, particularly in the first days after birth, traumatic nasal septum dislocations should be brought to their anatomical positions to avoid a more aggressive surgery in the future. All newborns should be routinely examined at birth to detect nasal septal deformity.

Ethics Committee Approval: The study was approved by the Beykoz State Hospital Ethics Committee (approval number: 45, date: 18.07.2018).

Informed Consent: The written informed consent was obtained from the parents of all patients.

Peer-review: Externally and internally peer-reviewed.

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

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İstanbul Med J 2022; 23(3): 229-35
DOI: 10.4274/imj.galenos.2022.44520

Neuroimaging Findings in Hemifacial Spasm: A Single-Center Experience

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ABSTRACT

Introduction: In this study, we documented the demographic, etiological, clinical and radiological features of our patients with primary hemifacial spasm (HFS). We also wanted to emphasize that there may be an association between idiopathic intracranial hypertension (IIH) and HFS.

Methods: Fifty-five patients diagnosed with HFS (28 women) who were followed up in the Movement Disorders Outpatient Clinics of the Department of Neurology University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital between January 2017 and January 2022 were included in this study. Demographic, clinical, and radiological findings were retrospectively reviewed. Depending on radiological findings, patients were divided into three groups: a) Normal findings, b) Incidental findings that did not appear to be related to clinical findings, and c) vascular abnormalities at the level of the brainstem.

Results: Only 23 patients had no atherosclerotic risk factors. While magnetic resonance imaging of the brain was normal in 23 patients, 19 patients had ischemic white matter changes, 5 patients had partial empty sella, 7 patients had dolichoectatic basilar artery, and 1 patient had a compression of the anterior segment of the left superior cerebellar artery to the 7th cranial nerve. Based on the history and clinical findings, lumbar puncture was performed in 4 patients, and 3 of them were diagnosed with idiopathic IIH with HFS, and they were treated with acetazolamide. Fifty-one patients were treated with botulinum toxin injections only.

Conclusion: Vascular compression is often noted on imaging of patients with primary HFS, but as in our case series, an empty sella finding in patients with chronic headache may be a sign of IIH and should not be overlooked. Also, HFS may be an uncommon presentation of IIH, and symptoms of HFS may improve with treatment of IIH. Additionally, the presence and history of Coronavirus disease-2019 infection should be questioned in newly admitted cases.

Keywords: Hemifacial spasm, brain magnetic resonance imaging, empty sella, intracranial hypertension

Introduction

Hemifacial spasm (HFS) is a hyperkinetic movement disorder characterized by involuntary, arrhythmic, painless, clonic, or tonic intermittent spasms on one side of the face that negatively affects the patient's daily life (1). There are two forms of HFS, primary and secondary. Primary HFS is more common in women than in men (1.5:1 ratio), and the mean age at onset is about 45-52 years, although the range is wide. 14.5 in 100,000 women and 7.4 in 100,000 men have HFS. Most cases occur sporadically, but there are some familial cases (2,3). Secondary HFS often occurs after peripheral facial paralysis, and less commonly because of facial nerve or brainstem damage after tumors, demyelinating diseases, trauma, and infections (3).

There are two theories regarding this pathogenesis. The first theory is that a phase or false synapse forms in the area of demyelination caused by compression. Other theory states that the abnormal signals originate from the nucleus of the facial nerve, which has rearranged itself due to the disorganized afferent information (2). Primary HFS is thought to be caused by vascular compression of the facial nerve at the level of the nucleus and in the regions where it exits the nucleus (4). Magnetic resonance imaging (MRI) is an important tool to exclude secondary etiologies and to demonstrate vascular compression in HFS (5).

Information on HFS comes from clinical, neurosurgical observations, and electrophysiological studies, although the exact cause is still unknown (6). The generally accepted view of the development of HFS is that compression of a vascular structure adjacent to the entry point of the facial nerve root and the focal demyelination resulting from this compression causes spasms the ephaptic transition. Such structures, thought to cause compression, can sometimes be visualized radiologically, but they are also sometimes not visible during surgical procedures or even autopsy (7-9).

