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Potential Immunological Treatments in COVID-19 Patients

COVID-19 Hastalarında Potansiyel İmmünolojik Tedaviler

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seemed in Wuhan, China in December 2019. SARS-CoV-2 infection in human was named as coronavirus disease 2019 (COVID-19). It has now infected more than 69 million people worldwide, becoming an epidemic responsible for more than 1,5 million deaths until 10th of December 2020. The epidemic still continues. This epidemic is the third epidemic caused by coronaviruses in the 21st century and may be the most important infectious disease representing a major public health threat to the whole world. Treatments against COVID-19 are constantly updated in the literature, based on evidence. Unfortunately, there is no definitive cure for COVID-19, and a number of drugs for use in severe cases of COVID-19 are now being studied in a number of nonrandomized or randomized trials. These include chloroquine, steroids, anti-inflammatory, and antiviral agents. Immunological treatments such as convalescent plasma, intravenous immunoglobulin, monoclonal antibodies (tocilizumab, eculizumab, itolizumab etc.), and anakinra treatments are tried in COVID-19 disease. Results from some trials look promising. Quite a few reports have also stood published so far on the use of immunological treatments for COVID-19 cases. In this review, we will discuss the key immunological treatments, mostly mentioned in the current literature, used in COVID-19 patients in detail.
Keywords: Anakinra; bamlanivimab; COVID-19; convalescent plasma; eculizumab; intravenous immunoglobulin; itolizumab; monoclonal antibodies; sarilumab; siltuximab; tocilizumab; svilobelimab.

ÖZ

Şiddetli akut solunum yolu sendromu koronavirüsü 2 (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) Aralık 2019'da Çin'in Wuhan kentinde görüldü. İnsanlarda SARS-CoV-2 enfeksiyonu koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) olarak adlandırıldı. Şu anda dünya çapında 69 milyondan fazla insanı enfekte etti ve 10 Aralık 2020'ye kadar 1,5 milyondan fazla ölümden sorumlu bir salgın haline geldi. Salgın hala devam ediyor. Bu salgın, 21. yüzyılda koronavirüslerin neden olduğu üçüncü salgındır ve tüm dünya için önemli bir halk sağlığı tehdidini temsil eden en önemli bulaşıcı hastalık olabilir. COVID-19'a karşı tedaviler, kanıta dayalı olarak literatürde sürekli olarak güncellenmektedir. Ne yazık ki, COVID-19 için kesin bir tedavi yoktur ve şiddetli COVID-19 vakalarında kullanılmak üzere bir dizi ilaç şu anda bir dizi randomize olmayan veya randomize çalışmada incelenmektedir. Bunlar arasında klorokin, steroidler, anti-enflamatuar ve antiviral ajanlar bulunmaktadır. COVID-19 hastalığında konvalesan plazma, intravenöz immünoglobulin, monoklonal antikorlar (tocilizumab, eculizumab, itolizumab vb.) ve anakinra tedavileri gibi immünolojik tedaviler denenmektedir. Bazı çalışmalardan elde edilen sonuçlar umut verici görünmektedir. COVID-19 vakalarında immünolojik tedavilerin kullanımı hakkında şimdiye kadar çok az sayıda rapor yayınlandı. Bu derlemede, COVID-19 hastalarında kullanılan ve çoğunlukla güncel literatürde bahsedilen temel immünolojik tedaviler ayrıntılı olarak tartışılacaktır.
Anahtar kelimeler: Anakinra; bamlanivimab; COVID-19; konvalesan plazma; eculizumab; intravenöz immünoglobulin; itolizumab; monoklonal antikorlar; sarilumab; siltuximab; tocilizumab; svilobelimab.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seemed in Wuhan, China in December 2019. SARS-CoV-2 is a positive (+) sense, single-stranded, and enveloped RNA virus. SARS-CoV-2 belongs to the genus Betacoronavirus of the Coronaviridae family. It has a characteristic genetic property with two subtypes (S and L) and more than 140 mutation points. Four major proteins in the structure of SARS-CoV-2 are responsible for intracellular replication and human cell interaction: membrane (M), nucleocapsid (N), envelope (E) and, spike (S) proteins (1-4). The SARS-CoV-2 infection in human was named as coronavirus disease 2019 (COVID-19).

COVID-19 first started in Wuhan city with unidentified cases of pneumonia seen in some adults and become a global pandemic. It has now infected more than 69 million people worldwide, becoming an epidemic responsible for more than 1,5 million deaths until the 10th of December 2020. The epidemic still continues (5-6). This epidemic is the third epidemic caused by coronaviruses in the 21st century and may be the most important infectious disease representing a major public health threat to the whole world. Treatments against COVID-19 are constantly updated in the literature, based on evidence. Unfortunately, there is no definitive cure for COVID-19, and a number of drugs for use in severe cases of COVID-19 are now being studied in a number of nonrandomized or randomized trials. These include chloroquine, steroids, anti-inflammatory and antiviral agents (7-10).

Quite a little reports have also stood published so far on the use of immunological treatments in COVID-19 cases. In this review, we will discuss the key immunological treatments, mostly mentioned in the current literature, used in COVID-19 cases in detail.

CONVALESCENT PLASMA TREATMENT FOR PATIENTS WITH COVID-19

Since COVID-19 was first detected, we still have no definitive treatment options. Lately, the use of human convalescent plasma (CP) is considered a potential choice for the therapy of COVID-19 (11-12). CP has been successfully used in SARS-CoV, Ebola, MERS, and H1N1 viral infections (13). Quite a lot of works have been published in the past decade to assess the clinical helpfulness of CP in relation to respiratory coronavirus infections. Nowadays there is a snowballing interest in the use of passive immunotherapy through transfusion of CP originating from healed COVID-19 patients documented by numerous ongoing studies and daily reviews/perspectives/comments.

Duan et al. (14) presented 10 serious COVID-19 patients who received a CP transfusion covering high neutralizing antibody titers (>1: 640) in the mean of 16.5th day (median) of admission/post-infection. Improvement in laboratory values and clinical symptoms were observed from the 3rd day after infusion. They observed improvement in oxygen saturation, neutralizing antibody titer, SARS-CoV-2 viral load, lung lesions, C-reactive protein (CRP), and lymphocyte count. Also, no serious side effects were observed in patients.

Shen et al. (15) reported a case series of 5 severely patients, all getting CP having SARS-CoV-2 antibodies (titer >1/1000) and a neutralization titer larger than 1:40, applied between day 10th and 22nd of admission. In 4 out of 5 patients, an increase in viral antibody titers, a decrease in SARS-CoV-2 viral loads, and an improvement in acute respiratory distress syndrome (ARDS) were observed. Zhang et al. (16) presented 4 critical patients transfused with 200-2400 mL CP from day 11 to day 18 of the admission. Improvement was observed in all patients including a pregnant woman. In two other studies evaluating CP treatment in COVID-19; Ye et al. (17) observed improvement in 6 patients and Ahn et al. (18) 2 patients after treatment.

The United States Food and Drug Administration (FDA) approved the use of CP on March 26, 2020. It triggered the planning of several trials regarding the use of CP to treat critical patients. When the search was conducted on the clinictrials.gov (https://www.clinicaltrials.gov) site on December 14th, 2020; 135 active ongoing studies were observed when the terms "convalescent plasma and COVID-19" were examined.

Rajendran et al. (19) studied five newly reported studies happening CP use in patients with COVID-19. The main results of this review observed that mortality was reduced in critical cases, SARS-CoV-2 RNA disappeared in most patients, clinical symptoms and radiological findings improved, and no significant side effects were seen secondary to CP treatment.

A systematic review and meta-analysis conducted in 2015 examined 32 studies on heavy influenza and SARS-CoV infection. These studies included 699 treated patient groups and 568 untreated control groups. In a pooled analysis of the data, the investigation revealed evidence of a reliable lessening in mortality in the group treated with CP compared to those who did not get a placebo (20).

In clinical situations characterized by hypercytokinemia, the timing of immunomodulatory treatments is very important, and early initiation of CP therapy seems to be associated with a better outcome. In heavy COVID-19 patients, inflammatory factors mainly associated with IL-6-related hypercytokinemia have been reported to increase significantly 7 to 14 days after onset, contributing to the aggravation of the disease (21). A study in 175 patients in China who improved from COVID-19 viral infection, obviously observes the formation of neutralizing antibodies specific for SARS-CoV-2 from 10-15 days when observed from infection. Peak neutralizing antibody levels were detected in all patients from 10 days and these antibodies remained stable thereafter (22).

The FDA classified the criteria for timing of COVID-19 CP collection as follows;

Scenario A (clinical results based): Donors' symptoms must have totally healed at least 28 days earlier donation.

Scenario B (clinical plus laboratory study based): Donors' symptoms must have totally healed at least 14 days earlier donation and COVID-19 PCR from nasopharyngeal swab must be negative.