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Cite this article as: Eyigürbüz T, Yıldırım Z, Korkut E, Akalın Akkaş E, Adatepe MT, Kale N. Neuroimaging Findings in Hemifacial Spasm: A Single-Center Experience. İstanbul Med J 2022; 23(3): 229-35.

Received: 20.06.2022
Accepted: 07.08.2022

Although in many patients brain imaging is unremarkable, some patients have non-specific findings, and in some patients, lesions that appear to be the cause of HFS can be detected by imaging. These lesions are vascular abnormalities in the vicinity of the facial nerve, as well as less common tumors and structural changes in this area. In some cases, mostly in middle and old age, incidental lesions far from the facial nerve region may be detected, such as cortical infarcts (7,9-14). Surgical microvascular decompression and botulinum toxin injections as symptomatic treatment are the mainstay of treatment for primary HFS (4).

This cross-sectional study documented the demographic, clinical, and radiological findings of patients with HFS. Additionally, this article discusses the very rare HFSs associated with intracranial hypertension (IIH).

Methods

The records of patients who were followed up in the Movement Disorders Outpatient Clinics of the Department of Neurology of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital between January 2017 and January 2022 were retrospectively reviewed. Fifty-five patients diagnosed with HFS were included in this study.

Demographic data such as age, sex, and comorbidities, including atherosclerotic risk factors (RF), as well as clinical data such as medications for HFS, MRI of the brain, and MRI angiography (MRA) of the brain or computed tomography angiography (CTA), and cerebrospinal fluid (CSF) results, when appropriate, were collected and recorded.

Hypertension, diabetes mellitus (DM), atrial fibrillation, coronary artery disease, heart failure, previous stroke, smoking, obesity, and dyslipidemia were the atherosclerotic RFs.

We classified RFs into 1 RF, 2 RFs, and >2 RFs. Because DM may result in cranial nerve involvement, the presence of DM was reported separately. Depending on radiological findings, patients were divided into three groups: a) Normal findings, b) Incidental findings that did not appear to be related to clinical findings, and c) Vascular abnormalities at the level of the brainstem.

According to a protocol approved by the Local Ethics Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 199, date: 17.06.2022), all participants gave written informed consent according to the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0 software (Armonk, NY: IBM Corp.). Normality of variables was analyzed using the Shapiro-Wilk test. The homogeneity of group variances was tested with Levene's test. The Mann-Whitney U test was used to compare continuous data that did not have a normal distribution. Pearson chi-square tests and Fisher's Exact tests were used to compare categorical data. Analysis results were expressed as the number of observations (n), percentage, minimum, maximum, mean, and standard deviation. A p-value < of 0.05 was considered statistically significant.

Results

Fifty-five patients with HFS were included in this study. 50.9% were female (n=28). The mean age was 58.16±9.7 years. 65.5% had left-sided HFS (n=36) and the remaining 34.5% (n=19) had right-sided HFS. The mean age was 57.5±9.3 and 59.5±10.6 years in the left HFS and right HFS groups, respectively. While men predominated in the left HFS group (n=19, 52.8%), women predominated in the right HFS group (n=11, 57.9%). There were no significant differences between the left and right HFS groups to age, sex, RFs, concomitant DM, history of chronic headache, disease duration, medications for HFS, and neuroimaging findings. Table 1 shows the findings of our patients.

One patient with left-sided HFS had otosclerosis and one patient with right-sided HFS had systemic amyloidosis. When asked about concomitant symptoms, one patient with right-sided HFS was found to have tinnitus and one patient with left-sided HFS was found to have cervical dystonia concomitant with HFS, whereas the other patients had isolated HFS symptoms.

We had a patient with HFS on the left side whose symptoms occurred after severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection. This patient, who had SARS-CoV-2 infection about two weeks before the onset of symptoms compatible with HFS and had not been vaccinated, also had pulmonary involvement but did not require hospitalization.