The FDA suggests two clinical indications for the present usage of CP treatment in COVID-19 patients;

Scenario A (severe disease) is demarcated as one or more of the undermentioned: Dyspnea, blood oxygen saturation ≤93%, respiratory rate ≥30/min, PaO_2/FiO_2 rate <300, and radiological deteriorating with the dawn of lung infiltrates >50% within 1 to 2 days.

Scenario B (life-threatening disease) is demarcated as one or more of the undermentioned: Septic shock, respiratory failure, or multisystem dysfunction.
Additionally, CP could be used prophylactically in some cases, although it is not recommended by the FDA. These conditions are healthcare providers, individuals exposed to approved COVID-19 cases, and patients with multiple medical conditions (11).
CP therapy for COVID-19 patients, its schematic representation and the main steps are shown in Figure 1.

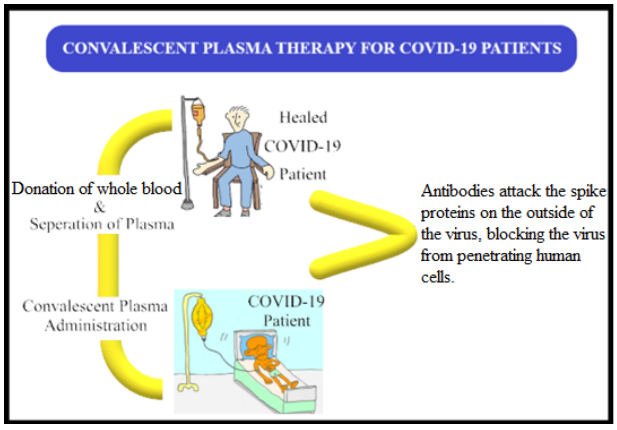

Figure 1. Schematic demonstration of convalescent plasma therapy in COVID-19 patients

Side Effects of Convalescent Plasma Treatment
Serious side impacts have not been reported in CP treatments for the Ebola infection, SARS, H1N1, and MERS outbreaks. The CP treatment was well tolerated in most cases. Some studies have reported minor side effects such nausea, chills, skin rashes, increased body temperature, and itching. These effects were solved spontaneously by symptomatic therapy or by dipping the transfusion ratio. Major side effects such as anaphylactic reactions, circulation overload, and transfusion-related acute lung injuries (TRALI) have also been reported in several cases. The incidence of TRALI is many rare (1 in 5000) and occurs only in severe cases (13,23).
General Warnings about Convalescent Plasma Treatment
The risk of transmitting hepatitis C virus, hepatitis B virus, and HIV from donated plasma must be fully examined. There is very limited information about the security of CP treatment in expectant patients; therefore, sufficient data are needed regarding the usage of CP treatment during pregnancy (13).
The amount and duration of treatment in the CP depends on the severity and viral load of COVID-19. It is trusted that virus-neutralizing antibodies, even in minor quantities, can be efficient when used for the avoiding or therapy of early symptoms of COVID-19. The resulting passive immunity can take weeks and months, but the exact duration needed is unknown. Antibodies should be used shortly after collection. CP treatment has the most potential to treat severe SARS-CoV-2 viral infections, as mutations that can change their properties may occur in the virus. Most of the previous studies were done with a small number of patients with co-morbidity such as diabetes or liver disease. Therefore, a larger scale of clinical studies are needed to produce statistically significant data in footings of the effectiveness of CP therapy and to study possible side effects united with it (13,23).
As a result, it is clear that until vaccines are available for COVID-19, new treatment options are urgently needed to reduce mortality and treat serious cases. CP should be imagined as a therapeutic option at the beginning of symptoms in severe COVID-19 patients.

INTRAVENOUS IMMUNOGLOBULIN TREATMENT FOR PATIENTS WITH COVID-19
Intravenous immunoglobulin (IVIG) was first licensed in the USA in 1980. It is a very effective treatment for preventing life-threatening infections in cases with primary and secondary immune deficiencies. IVIG has also been administered as adjuvant therapy for critically ill patients. A blood product purified off mixed plasma of healthy individuals, globulins is the parent component and wealthy in anti-bacterial and anti-viral IgG antibodies (24-27).
IVIG is a pool of IgG from thousands of healthy donors and exposed individual donors to endemic infectious illness, vaccines, and ubiquitous microorganisms contributing to the manufacture of IgG antibodies versus distinct microorganisms and their antigens (28-31). IVIG from healthy donors is used not only to treat autoimmune diseases such as vasculitis, ITP, Guillain Barrè Syndrome, and Kawasaki but also some difficult, bacterial, and viral infections.
IVIG infusion can effectively neutralize pathogens in the respiratory tract by increasing the serum IgG level, thus promoting improvement from disease and shortening the duration of the illness. IVIG can also cure the body's defenses, block target cell-related host receptors, and avert the pathogen from more damaging the target cell (24). In supplement, the usage of IVIG may also affect the differentiation and maturation process of lymphocytes, inhibit the immune reply of white blood cells, inhibit the manufacture of inflammatory factors, and thus decrease the inflammatory lesion met by the sick. Lately, it has been reported to be involved in improving the hyperinflammatory condition as well as coagulation abnormity in septic cases (32-36).
Various mechanisms of action have been attributed to the beneficial effects of IVIG. Thus, these therapeutic properties of IVIG appear to be particularly suitable for COVID-19 severe infection, where inflammation with an untargeted adaptive immune activation and resulting clotting abnormalities playing a role in the pathogenesis of the disease (37,38).
Virgo (S) glycoprotein forms a homotrimer protruding off the viral surface. The angiotensin-converting enzyme 2 (ACE2) receptor is highly expressed on the apical surface of many cell types, including airway epithelium. The S protein mediates the entry of SARS-CoV-2 into host cells by binding to the ACE2 receptor. Although the mechanisms by which IVIG acts against COVID-19 are not fully understood, two mechanisms are being discussed (Figure 2). First, neutralizing antibodies prevent the binding of the SARS-CoV-2 spike protein at the ACE2 receptor, preventing viral inlet into the cell. Second, IVIG is thought to have an anti-inflammatory effect by binding the FCy receptor (39-40).

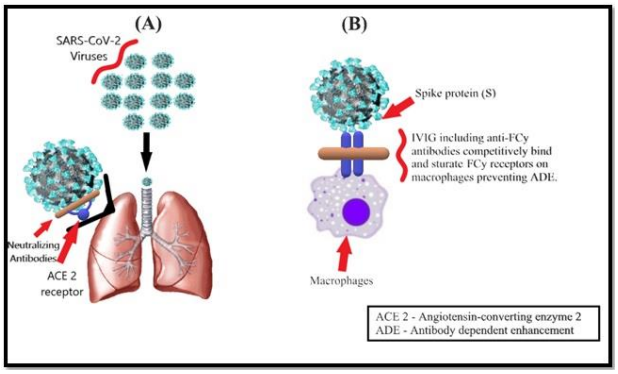

Figure 2. (A) Neutralizing antibodies prevent SARS-CoV-2 spike protein from binding to the ACE2 receptor, preventing viral entry into the cell. **(B)** Among the proposed mechanisms by which IVIG exerts an anti-inflammatory effect, the binding of the FCγ receptor is shown

A meta-analysis of the use of IVIG in SARS infection finalized that it is not net whether it improves prognosis. When we look at the literature regarding the use of IVIG in MERS infection, there is no evidence that IVIG has anti-MERS efficacy has not been reported. Investigations on infection with influenza viruses such as H1N1 have shown that IVIG can prohibit a serious infection with pandemic influenza. In 2009, a multicenter, randomized, double-blind, controlled hyperimmune globulin used to treat cases with heavy H1N1 infection found that the use of hyperimmune globulin in the therapy of heavy H1N1 within 5 days of start of symptoms was associated with a decline in viral burden and death rate (35,41).

There are some studies on COVID-19 patients treated with IVIG reported. Cao et al. (42) reported remission in 3 severe cases of COVID-19 after IVIG treatment. Lin et al. (43) recommended early initiation of high-dose IVIG associated with anticoagulant therapy. Quinti et al. (44) demonstrated that the patients were protected with IVIG therapy in seven patients with primary immunodeficiency. Lanza et al. (45) successfully treated a 42-year-old patient with heavy COVID-19 pneumonia with IVIG infusion. In a study conducted by Xie et al. (46) with 58 severe COVID-19 cases who received IVIG therapy followed in the ICU, initiating IVIG as adjuvant therapy for COVID-19 pneumonia within 2 days after acceptance to the ICU reduced the usage of mechanical ventilation, shortened the time of hospital remain and significant clinical efficacy was found. Mohtadi et al. (47) observed improvement in all patients after high-dose IVIG (0.3-0.5 g/kg) treatment for 5 alternate days in 5 severe COVID-19 patients who failed standard treatments.

Early usage of IVIG as an adjuvant therapy aimed at COVID-19 pneumonia in selected patients may decrease the rates of mechanical ventilation and hospitalization. Although a definitive treatment for COVID-19 is not available, the combination of IVIG and other alternative therapeutic modalities such as dexamethasone can be used as a treatment protocol against COVID-19.

MONOCLONAL ANTIBODY TREATMENT FOR THE PATIENT WITH COVID-19

Monoclonal antibodies (MAB) are antibodies that react against only one epitope and are derived from only one B-lymphocyte-based cell clone. The method of obtaining MABs was described by César Milstein, Georges Köhler, and Niels Jerne in 1975. MABs are especially used to avert and treat diseases from the immune system and cancer illnesses. Only seven out of 100 licensed MABs are for treating and preventing infectious diseases. MABs are not chemical mixes like most drugs. It is based on natural antibodies, which are proteins that the body breeds to advocate itself against illnesses. However, it is constituted in the laboratory and mass-produced in factories. Because of these properties, they are named "designer antibodies" (48,49).

It is being tested whether various MABs licensed for other diseases or under development have an impact on COVID-19 cases. Researchers have besides been swiftly detecting MABs that particularly goal SARS-CoV-2. More than 70 MAB products are currently under progress for COVID-19. MAB agents that have been tried in the literature to treat COVID-19 so far are tocilizumab (a MAB against IL-6), siltuximab (a MAB against IL-6), sarilumab (a MAB against IL-6), eculizumab (anti-C5 MAB), bamlanivimab (LY-Cov555), vilobelimab (Anti-C5a antibody IFX- 1) and itolizumab (Anti-CD6 MAB) (49-56).

Tocilizumab

Interleukin-6 (IL-6) is a cytokine that moves significant role in inflammatory reaction and immune reply. New clinical experience shows that IL-6 is one of the greatest significant cytokines involved in COVID-19 induced cytokine storms. Therefore, tocilizumab (TCZ), a recombinant humanized anti-human IL-6R monoclonal antibody of the IgG1 subtype, is being tested in the therapy of COVID-19 cases. TCZ is soluble and specifically binds membrane connected IL-6 receptors (mIL-6R and sIL-6R) and inhibits mIL-6R and sIL-6R mediated signal transduction. It is certified for the therapy of systemic juvenile idiopathic arthritis and rheumatoid arthritis. It has withal been reported to show part in Crohn's disease and Castleman disease (57-61).

Let's evaluate the results of the studies with TCZ in order. Luo et al. (50) included 15 patients diagnosed with COVID-19 in the study. While 10 patients showed a reduction in IL-6 levels and clinical improvement, 4 patients did not show a reduction in IL-6 levels or clinical recovery. In addition, 1 patient also aggravated the clinic. No adverse drug reactions were reported during TCZ treatment. Xu et al. (62) included 21 severe COVID-19 patients in the study. The body temperature of all patients returned dramatically to normal on the first day after taking TCZ and remained stable thereafter. In the following days, clinical symptoms improved simultaneously. After TCZ treatment, 13 patients recovered within 2 weeks and were discharged. 6 patients recovered between the 2nd and 3rd weeks and were discharged. No death was reported. Toniati et al. (63) included 100 severe cases of COVID-19 pneumonia in the study. 24-72 hours after TCZ treatment, 58 patients presented a fast recovery in the respiratory tract and clinical, 37 patients were stabilized compared to the pre-TCZ status, and 5 patients deteriorated. 77 patients improved on the 10th day; 61 of them were observed that diffuse two-sided opacities disappeared significantly on lung X-ray, and 15 were discharged from the hospital. In addition, 20 patients died and 3 patients' clinic

deteriorated. Serious adverse events in three patients were observed during the 10-day follow-up; two patients improved septic shock and one died. One patient developed gastrointestinal perforation necessitating emergency surgery.

Sciascia et al. (64) enrolled 63 patients with COVID-19 in the study. In this study, the cases were treated with intravenous (IV) and subcutaneous (SC) routes. There was no difference between the routes of administration in terms of mortality. Fever regressed in the first 24 hours in 24 of 25 patients who had fever after TCZ treatment. An improvement was observed in clinical and laboratory values. Seven patients died. In this study, TCZ application within 6 days after hospitalization was found to be associated with an increased probability of survival. Campochiaro et al. (65) enrolled 65 cases with COVID-19 in the study. Thirty two patients were followed with TCZ and 33 cases with standard therapy. Although mortality was determined as 15% in the TCZ group and 33% in the standard therapy group, there was no statistically significant difference between the two groups in terms of clinical recovery and mortality. Klopfenstein et al. (66) enrolled 45 cases with COVID-19 in the study. Twenty cases were followed up with TCZ and 25 cases with standard therapy. As a result of the study, they found that TCZ can reduce the number of admissions to the ICU and/or mortality in cases with severe SARS-CoV-2 pneumonia. Tleyjeh et al. (67) evaluated the results of 11,775 patients in their meta-analysis with the data of 24 studies. As a result of the meta-analysis, it was determined that TCZ decreased the risk of mechanical ventilation in hospitalized COVID-19 cases and did not decrease short-term mortality in randomized controlled studies. On the other hand, a relationship between TCZ and low mortality was found in cohort studies.

When the results of the studies in the literature were evaluated, it was observed that TCZ prevented the fever seen due to COVID-19 disease for the first 24 hours. Respiratory and clinical recovery has been observed in many patients. It is not known exactly how much TCZ prevents mortality. Although TCZ did not provide definitive treatment for COVID-19 patients, it was observed that the rate of recovery was high in patients. A clinical trial with TCZ according to current data in patients who do not respond with standard treatments may be promising.

Siltuximab

Siltuximab is an anti-IL-6 chimeric MAB, used in Castleman disease. It can be considered as a therapeutic strategy for treating severe cases of SARS-CoV-2 infection, as it benefits COVID-19 patients with TCZ therapy. Currently, there are no studies with completed data for COVID-19, the drug is in the trial phase (51).

Sarilumab

Another anti-IL-6 MAB is sarilumab. Right now, information on the usage of sarilumab in COVID-19 is limited. Let's evaluate one by one of the studies performed with sarilumab treatment in COVID-19 cases. Della-Torre et al. (68) administered sarilumab to 28 cases with heavy COVID-19 pneumonia and inflammatory phenotype in addition to standard treatments. Patients who were given sarilumab were compared with 28 patients with similar demographic, laboratory, and respiratory parameters who received only standard therapy. The mortality rate was lower in the sarilumab group (2/28 patients, 7%) compared to the standard treatment group (5/28 patients, 18%), but this difference was not statistically significant. Treatment with sarilumab in this study was associated with a significantly earlier reduction in serum CRP and fever.

Benucci et al. (69) gave sarilumab treatment to eight patients hospitalized for COVID-19. Aggressive and early treatment with sarilumab resulted in discharge within 14 days of hospital admission, with seven of eight cases showing negatory results on the molecular test. One case died. Montesarchio et al. (70) enrolled 15 laboratory-confirmed COVID-19 cases in the study. After administration of sarilumab, fast healings in respiratory parameters were reported in 67% of cases and 34% died. Nine of the eleven cases with CT findings replied to the therapy. However, all cases, with the inclusion of those who responded to sarilumab, referred with serum IL-6 levels at least ten times the top limit of normal.

Considering the available data, more randomized controlled works are needed to understand whether sarilumab treatment is effective in COVID-19.

Eculizumab

Eculizumab is anti-C5 (fifth element of complement pathway) MAB. It is a drug used to cure neuromyelitis optica, atypical hemolytic uremic syndrome, and paroxysmal nocturnal hemoglobinuria. There are studies that have been treated with eculizumab in COVID-19 patients, the data of these studies are evaluated below one by one.

Diurno et al. (52) successfully treated four COVID-19 cases admitted to the ICU for heavy pneumonia or ARDS with eculizumab. The average disease duration was observed as 12.8 days. Laurence et al. (71) administered eculizumab therapy to three critical COVID-19 patients. It resulted in a significant decrease in neutrophil and D-dimer levels in all three patients, and normalization of creatinine and liver function values in two patients. One case with heavy cardiac insufficiency had a whole remission. Partial remission was observed in the other two cases. Annane et al. (72) compared the data of 35 cases who received eculizumab and 45 cases who received standard treatment. The mortality rate was found inferior in the eculizumab group (17.1%) compared to the standard treatment group (37.8%). Cases treated with eculizumab had a meaningfully faster fall in total and conjugated bilirubin, blood urea nitrogen, and lactate levels compared to patients receiving standard therapy. A significantly faster rise in prothrombin time, platelet count, and oxygen saturation was observed. In this study, it was concluded that eculizumab could reduce hypoxia and improve survival in patients with severe COVID-19.