Brain MRI was normal in 41.8% patients (n=23). 34.5% of patients (n=19) had non-specific ischemic white matter changes, 9.1% of patients (n=5) had partial empty sella, and 12.7% of patients (n=7) had a dolichoectatic basilar artery. A patient had a compression of the anterior segment of the left superior cerebellar artery on the 7th cranial nerve, accompanied by partial empty sella. MRA and CTA were used only to confirm vascular abnormalities when necessary. MRA and CTA were used only to confirm vascular abnormalities when necessary. The results of these examinations were consistent with MRI findings of the brain. There was no significant difference between the left and right HFS groups in neuroimaging findings (Table 1).

Radiologically, the study population was divided into three groups: Normal, incidental findings, and vascular abnormalities (one patient with compression of the anterior segment of the left superior cerebellar artery on the 7th cranial nerve associated with partial empty sella was included in this group). The mean ages of the three groups were 54.7 10.3, 62.3 7.1, and 55.6 11.2 years, respectively (p=0.014). Pairwise comparisons showed that the mean age of patients with incidental findings was significantly higher than that of patients with normal MRI findings (p=0.012). While male predominance was observed in patients with normal MRI findings and patients with vascular findings (n=13, 56.5% and n=6, 75%, respectively), females predominated in patients with incidental findings (n=16, 66.7%). There were no significant differences in the other pairwise comparisons (Table 2).

RF were significantly different among the three groups. Patients without RFs were predominant in the group with normal MRI findings and group with vascular findings, while patients with 1 RF and 2 RFs were predominant in the group with incidental findings (Table 2).

Table 1. Demographics and clinical data of the left and right HFS groups

(n=55)	Left HFS (n=36)	Right HFS (n=19)	p
Age mean ± SD	57.5±9.3	59.5±10.6	0.32*
Sex, female:male, n (%)	17:19 (47.2%:52.8%)	11:8 (57.9%:42.1%)	0.32**
Risk factors, n (%)			
None	17 (47.2%)	6 (31.6%)	0.29***
1 RF	11 (30.6%)	4 (21.1%)	-
2 RFs	6 (16.7%)	7 (36.8%)	-
>2 RFs	2 (5.6%)	2 (10.5%)	-
Diabetes mellitus, n (%)	5 (13.9%)	2 (10.5%)	1.0**
Chronic headache, n (%)	7 (19.4%)	2 (10.5%)	0.47**
Disease duration, mean ± SD (months)	53.8±59.3	75.4±59.9	0.29*
Medication against HFS, n (%)			
None	28 (77.8%)	18 (94.7%)	0.14**
Carbamazepine/oxcarbazepine	8 (22.2%)	1 (5.3%)	-
Neuroimaging, n (%)			
Normal	17 (47.2%)	6 (31.6%)	0.33***
Non-specific ischemic white matter changes	9 (25%)	10 (52.6%)	-
Dolichoectatic basilar artery	5 (13.9%)	2 (10.5%)	-
Partial empty sella	4 (11.1%)	1 (5.3%)	-
Vascular abnormalities and empty products	1 (2.8%)	0	-
Treatment, n (%)			
Botulinum toxin	32 (88.9%)	19 (100%)	0.52***
Acetazolamide + botulinum toxin	2 (2.8%)	0	-
Acetazolamide	1 (5.6%)	0	-
No treatment	1 (2.8%)	0	-

n: Number, SD: Standard deviation, HFS: Hemifacial spasm, RF: Risk factor, *Mann-Whitney U test, **Fisher's exact test, ***Pearson chi-square test

Table 2. Demographics and clinical data of the groups according to neuroimaging findings