When the available data were evaluated, it was observed that eculizumab treatment was effective compared to standard therapy. More randomized controlled studies are needed to make a decision on this treatment.

Bamlanivimab

Bamlanivimab (LY-CoV555) has been authorized by the FDA for emergency use for mild to moderate COVID-19 therapy in adults and children who are not admitted to the hospital. It has been stated that bamlanivimab reduces the rates of hospitalization with early treatment in COVID-19 patients with chronic illness (53). But for now, there are no

studies with finalized data for COVID-19, the drug is in the trial phase.

Vilobelimab

Vilobelimab is anti-C5a is IFX-1 MAB. Vlaar et al. (55) among whole data of 15 patients with COVID-19 who received vilobelimab and 15 patients who received standard therapy were compared. Six patients died out of 30 patients comprised in the work. Two patients were in the group receiving vilobelimab, the other 4 patients were in the group receiving standard therapy. Considering the available data, more randomized controlled studies are needed to understand whether vilobelimab treatment is effective.

Itolizumab

Itolizumab is anti-CD6 MAB. Atal et al. (73) conducted a multi-center, two-arm, open-label, randomized, phase II, and pivotal clinical trial in 30 cases in India. While 20 patients were treated with itolizumab and supportive care, 10 patients in the control group received only supportive care. In this way, the study was performed with a 2:1 randomization. All patients who received itolizumab fully recovered and were discharged from the hospital, while three (30%) of ten cases in the control group died. All patients who received itolizumab were weaned from oxygen on the 30th day, and unlike the control group, none of them needed ventilator support. Clinical markers of inflammation such as CRP, lactate dehydrogenase (LDH), D-dimer, serum ferritin, TNF-a, and IL-6 presented clinically significant normalization after itolizumab administration and correlated well with radiological and clinical recovery in symptoms. In another investigation, the authors deduced that in 19 senior COVID-19 cases who were moderately ill, itolizumab therapy was associated with an importantly cut risk of ICU admission and a 10-fold lower death risk (74).

When current studies are evaluated, it is seen that itolizumab treatment will give promising results in severe COVID-19 disease. It can be said that itolizumab is the second most effective MAB agent used in the treatment of COVID-19 after TCZ, which is observed to be the most effective. However, randomized controlled studies are required to obtain a clear conclusion on itolizumab treatment.

ANAKINRA

Anakinra is a human recombinant IL-1 receptor (IL-1R) antagonist. It is used as a second-line therapy to manage rheumatoid arthritis symptoms after treatment with a disease modifying anti-rheumatic drug (DMARD) has failed. It is used to treat anyone from infants to adults with periodic syndrome associated with cryopyrin, including neonatal-onset multisystem inflammatory disease. It also appears to be forceful in the therapy of macrophage activation syndrome (MAS), a type of cytokine storm. It has been shown to aid the treatment of secondary hemophagocytic lymphohistiocytosis (HLH), especially in pediatric patients with other rheumatologic disorders. Coronaviruses can induce the manufacture of tumor necrosis factor, IL-6, IL-1β, and other cytokines related in autoinflammatory disorders. It has been suggested that anakinra, may help neutralize the hyperinflammatory state associated with COVID-19, which is considered to be a reason for ARDS (75-79).

Cavalli et al. (80) compared the data of 29 patients diagnosed with COVID-19 treated with anakinra and 16 patients treated with standard therapy. A decrease in serum CRP and progressive improvement in respiratory parameters were observed in 21 (72%) of 29 cases who received anakinra treatment on day 21. The 3 (10%) patients died and 5 (17%) cases were due to mechanical ventilation. In the standard of therapy arm, 8 (50%) of 16 cases presented respiratory parameters recovery; one (6%) case was on mechanical ventilation and seven (44%) cases died. Mortality was inferior in the anakinra arm (10%) compared to the standard treatment arm (44%) on day 21. Survival without mechanical ventilation was 72% in the anakinra arm and 50% in the standard therapy arm. Huet et al. (79) compared the data of 52 cases with COVID-19 treated with anakinra and 44 cases on standard therapy. Thirteen (25%) cases in the anakinra arm and 32 (73%) cases in the standard therapy arm had a history of acceptance to the ICU for death or invasive mechanical ventilation. As a result of this study, in severe forms of COVID-19-related pneumonia necessitating oxygen treatment, a 10-day therapy with SC anakinra was associated with a decrease in both the necessity for mechanical ventilation and death rate compared to an arm receiving standard therapy with like features.

Navarro-Millán et al. (81) examined the data of 14 patients diagnosed with COVID-19 who were given anakinra therapy. Seven of those who received anakinra therapy within 36 hours after the onset of ARDS did not need mechanical ventilation and all were discharged home. As a result of this study, it was concluded that anakinra, when introduced early after the inception of ARDS, may be useful in COVID-19 cases with signs of cytokine storm syndrome.

CONCLUSION

CP and IVIG could open a new window to the method of COVID-19 treatment, especially applied at the beginning of the disease at accurate doses, according to current literature. To decide on the role of MAB therapy in COVID-19, it seems to require further randomized controlled clinical trials.

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Intravenous Diltiazem or Metoprolol Administration in the Emergency Department for Acute Rate Control of Atrial Fibrillation Patients with Rapid Ventricular Response with Unknown Ejection Fraction

Acil Serviste Ejeksiyon Fraksiyonu Bilinmeyen Hızlı Ventriküler Yanıtlı Atriyal Fibrilasyon Hastalarının Akut Hız Kontrolünde İntravenöz Diltiazem veya Metoprolol Uygulaması

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ABSTRACT

Aim: Atrial fibrillation (AF) is the most widespread persistent cardiac arrhythmia in adults. There is no standard procedure applied in AF patients with rapid ventricular response with unknown ejection fraction (EF) in the emergency department. This study aimed to compare the effectiveness and side effects of diltiazem and metoprolol treatments without knowing the EF in AF patients with rapid ventricular response in the emergency department.

Material and Methods: Patients with a ventricular response ≥110/min were selected as having AF with rapid ventricular response. The patients first received 25 mg intravenous diltiazem as a rate control drug were compared with those first received 5 mg metoprolol. A total of 50 patients whose EF were not registered before the admission date and was measured after being consulted for cardiology following acute rate control in emergency department were included in this study.

Results: For the first drug treatment, diltiazem was given to 56% (n=28) of the patients and metoprolol to 44% (n=22). Moreover, 44% (n=22) of the patients needed a second drug infusion. The proportion of patients received diltiazem in those with preserved EF was significantly higher than those with reduced EF (p=0.032). No statistically significant difference was found between the rates of needing a second administration based on the EF (p=0.157).

Conclusion: Diltiazem was found to reduce heart rate earlier than metoprolol. While updating the guidelines for drug selection in acute rate control of AF with rapid ventricular response, rural emergency departments, where EF measurement cannot be achieved, should also be considered.

Keywords: Atrial fibrillation; diltiazem; ejection fraction; emergency department; metoprolol; rapid ventricular response.

ÖZ

Amaç: Atriyal fibrilasyon (AF) erişkinlerdeki en yaygın inatçı kardiyak aritmidir. Acil serviste, ejeksiyon fraksiyonu (EF) bilinmeyen hızlı ventrikül yanıtlı AF hastalarında uygulanan standart bir tedavi prosedürü yoktur. Bu çalışmanın amacı acil serviste EF'si bilinmeyen hızlı ventrikül yanıtlı AF hastalarında diltiazem ve metoprolol tedavilerinin etkinliğinin ve yan etkilerinin karşılaştırılmasıdır.

Gereç ve Yöntemler: Ventriküler yanıtı ≥110/dk olan hastalar, hızlı ventrikül yanıtlı AF olarak kabul edildi. Hız kontrolünde ilk ilaç olarak 25 mg intravenöz diltiazem almış olan hastalar, ilk ilaç olarak 5 mg metoprolol almış olanlar ile karşılaştırıldı. Başvuru tarihinden önce EF değeri kaydedilmemiş olan ve acil serviste akut hız kontrolünü takiben kardiyoloji bölümüne konsülte edildikten sonra ölçülen toplam 50 hasta bu çalışmaya dahil edildi.

Bulgular: İlk ilaç tedavisi olarak hastaların %56 (n=28)'sına diltiazem ve %44 (n=22)'üne metoprolol verilmişti. İkinci bir ilaç tedavisine ihtiyaç duyan hastaların oranı ise %44 (n=22) idi. EF korunmuş olanlarda diltiazem alan hastaların oranı, EF azalmış olanlara göre anlamlı derecede daha yüksek idi (p=0.032). Hastaların EF'sine göre ikinci bir uygulama ihtiyacı olma oranları arasında istatistiksel olarak anlamlı bir fark bulunmadı (p=0.157).

Sonuç: Diltiazemin kalp atım hızını metoprolole göre daha erken düşürdüğü görüldü. Hızlı ventrikül yanıtlı AF'nin akut hız kontrolünde ilaç seçimi için kılavuzlar güncellenirken, EF ölçümünün elde edilemediği kırsal acil servisler de göz önünde bulundurulmalıdır.

Anahtar kelimeler: Atriyal fibrilasyon; diltiazem; ejeksiyon fraksiyonu; acil servis; metoprolol; hızlı ventriküler yanıt.

INTRODUCTION

Atrial fibrillation (AF) is a supraventricular arrhythmia diagnosed with the presence of irregular RR intervals and the absence of regular repetitive P waves for ≥30 seconds or longer with 12-lead electrocardiography (ECG) or single-lead ECG (1).

AF is the most widespread persistent cardiac arrhythmia in adults (2). The incidence of AF in men and women increases with age (3). According to estimates based on the increase in the elderly population, it is expected that there will be 12.1 million AF patients in the United States by 2030. The incidence of symptomatic patients who admitted to the emergency department (ED) due to the increased prevalence of AF patients has also risen (4).

There is still uncertainty in AF treatment management, which is seen as a critical public health issue (5). No significant difference in mortality has been found between rate control and rhythm control in AF (6). The treatment used for rate control in AF changes according to the patient's condition, symptoms, ejection fraction (EF), and hemodynamics (1).

It is not always possible to estimate the EF in the rural ED. There is no standard procedure applied in AF patients with the rapid ventricular response (RVR) without knowing the patient's EF in the ED. Drug selection according to EF is recommended in current AF guidelines (1,7 8). There is no suggestion on the drug that can be used as the first choice in patients with unknown EF. Therefore, studies comparing the efficacy and side effects of treatment are needed to determine a standard treatment protocol for AF patients with RVR in the ED. Thus, our study aimed to compare the effectiveness and side effects of diltiazem or metoprolol treatment without knowing the EF in patients with AF with RVR in the ED.

MATERIAL AND METHODS

Study Design and Setting

The research study was planned as a retrospective cohort. Between January 1, 2018 and December 31, 2018, patients with AF with RVR admitted to a rural secondary care ED in Turkey were examined using the hospital data processing system and the ED records.

This study was approved by the non-interventional clinical researches ethics committee of Samsun Training and Research Hospital (09.12.2020, 2020/16/16). This ED treats 80,000 patients each year. Patients admitted with rhythm and rate problems had high priority and were followed up in the monitored area in the resuscitation room in the ED. Any medication or intervention administered to patients in the ED's monitored area is immediately noted on the patient follow-up form. Also, patients are in continuous ECG monitoring, and as a rule, their vital signs are recorded every half hour on the patient observation form. Emergency medicine specialists were working 24/7 in this rural ED.

Selection of the Participants

Patients aged 18 years and older, who were diagnosed with AF with RVR at the time of admission to the ED, were included in the study. Patients with a ventricular response ≥110/min were selected as AF with RVR. The patients whose EF were not registered before the admission date and whose EF was measured after being consulted for cardiology following acute rate control were retrospectively and randomly selected.

The following exclusion criteria were applied: those whose data could not be accessed through the hospital data processing system and ED records or whose information was incomplete or pregnant patients. Patients with B-type natriuretic peptides (BNP) values ≥100 pg/ml and N-terminal fragment brain natriuretic peptides (NT-Pro-BNP) ≥300 pg/ml were also excluded from the study, considering that they may have acute heart failure (9). Because the BNP and NT-Pro BNP values may not increase in obese patients, those patients' oxygen saturation levels were also examined. In the study conducted by Masip et al. (10), oxygen saturation <93% for acute heart failure was accepted as the cut-off. Based on this, patients with oxygen saturation <93% were not included in the present study. Moreover, patients presenting with unstable ventricular tachycardia, patients referred from another hospital, those with traumatic injuries, severely dehydrated patients, those with body temperature >38°C, those with an infectious disease, intoxicated patients, and those who received rate-control medications while in the ambulance, were excluded.

Measurements and Outcomes

The patients who first received 25 mg intravenous (IV) diltiazem (n=28) as a rate control drug were compared with those who first received 5 mg IV metoprolol (n=22). A total of 50 patients eligible for the study were included. Age, gender, chronic diseases, heart rate and blood pressure at presentation, EF, previous history of AF, and drugs used were analyzed.

Thirty minutes after the first drug administration, the following data recorded for each patient were examined: heart rate, blood pressure, return to sinus rhythm after medical treatment, and the treatment administered as the second drug. The following factors were also analyzed: hospitalization status, need for intensive care, complications, such as hypotension or bradycardia after medical treatment, and re-admission to the ED within seven days.

Statistical Analysis

Statistical analyses were performed using the Jamovi software program (Version 1.2.22). Descriptive statistics of the categorical variables were presented as numbers and percentages. Descriptive statistics of the numerical variables were presented as mean±standard deviation or median (interquartile range) [minimum-maximum] depending on the distribution. Mann-Whitney U test was used to compare two independent groups when the numerical variables did not show normal distribution. Pearson chi-square and Fisher's exact test were used in 2x2 tables to compare the differences between the categorical variables. Statistical significance level was accepted as 0.05.

RESULTS

The mean age of the 50 patients included in the study was 71.4±12.3 years, and 58% (n=29) were female. The mean systolic blood pressure at arrival in the ED was 124.30±26.30 mm/Hg, and the mean heart rate at arrival was 153.8±16.2 bpm. When the EF levels were examined, the levels were only reduced in 9 (18%) patients. The presence of chronic diseases and the use of medications were also examined. It was found that 82% (n=41) of the patients had chronic diseases, as follows: hypertension (HT, 42%, n=21), diabetes mellitus (DM, 26%, n=13),

history of AF (42%, n=21), coronary artery disease (CAD, 24%, n=12), and chronic obstructive pulmonary disease (COPD)-Asthma (18%, n=9). One patient (2%) had a history of stroke. Likewise, 68% (n=34) of the patients were using drugs routinely; 38% (n=19) of these drugs were beta-blockers (BBs), 22% (n=11) were calcium channel blockers (CCBs), 6% (n=3) were digoxin, and 2% (n=1) were amiodarone.

As the first drug treatment, diltiazem was given to 56% (n=28) of the patients and metoprolol to 44% (n=22). The proportion of patients who needed a second drug infusion was 44% (n=22). For the second drug infusion, 13 (59.1%) patients received metoprolol, two (9.1%) received diltiazem, three (13.6%) received digoxin, and four (18.2%) received amiodarone. Ten (20%) patients required hospitalization to the wards, and 3 (6%) patients required intensive care. Hypotension developed in 5 (10%) of the patients. Bradycardia was not observed in any of the patients. Five (10%) of the patients revisited the ED within seven days with the diagnosis of AF-RVR.

According to the patients' EF, a statistically significant difference was found between the rates of the drugs used in the first administration. Accordingly, the proportion of patients that received diltiazem in those with preserved EF was significantly higher than those with reduced EF (p=0.032). The proportion of patients that received metoprolol with reduced EF was significantly higher than those with preserved EF. However, no statistically significant difference was found between the rates of needing a second administration based on the EF of the patients (p=0.157, Table 1).

When considered separately according to EF, no statistically significant difference was found between the drugs used in the first administration and the need for the second administration (for preserved EF p=0.923; for reduced EF p=0.999). However, in patients with preserved EF, diltiazem was first administered to 26 patients, and a second administration was needed for 10 (38.5%) patients. In patients with preserved EF, metoprolol was administered to 15 patients first, and a second medication was required for 6 (40%) patients. In patients with reduced EF, diltiazem was first administered to 2 patients, and a second medication was needed for 1 (50%) patient. Metoprolol was first administered to 7 patients with reduced EF, and a second drug treatment was necessary for 5 (71.4%) patients (Table 2).

There was no statistically significant difference between hospitalization rates (p=0.665), the need for an intensive care unit (p=0.456), and the maximum heart rate change (p=0.791) according to the patients' EF (Table 3).

The median heart rate change in 30 minutes was significantly higher in patients who received diltiazem at the first administration than patients treated with metoprolol (p=0.048). When other comparisons were examined, there was no statistically significant difference in the need for second infusion (p=0.449), the occurrence of hypotension (p=0.643), and the rates of return to sinus (p=0.462) based on the drugs given (Table 4).

Table 5 demonstrates the difference between the need for second drug treatment and previous CCBs use, BBs use, or AF history. The proportion of patients with a history of AF who needed a second medication was significantly higher than those without an AF history (p=0.030).