(n=55)	Normal (n=23)	Incidental (n=24)	Vascular (n=8)	p
Age mean ± SD	54.7±10.3[a]	62.3±7.1[b]	55.6±11.2[ab]	**0.014***
Sex, female:male, n (%)	10:13 (43.5%:56.5%)	16:8 (66.7%:33.3%)	2:6 (25%:75%)	**0.08****
Side, L:R, n (%)	17:6 (73.9%:26.1%)	13:11 (54.2%:45.8%)	6:2 (75%:25%)	0.3**
Risk factors, n (%)				
None	16 (69.6%)	3 (12.5%)	4 (50%)	**0.01****
1 RF	3 (13%)	10 (41.7%)	2 (25%)	-
2 RFs	3 (13%)	9 (37.5%)	1 (12.5%)	-
>2 RFs	1 (4.3%)	2 (8.3%)	1 (12.5%)	-
Diabetes mellitus, n (%)	1 (4.3%)	5 (20.8%)	1 (12.5%)	0.24**
Chronic headache, n (%)	2 (8.7%)	4 (16.7%)	3 (37.5%)	0.17**
Disease duration, mean ± SD (months)	62.4±53.2	62.9±69.6	23±14.7	0.47*
Medication against HFS, n (%)				
None	18 (78.3%)	21 (87.5%)	7 (87.5%)	0.66**
Carbamazepine/oxcarbazepine	5 (21.7%)	3 (12.5%)	1 (12.5%)	-
Treatment, n (%)				
Botulinum toxin	23 (100%)	21 (87.5%)	7 (87.5%)	0.5**
Acetazolamide + botulinum toxin	0	1 (4.2%)	1 (12.5%)	-
Acetazolamide	0	1 (4.2%)	0	-
No treatment	0	1 (4.2%)	0	-

n: Number, SD: Standard deviation, F: Female, M: Male, HFS: Hemifacial spasm, RF: Risk factor, *Kruskal-Wallis test, [ab]There is no difference between groups with the same letter, **Pearson chi-square test

There were no significant differences among these three groups on the side of HFS, presence of DM, presence of chronic headache, duration of illness, medication for HFS, and treatment (Table 2).

Electromyography was performed in 5.45% patients (n=3), and short-term, frequently recurrent spasm activity was observed when recorded with simultaneous superficial disc electrodes from the orbicularis oculi and orbicularis oris muscles.

A lumbar puncture was performed in 11.1% of patients with left-sided HFS (n=4) because of a history of chronic headache with partial empty sella. CSF opening pressure, cell count, biochemistry (including sodium, potassium, chloride, glucose, and protein levels), IgG and oligoclonal banding pattern, bacterial culture, and viral polymerase chain reaction panel were evaluated. One patient had normal CSF findings. Three patients had increased opening pressure, whereas the other CSF findings were completely normal, and they were diagnosed with idiopathic IIH.

IIH patients (n=3) were treated with acetazolamide. While one of them improved only with acetazolamide treatment, two of them also required botulinum toxin injection. 92.72% patients (n=51) were treated with botulinum toxin injections only. One patient with left-sided HFS refused botulinum toxin injection and was followed up without treatment. Various MRI examples of some patients are shown in Figures 1-5.

Discussion

The mean age and left-sided predominance of our patients with primary HFS was consistent with the literature (1,2). Although females predominate in the literature in patients with primary HFS, there was no significant difference in the female-to-male ratio in our patient series.