Table 1. Distribution of diltiazem and metoprolol use and need for a second drug infusion according to EF, n (%)

	Reduced (n=9)	**Preserved (n=41)**	**p**
First drug treatment			
Diltiazem	2 (22.2)	26 (63.4)	**0.032**
Metoprolol	7 (77.8)	15 (36.6)	
Need for second drug infusion	6 (66.7)	16 (39.0)	0.157

EF: ejection fraction

Table 2. Examination of the second drug infusion need according to the first drug treatment used and the EF status

EF: Preserved	**Diltiazem (n=26)**	**Metoprolol (n=15)**	**p**
Need for second drug infusion	10 (38.5)	6 (40.0)	0.923
EF: Reduced	**Diltiazem (n=2)**	**Metoprolol (n=7)**	**p**
Need for second drug infusion	1 (50.0)	5 (71.4)	0.999

EF: ejection fraction

Table 3. The distribution rate of hospitalization, intensive care need and maximum heart rate change according to EF, n (%)

	Reduced (n=9)	**Preserved (n=41)**	**p**
Hospitalization	1 (11.1)	9 (22.0)	0.665
Need for intensive care unit	1 (11.1)	2 (4.9)	0.456
Maximum heart rate change, median (IQR) [min-max]	60 (20) [32-99]	58 (31) [8-110]	0.791

EF: ejection fraction, IQR: interquartile range

Table 4. The distribution between the first drug treatment used and the need for the second drug infusion, change in heart rate at 30 minutes, hypotension, and return to sinus rhythm, n (%)

	Diltiazem (n=28)	**Metoprolol (n=22)**	**p**
Heart rate change at 30 min, median (IQR) [min-max]	48 (42) [0-100]	28 (33) [0-78]	0.048
Need for second drug infusion	11 (39.3)	11 (50.0)	0.449
Hypotension	2 (7.1)	3 (13.6)	0.643
Return to sinus	9 (32.1)	5 (22.7)	0.462

IQR: interquartile range

Table 5. The distribution between the need for second drug infusion and previous calcium channel blocker use, beta-blocker use, and AF history, n (%)

Calcium Channel Blocker	**Yes (n=11)**	**No (n=39)**	**p**
Need for second drug infusion	4 (36.4)	18 (46.2)	0.734
Beta-Blocker	**Yes (n=19)**	**No (n=31)**	**p**
Need for second drug infusion	10 (52.6)	12 (38.7)	0.336
AF History	**Yes (n=21)**	**No (n=29)**	**p**
Need for second drug infusion	13 (61.9)	9 (31.0)	**0.030**

AF: atrial fibrillation

DISCUSSION

Diltiazem and metoprolol are the drugs most often used to treat AF with RVR in ED. The purpose of this study was to compare the efficacy and side effects of emergency medicine physicians' treatments in patients with AF with RVR with unknown EF. This research is also a call for preparing a standard treatment protocol to be used safely in AF patients with RVR with unknown EF for supporting the physicians working in rural EDs where cardiologists are unavailable.

Current guidelines for rate control of AF recommend using diltiazem or metoprolol, taking into account the patient's EF, decompensation, and hypotension status (1,7,8). Although physicians working in the ED can recognize the patient's decompensation and hypotension state alone, it may be difficult for them to predict the patient's EF. Experts reported that more than one-third of patients diagnosed with heart failure by primary care physicians without echocardiography was inaccurate according to the European Society of Cardiology (ESC) guidelines (11).

In our study, diltiazem was found to decrease the patients' heart rate more effectively at 30 minutes than metoprolol, but there was no significant difference in the need for a second drug. No significant difference was observed between diltiazem and metoprolol in terms of side effects. In the study of Hines et al. (12), there was no significant difference between the groups in the frequency of hypotension. Demircan et al. (13), showed that diltiazem and metoprolol were similar in efficacy and side effects in patients, regardless of EF; diltiazem also decreased the heart rate more quickly than metoprolol, and the reduction was greater at different time intervals. In support of this study, Memiş et al. (14), showed that diltiazem was more effective in RVR and the EF value was the determining factor for maintaining rate control. Martindale et al. (15), showed that diltiazem provides more effective rate control than metoprolol.

Heart rate control is an essential part of treatment in patients with AF. The target heart rate is still uncertain in AF rate control. AF acute rate control treatment recommendations in the 2020 ESC AF guideline are based on the patient's EF status (1). It is recommended to have a target heart rate <80 bpm during resting and a target heart rate <110 bpm during moderate exercise. In AF, BBs, CCBs, and in specific cases, digoxin and amiodarone, are the agents that can be used for acute heart rate control. According to the 2020 ESC AF guideline, in patients with an EF ≥ of 40%, metoprolol, diltiazem, or verapamil are the drugs of the first choice for acute heart rate control. BBs or digoxin use is recommended for patients with EF <40% (1). Although heart failure preserved EF patients accounted for more than half of patients with heart failure, randomized controlled trials comparing BBs to placebo are few (16). A meta-analysis published in 2014 stated that BBs should not be used as standard therapy in patients with heart failure with reduced EF plus AF (17).

The 2020 Canadian Cardiovascular Society / Canadian Heart Rhythm Society comprehensive guidelines have taken into account the patient's EF status in drugs to be used to control heart rate in AF. Unless contraindicated, BBs or CCBs in case of EF ≥40%, and BBs if EF <40% are recommended as the first choice. It also reported that metoprolol might be preferred in patients with AF with acute coronary syndrome who need rate control (7).

The American Heart Association (AHA) / American College of Cardiology (ACC) / Heart Rhythm Society (HRS) guideline recommends which agent to choose in AF rate control, paying attention to whether there is heart failure or not. The use of nondihydropyridine CCBs or BBs for acute rate control of AF patients without heart failure is suggested as a class-1 recommendation in the guideline. It reported that BBs are the most commonly used agent in acute rate control. It also emphasized that BBs' use should be avoided, especially in decompensated heart failure or acute COPD attack (8).

Atzema et al. (18), showed that the choice of drug used in RVR rate control changes depending on the size of EDs and the presence of a teaching hospital, but overall, CCBs were used more than BBs. Our investigation revealed that emergency medicine specialists tend to use diltiazem more often than other drugs.

According to the patients' EF, we found a statistically significant difference between the rates of the drugs used in the first drug treatment. The proportion of the patients with preserved EF that received diltiazem was significantly higher than those with reduced EF. On the other hand, the proportion of patients with reduced EF who received metoprolol was significantly higher than those with preserved EF.

No statistically significant difference was found between needing a second administration based on the patients' EF. Moreover, no statistically significant difference was observed between hospitalization rates and the need for an intensive care unit according to EF and the median maximum heart rate change.

It was observed that emergency medicine physicians tend to use diltiazem independently from EF. Studies can be conducted to determine why they use diltiazem. Regular training should be provided to ensure that their knowledge is up to date.

Demircan et al. (13), reported no significant difference in hypotension between patients using diltiazem and metoprolol. In our study, hypotension was observed in patients using diltiazem and metoprolol, but no statistically significant difference was observed between the groups.

As explained what to do in patients with ST-segment elevation myocardial infarction in a center without primary percutaneous intervention capacity; it should not be neglected to include additional recommendations in the guidelines for the drug treatment to be selected for rate control of AF patients with RVR in centers where EF measurement cannot be performed. For this purpose, disseminating echocardiography in emergency services and providing emergency physicians with basic eco skills may also contribute to the solution.

Most of the patients with AF in the ED investigated in this study could not be included in the data because we conducted the study in patients with unknown EF. Another limitation of the study was that it was a retrospective design. There is a need for randomized, controlled, prospective studies involving more patients.

CONCLUSION

There is a lack of information in the guidelines on how emergency physicians, especially in rural hospitals, should achieve acute rate control in AF patients with RVR with

unknown EF. We observed that emergency medicine physicians tend to use diltiazem for heart rate control more than metoprolol. We concluded that patients treated with diltiazem had a heart rate decrease more quickly than those treated with metoprolol. Furthermore, patients with a history of AF needed to be administered a second drug infusion for heart rate control.

Ethics Committee Approval: The study was approved by the Ethics Committee of Samsun Training and Research Hospital (09.12.2020, 2020/16/16).