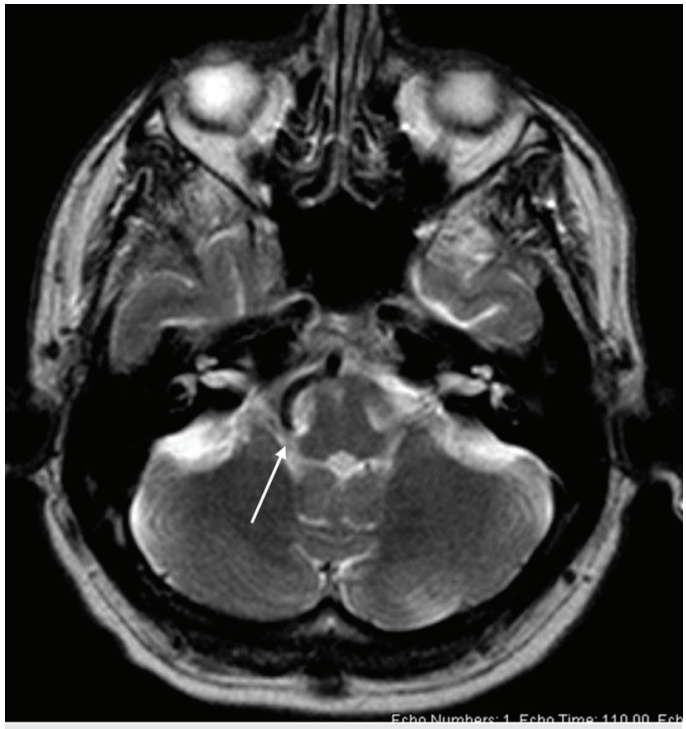

Figure 1. Dolichoectasia at the right vertebral artery and the proximal part of the basilar artery on axial T2 weighted image and compression to the lower cranial nerves on the right

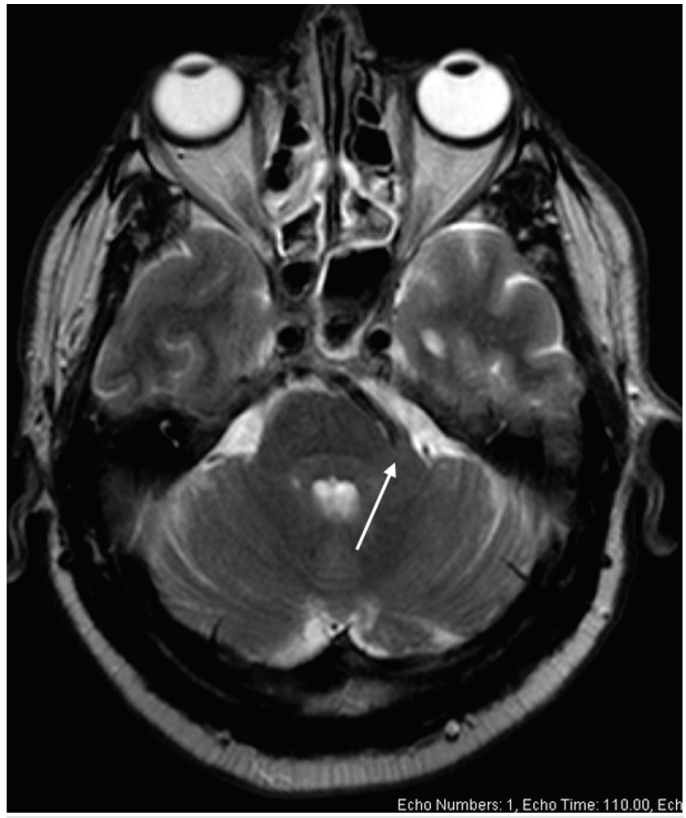

Figure 2. Dolichoectasia at the left vertebral artery on axial T2 weighted image and compression on left 7th and 8th cranial nerves

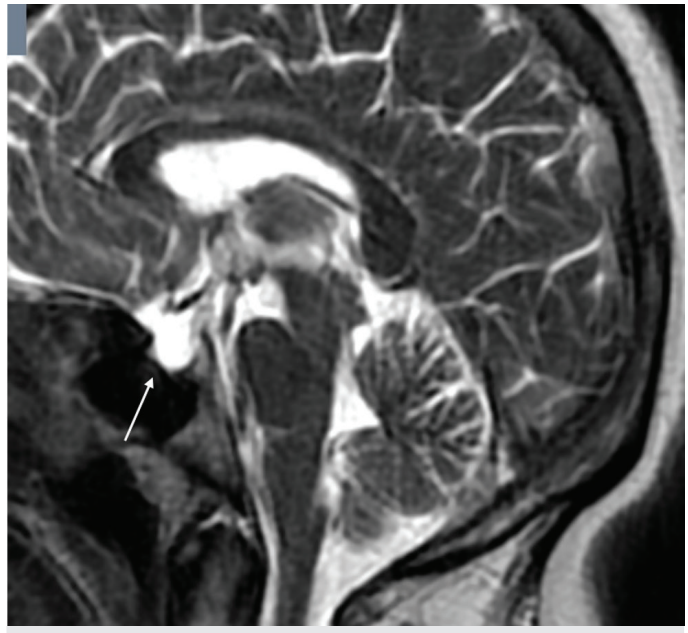

Figure 3. Empty sella on sagittal T2 weighted image

HFS is a clinical picture of unknown causes but is thought to be triggered by arteriosclerosis by many authors. This relationship was first described by Schultze in 1875 during the autopsy of a patient with an aneurysm compressing the facial nerve (15). In the 1960s, Gardner and Sava drew attention to this relationship and pointed out that the symptoms would be relieved by surgically relocating the vessel (9,14). In our series, 7 of

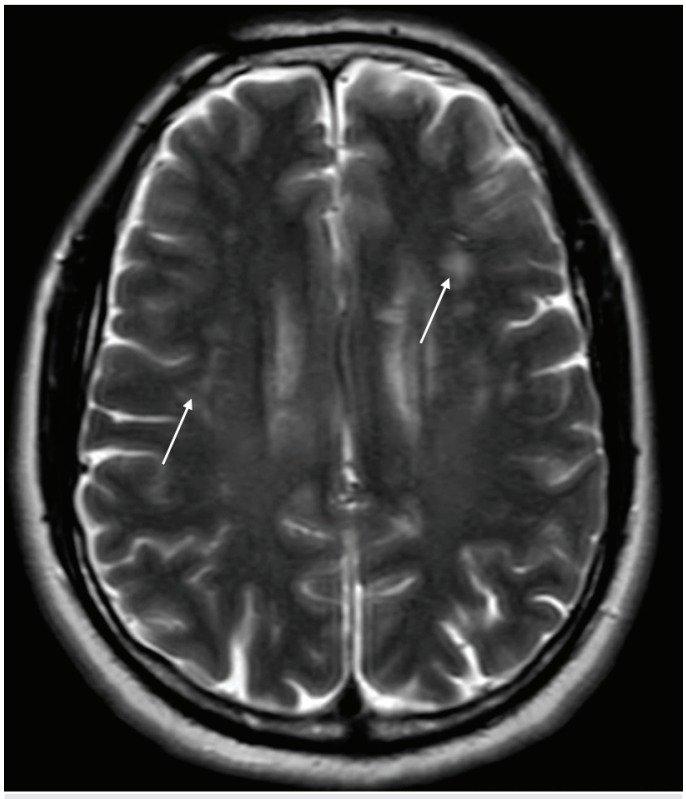

Figure 4. Bilateral hyperintense ischemic-gliotic lesions on axial T2 weighted image