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The Diagnostic Value of Soluble Urokinase Plasminogen Activator Receptor in Crimean-Congo Hemorrhagic Fever Disease in the Emergency Department

Acil Serviste Kırım Kongo Kanamalı Ateş Hastalığında Soluble Ürokinaz Plazminojen Aktivatör Reseptörünün Tanısal Değeri

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ABSTRACT

Aim: The urokinase-type plasminogen activator (uPA) system consists of a protease, a receptor (urokinase-type plasminogen activator receptor, uPAR), and inhibitors that can be expressed on various cell types. Previous literature shows that the amount of soluble urokinase-type plasminogen activator receptor (suPAR) secreted from affected cells is higher in Crimean-Congo hemorrhagic fever (CCHF) patients than in healthy controls. Thus, we aimed to investigate the diagnostic value of suPAR in the differential diagnosis of CCHF in emergency services.
Material and Methods: Individuals over 16 years old with a preliminary diagnosis of CCHF disease were divided into two groups as real time-polymerase chain reaction (RT-PCR) and/or IgM positive (CCHF group) and RT-PCR and/or IgM negative (control group).
Results: Eighty patients were included in this study. Forty patients with CCHF virus PCR and/or CCHF virus IgM were identified as CCHF group and 40 patients included as negative control group. The median age of the patients was 45 (range, 16-91) years, and 49 patients (61.3%) were male. Leukocyte, platelet, and fibrinogen levels were significantly lower, while creatinine kinase, aPTT, and D-dimer levels were significantly higher in CCHF group. There was no statistically significant difference between the control group and CCHF group for SuPAR (p=0.386). In addition, control group patients not diagnosed with CCHF were examined, brucellosis, influenza, and pneumonia were found to be the most common.
Conclusion: The use of suPAR as a biomarker in the differentiation of patients with similar findings in emergency services was investigated and found to have no diagnostic value.
Keywords: Hemorrhagic fever virus; Crimean-Congo; receptors; urokinase plasminogen activator; diagnosis.

ÖZ

Amaç: Ürokinaz tipi plazminojen aktivatör (uPA) sistemi, çeşitli hücrelerden salınan proteaz, reseptör (ürokinaz tipi plazminojen aktivatör reseptör, uPAR) ve inhibitörlerden oluşur. Literatürde, enfekte olan hücrelerden salgılanan solüble ürokinaz plazminojen aktivatör reseptörü (suPAR) düzeyinin Kırım-Kongo kanamalı ateşi (KKKA) hastalarında sağlıklı kontrollere göre daha yüksek olduğu gösterilmiştir. Bu çalışmada acil serviste KKKA'nın ayırıcı tanısında suPAR'ın tanısal değerinin araştırılması amaçlanmıştır.
Gereç ve Yöntemler: KKKA hastalığı ön tanısı alan 16 yaşın üstündeki bireyler gerçek zamanlı polimeraz zincir reaksiyonu (real time-polymerase chain reaction, RT-PCR) ve/veya IgM pozitif (KKKA grubu) ve RT-PCR ve/veya IgM negatif (kontrol grubu) olarak iki gruba ayrıldı.
Bulgular: Bu çalışmaya 80 hasta dahil edildi. KKKA virus PCR ve/veya KKKA virus IGM'li 40 hasta KKKA grubu olarak ve 40 hasta negatif kontrol grup olarak belirlendi. Hastaların ortanca yaşı 45 (aralık, 16-91) yıldı ve 49 (%61,3) hasta erkekti. KKKA grubunda lökosit, trombosit ve fibrinojen seviyeleri istatistiksel anlamlı olarak düşüktü, kreatinin kinaz, aPTT ve D-dimer seviyeleri ise istatistiksel anlamlı olarak yüksekti. SuPAR için kontrol grubu ile KKKA grubu arasında istatistiksel olarak anlamlı bir farklılık yoktu (p=0.386). Ayrıca KKKA tanısı almayan kontrol grubu hastaları incelendiğinde, en sık bruselloz, influenza ve pnömoni olduğu bulundu.
Sonuç: Acil serviste benzer bulgulara sahip hastaların ayırt edilmesinde bir biyobelirteç olarak suPAR'ın kullanımı araştırıldı ve tanısal değeri olmadığı bulundu.
Anahtar kelimeler: Hemorajik ateş virüsü; Kırım-Kongo; reseptörler; ürokinaz plazminojen aktivatör; tanı.

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INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a viral infectious disease with fatal outcomes that currently affects a wide geographical region, including Africa, Asia, Europe, and the Middle East (1,2). Within the last 15 years, epidemics have been reported in Turkey at levels second only to those in Iran, Afghanistan, and Russia (3).

CCHF is a viral hemorrhagic fever disease that may present with fever and bleeding that can progress to shock and severe disease with high (10-40%) mortality (4,5). CCHF virus infection first leads to virus proliferation in dendritic cells and other local tissues, followed by virus migration to regional lymph nodes and subsequent spread to a wide variety of tissues and organs, including the liver, spleen, and lymph nodes via lymphocytes and monocytes (1). Biomarkers are urgently needed for the diagnosis and prognosis of this high-mortality disease, and many studies in the literature have addressed this purpose (5-10).

One potential biomarker is the urokinase-type plasminogen activator (uPA) system. The uPA system consists of a protease, a receptor (urokinase-type plasminogen activator receptor, uPAR), and inhibitors that can be expressed on various cell types, including neutrophils, lymphocytes, monocytes/macrophages, and endothelial and tumor cells. The soluble form of the uPAR is defined as the soluble urokinase-type plasminogen activator receptor (suPAR) and involved in immunological functions ranging from cell adhesion, migration, and chemotaxis to proteolysis, immune activation, tissue remodeling, invasion, and signal transduction (11,12). The available literature indicates that suPAR secretion is greater from lymphocytes and monocytes from patients with CCHF than from healthy controls (13).

The aim of the present study was to investigate the diagnostic value of suPAR in differential diagnosis of CCHF at emergency medicine.

MATERIAL AND METHODS

The participants in this study, held from June to September 2018, were over the age of 16 admitted to our hospital's emergency service who had received a preliminary diagnosis of CCHF disease, had been diagnosed in the relevant clinics, and agreed to participate. Patients less than 16 years of age and who did not agree to participate in the study were not included. The study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital (05.03.2018, 05-35).

The medical records of the patients were retrospectively reviewed. Clinical findings and laboratory results upon admittance to emergency services were recorded. Real-time polymerase chain reaction (RT-PCR) (QiagenR CCHFV viral RNA KIT, Qiagen, Hilden Germany) and CCHF virus IgM tests with the indirect immunofluorescence test method were studied in the reference laboratory. RT-PCR and/or IgM positivity were used for the definitive diagnosis of the CCHF cases. Patients were classified as serum RT-PCR and/or IgM positive (CCHF group) and RT-PCR and/or IgM negative (control group). The clinical diagnoses made for the patients in the control group were recorded.

The serum and plasma of the blood samples taken for the examination of the patients in the emergency service were used. After the samples were taken into tubes without anticoagulants, they were kept at room temperature for 30 minutes in line with the ELISA kit instructions we used. Then they were centrifuged at 3000 rpm for 20 minutes at +4 °C. Serum remaining on top of tubes was transferred to Eppendorf tubes and lifted to -80 °C by the date of the study. SuPAR was manually studied using the ELISA Kit (cat.no: SL2132Hu) according to the kit procedures. ELISA reader was performed with ChemWell® 2910 Automated EIA and Chemistry Analyzer (Awareness Technology, USA).

Statistical Analysis

Statistical analyses were made with the SPSS v.20.0 package. Normality assumption was evaluated using histograms and the Kolmogorov-Smirnov test. Differences between two groups were analyzed using Student's t-test for the quantitative variables and the Mann-Whitney U test for the variables not distributed normally. Categorical variables were analyzed with Pearson chi-square and Fisher's exact test. Spearman correlation analysis was performed to evaluate the correlation between suPAR and other parameters. Quantitative data were expressed as median, 25%-75% quartiles, minimum-maximum or mean±standard deviation, and categorical variables as frequency (percentage). A two-sided p value of 0.05 was considered as statistically significant.

RESULTS

Two-hundred and nine patients who met the inclusion criteria were identified. While 71 of these 209 patients were diagnosed with CCHF, 138 of them were not. According to the number of kits, 80 patients admitted to the emergency service with a CCHF pre-diagnosis, 40 patients for the CCHF group and 40 for the control group, were selected randomly.

The median age of the patients was 45 (range, 16-91) years, and 49 (61.3%) patients were male. Forty patients with positive PCR and/or IgM results for CCHF virus were identified as the CCHF group, and 40 patients with disease symptoms but without CCHF positivity were designated as the negative control group. Median age was 50.5 (range, 16-91) years in control group, and 27 (67.5%) of the patients were male and 13 (32.5%) were female; while median age was 43.5 (range, 16-91) years in CCHF group, and 22 (55.0%) of the patients were male and 18 (45.0%) were female. There was no statistically significant difference between CCHF and control groups in terms of age (p=0.988) and gender (p=0.251).

The comparison of laboratory values between these two groups is presented in Table 1. Creatinine kinase, D-dimer, aPTT, fibrinogen, leukocyte, and platelet values were found to be significantly different between the CCHF and control groups. Leukocyte, platelet, and fibrinogen levels were significantly lower in the CCHF group ($p<0.001$, $p=0.006$, $p<0.001$; respectively). Creatinine kinase, activated partial thromboplastin time (aPTT), and D-dimer levels were significantly higher in the CCHF group ($p=0.001$, $p=0.005$, $p=0.013$; respectively). No statistically significant difference was detected in the SuPAR levels between the control group and the CCHF group (p=0.386). The suPAR levels of the CCHF patients were positively correlated with alanine aminotransferase (ALT) and aPTT levels ($r=0.295$, $p=0.008$, and $r=0.309$, $p=0.006$, respectively).