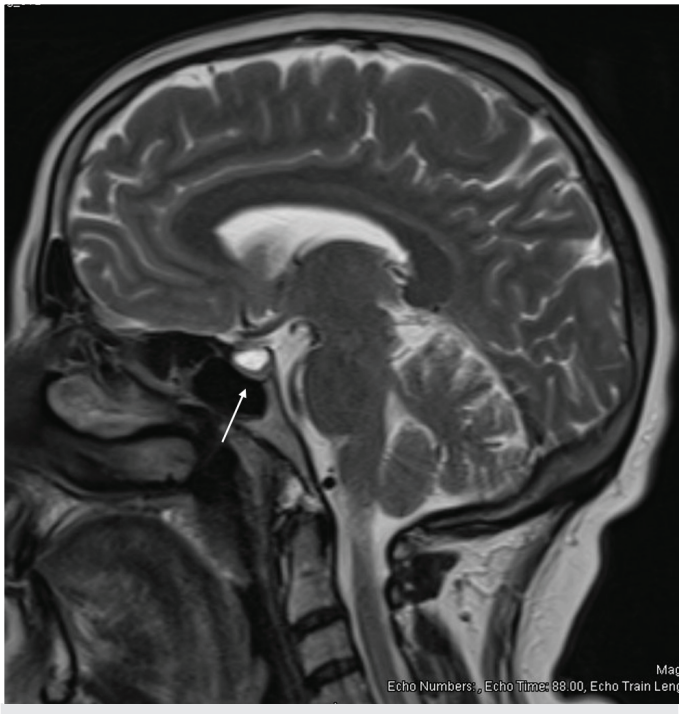

Figure 5. Partial empty sella on sagittal T2 weighted image

55 patients (12.7%) had vascular abnormalities at the brainstem level, revealed radiologically. In the literature, space-occupying lesions in the cerebellopontine corner or congenital causes of bone structure are considered more rarely (9,14). In our series, there were no such lesions. Non-specific ischemic white matter changes were considered incidental in 19 (34%) cases and partial empty sella in 5 cases (9.1%). Additionally, a case of empty sella was accompanied by brain stem vascular abnormality. In our cases, vascular abnormalities included the dolichoectatic basilar artery. The radiological findings in our study agree with the literature (16).

Neuroradiological examinations with various MRI techniques may show a compression of vascular structures passing through the facial nerve root entry zone (2).

HFS is a movement disorder thought to be caused mainly by compression of the facial nerve by vascular structures in the root exit region (17). Mostly, the posterior inferior cerebellar artery, anterior inferior cerebellar artery, and vertebral arteries are held responsible. Space-occupying lesions such as epidermoid tumors, neuroma, meningioma, astrocytoma, and parotid gland tumors are observed in approximately 5% of HFS patients (2).

Vascular abnormalities at the level of the brain stem are defined by many researchers as the triggering factor for developing HFS (6,8-10,14,18-20). It has been reported that the vessel causing compression may be as large as those found in the vertebral artery or as thin as in the posterior inferior cerebellar artery, anterior inferior cerebellar artery or cochlear artery (9,14). It is stated that in classical HFS, the compressing artery almost always crosses the nerve from front to back at the level of the exit point or the intrapontine section of the facial nerve, and the compressing veins cause a similar picture in many patients with a close relationship (8,10,20). Some cases had more than one vascular abnormality. MRI showed neurovascular compression of the seventh cranial nerve in 43% of patients (21).

The rates of etiological causes vary in the literature, in line with the examination methods or whether the series is clinical or surgical. In a literature review that included 1688 cases, 509 had vascular abnormalities, 19 tumors, 7 bone abnormalities, and 986 cases had an undetermined cause, and in 163 cases, radiologic, surgical, or autopsy studies failed to demonstrate a cause (22). Barker et al. (23) reported that all 703 cases with HFS in which they applied microvascular decompression were due to vascular abnormality and that microvascular decompression is a safe and definite treatment for HFS with proven long-term efficacy.

Some case-control studies found a significantly higher prevalence of hypertension among patients with primary HFS than among patients with other neurological diseases or healthy controls (21,24), other series failed to find a significant difference in the prevalence of arterial hypertension in patients with primary HFS (24-26). In our study 58.2% of the patients (n=32) had at least one RF and 30.9% had hypertension (n=17).

Symptoms of HFSs can be relieved by medication, injections, or surgery. Plenty of rest and stress reduction are also recommended. But there is no cure for HFS. Anticonvulsants, baclofen, anticholinergics, and clonazepam have been used in treatment for many years. However, it is a critical development that patients with botulinum toxin treatment show almost complete recovery, albeit temporary (2).

Partial empty sella may be an incidental radiological finding in an asymptomatic patient with preserved pituitary function, or it may be a sign of IIH with increased CSF pressure (27). In our study, an empty sella image was detected in 5 cases. Three of these patients had high opening pressure at the lumbar puncture, while other CSF findings were completely normal, and these three patients were also diagnosed with IIH. One recovered with acetazolamide treatment alone, while the other two required botulinum toxin injections. We can interpret the empty sella as an incidental finding in this series because empty sella is a very common anatomical variation. But since empty sella may be a sign of IIH, we can also interpret that one patient in our series had HFS due to elevation of intracranial pressure and improved well with acetazolamide treatment.

MRI features of IIH in patients with unilateral facial spasm association have already been described in the literature and recently called "IIH-spasm syndrome" (28-30).

The etiology of IIH is unknown. It can cause headaches, pulsatile tinnitus, double vision, papilledema, and sixth cranial nerve palsy. In addition to these typical findings, atypical presentations, including HFS, have been reported. Chen et al. (31) reported a 43-year-old female patient who presented with a 2-year history of left-sided HFS. MRI demonstrated bilateral anterior inferior cerebellar artery vascular loops involving the internal auditory canals as well as IIH-associated findings. After the lumbar puncture, which revealed an elevated CSF opening pressure, the patient was put on acetazolamide treatment, resulting in complete resolution of the HFS (31). In our study, we had four patients with a history of chronic headaches accompanied by partial empty sella. Three of them had elevated CSF opening pressure. One of these patients improved after 5 days with only acetazolamide treatment. An empty sella sign is usually seen in middle-aged, obese women with hypertension. Additionally, these patients may also have headaches, endocrine disorders, and visual disturbances if the sella is enlarged (32). If HFS is accompanied by headache and an empty sella finding, IIH should be suspected. Another case that should be considered was HFS with pulmonary involvement after a SARS-CoV-2 infection two weeks ago. There are several neurological symptoms after the global outbreak of Coronavirus disease-2019 (COVID). The central nervous system, meninges, cranial nerve, spinal cord, and peripheral nerve involvement have been reported. HFS cases that developed after the COVID pandemic began to be published as case reports (33,34). Where these patients will evolve in the long-term follow-up is one of the medical concerns, and in this respect, it would be appropriate to deepen the anamnesis in newly admitted HFS cases.

Conclusion

Botulinum toxin injection is the most common symptomatic treatment of HFS in the clinical practice of neurology. Vascular compression is frequently noted in the imaging of patients with primary HFS, however, as in our case series, partial empty sella finding, which may be a sign of IIH, should not be overlooked.

Although rare, IIH may occur with HFS findings. Here, HFS symptoms may also improve when IIH is treated.

Ethics Committee Approval: According to a protocol approved by the Local Ethics Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 199, date: 17.06.2022), all participants gave written informed consent according to the Declaration of Helsinki.

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept - T.E., Z.Y., M.T.A., N.K.; Design - T.E., Z.Y., M.T.A., N.K.; Data Collection or Processing - T.E., Z.Y., E.K., E.A.A., M.T.A., N.K.; Analysis or Interpretation -T.E., Z.Y., E.K., E.A.A.; Literature Search - T.E., Z.Y., N.K.; Writing - T.E., Z.Y., M.T.A., N.K.

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

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

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İstanbul Med J 2021; 22(1): 1-7.

DOI: 10.4274/imj.galenos.2020.34538

Retraction: *Bulgur İ, Piyal B. Exposures Moved from Work to Home as a Public Health Hazard. İstanbul Med J 2021; 22(1): 1-7.*

The journal has taken the decision to retract this paper following concerns expressed by Diana Ceballos (PhD, MS, CIH, Assistant Professor, Director Exposure Biology Research Laboratory, Department of Environmental Health, Boston University School of Public Health). After investigation, it has been concluded that the article shows considerable overlap with a previously published article by D.Ceballos et al (Kalweit A, Herrick RF, Flynn MA,

Spengler JD, Berko JK, Levy JI and Ceballos DM. Eliminating Take-Home Exposures: Recognizing the Role of Occupational Health and Safety in Broader Community Health. Ann Work Expo Health 2020; 64: 236-49) without proper citation and presents novel ideas from this paper without the authors' consent. The decision is made according to the Committee on Publication Ethics (COPE) guidelines as citation correction alone would not sufficiently address potential intellectual property concerns.

The Editors of Istanbul Medical Journal take issues of research and publication misconduct seriously in order to preserve the integrity of the academic record. Our apologies are extended to the readers and to the authors of the previously published article that this was not discovered before publication.

The retracted article will be digitally watermarked on each page as "Retracted" to maintain the scholarly record.

Kind regards,

İstanbul Medical Journal *Editorial Board*