Table 1. Comparison of laboratory parameters in CCHF and control groups

	Control (n=40)			CCHF (n=40)			p
	Median	**Q1 - Q3**	**Min - Max**	**Median**	**Q1 - Q3**	**Min - Max**	
AST (IU/L)	47.5	22.5 - 97.5	15 - 913	53.5	32.0 - 148.75	18 - 914	0.105
ALT (IU/L)	36.5	16.0 - 79.5	7 - 853	36.5	19.0 - 88.5	15 - 604	0.516
LDH (IU/L)	303.0	235.75 - 376.25	125 - 1426	298.0	229.5 - 496	116 - 1995	0.427
CPK (mg/dL)	108.5	66 - 203	20 - 2492	247.5	128.25 - 474.75	38 - 4003	**0.001**
WBC (10^3/μL)	6539	4001 - 9750	2254 - 14440	2736	2268.5 - 3549.5	802 - 13490	**<0.001**
PLT (10^3/μL)	130100	106400 - 148800	33000 - 307200	107950	61500 - 128900	6780 - 263500	**0.006**
CRP (mg/dL)	6.33	1.33 - 11.80	0.32 - 21.00	1.61	0.32 - 3.63	0.30 - 8.08	**<0.001**
aPTT (second)	26.4	24.0 - 28.6	20.3 - 38.3	28.3	26.1 - 33.9	22.0 - 53.5	**0.005**
D-dimer	1.33	0.92 - 3.34	0.19 - 32.7	2.45	1.4 - 5.09	0.19 - 80.0	**0.013**
INR	1.13	1.05 - 1.22	0.89 - 1.90	1.1	1.01 - 1.19	0.92 - 1.82	0.128
Creatinine (mg/dl)	0.89	0.77 - 1.2	0.45 - 3.57	0.79	0.73 - 0.95	0.63 - 2.72	0.067
Fibrinogen (mg/dl)	298	250 - 384	177 - 605	223	204.75 - 265	118 - 900	**<0.001**
suPAR (pg/ml)	4740	3775 - 6905	2615 - 13935	4477.5	3108.75 - 6410	1885 - 12985	0.386

CCHF: Crimean-Congo Hemorrhagic Fever, Q1-Q3: 25th - 75th percentile, Min-Max: Minimum-Maximum, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CPK: creatine phosphokinase, WBC: white blood cells, PLT: thrombocyte count, CRP: C-reactive protein, aPTT: activated partial thromboplastin time, INR: international normalized rate, suPAR: soluble urokinase-type plasminogen activator receptor

A weak positive correlation was also found between suPAR and both aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels (r=0.264, p=0.018, and r=0.231, p=0.040, respectively, Table 2).

The most common clinical findings in CCHF patients were fever (n=38, 95.0%), headache (n=37, 92.5%), myalgia (n=37, 92.5%), weakness, nausea and vomiting (n=23, 57.5%). However, no statistically significant difference was found between the control group and CCHF patients in terms of clinical findings (Table 3).

The control group patients had brucellosis (n=7, 17.5%), influenza (n=7, 17.5%), and pneumonia (n=7, 17.5%) as the most common diagnoses (Table 4).

Table 2. Correlation of suPAR value and some biomarkers in CCHF patients

	suPAR	
	r	**p**
AST	**0.264**	**0.018**
ALT	**0.295**	**0.008**
LDH	**0.231**	**0.040**
CPK	0.048	0.670
WBC	0.051	0.656
PLT	-0.034	0.762
CRP	0.053	0.641
aPTT	**0.309**	**0.006**
D-dimer	0.030	0.792
INR	0.013	0.911
Creatinine	0.126	0.267
Fibrinogen	-0.040	0.726
Sedimentation	-0.094	0.407

suPAR: soluble urokinase-type plasminogen activator receptor, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CPK: creatine phosphokinase, WBC: white blood cells, PLT: thrombocyte count, CRP: C-reactive protein, aPTT: activated partial thromboplastin time, INR: international normalized rate

Table 3. Comparison of clinical findings in CCHF and control groups, n (%)

Clinical findings	**Control (n=40)**	**CCHF (n=40)**	**p**
Fever	35 (87.5)	38 (95.0)	0.432*
Hypotension	6 (15.0)	3 (7.5)	0.481*
Tachycardia	3 (7.5)	3 (7.5)	0.999*
Blur of Consciousness	2 (5.0)	3 (7.5)	0.999*
Ecchymosis	2 (5.0)	2 (5.0)	0.999*
Bleeding	4 (10.0)	3 (7.5)	0.999*
Headache	36 (90.0)	37 (92.5)	0.999*
Myalgia	32 (80.0)	37 (92.5)	0.105**
Nausea-vomiting	19 (47.5)	23 (57.5)	0.370**
Diarrhea	8 (20.0)	12 (30.0)	0.302**
Abdominal pain	13 (32.5)	16 (40.0)	0.485**

*: Fisher's exact test, **: Pearson chi-square test

Table 4. Distribution of final diagnoses of the control group

Final diagnose	**n (%)**
Brucellosis	7 (17.5)
Flu	7 (17.5)
Pneumonia	7 (17.5)
Isolated thrombocytopenia	4 (10.0)
Lower gastrointestinal tract bleeding	3 (7.5)
Acute gastroenteritis	2 (5.0)
Drug intoxication	2 (5.0)
Urinary tract infection	2 (5.0)
Acute hepatitis A	1 (2.5)
Acute pancreatitis	1 (2.5)
Q Fever	1 (2.5)
ITP	1 (2.5)
Acute cholecystitis	1 (2.5)
Skin, soft tissue infection	1 (2.5)

DISCUSSION

CCHF cases may show some clinical symptoms, including leukopenia, leukocytosis, anemia, thrombocytopenia, elevated AST and ALT levels, increased LDH levels, raised creatine phosphokinase (CPK) levels, prolonged prothrombin time (PT), aPTT levels, increased D-dimer levels, and elevated amounts of fibrin breakdown products (5-10). We detected elevations in thrombocytopenia, leukopenia, CK, and D-dimer levels in our CCHF group, as well as reduced levels of fibrinogen. However, these laboratory findings are known to be non-specific for CCHF (14).

There is need for an easy, inexpensive, and perfect single biomarker for the diagnosis, follow-up, and prognosis of diseases like CCHF, and studies have been conducted to search for this type of biomarker (15,16). The aim of our study was to determine the diagnostic value of suPAR in the differential diagnosis of laboratory samples and clinically similar patients in the emergency department.

Activation of lymphocytes, monocytes, and macrophages and the resulting excess secretion of cytokines have been reported to play an important role in the pathogenesis and prognosis of CCHF, as in other viral hemorrhagic diseases. Infected monocytes and lymphocytes have been shown to express high cell surface uPAR levels, and the serum suPAR level may be associated with the number of infected cells in the organism. For this reason, suPAR levels are predicted to be higher in CCHF-virus-infected patients than in healthy controls (17,18).

The expression of suPAR has been shown to increase during endotoxemia (19). Also, although its diagnostic value is lower than that of procalcitonin and C-reactive protein in sepsis (61.2% of which is bacterial), the suPAR level has been reported to increase during sepsis (20). In the cerebrospinal fluids of patients with proven central nervous system infections, suPAR levels were statistically significantly higher than in patients without infection (21). In patients with CCHF infection, compared to healthy controls, suPAR levels were found to be significantly higher, and a high diagnostic value was obtained with a threshold value of 3.06 ng/ml (13).

Biomarkers are also known to be important in predicting morbidity and mortality. In the study conducted by Yılmaz et al. (13), they found that suPAR levels were shown to have a prognostic significance (cut-off, 10.6 ng/mL) for mortality in patients with CCHF. Also, suPAR concentrations may reflect the severity of infection in other diseases. For example, suPAR levels are associated with poor outcomes in communicable and non-communicable diseases and with high mortality in malaria, tuberculosis, human immunodeficiency virus infection, and some forms of cancer (22-25). As seen in our study, no specific clinical findings and biomarkers were identified for CCHF, although suPAR has been indicated in the literature to be a diagnostic and prognostic biomarker that differentiates between patients with CCHF and healthy individuals (13). However, we found no statistically significant difference between our patients with CCHF and the control group in terms of suPAR. This situation is thought to reflect the fact that the control group selected in our study had diseases that could be confused with CCHF, both clinically and in laboratory findings. In our study, the most common diagnoses in the control group were again infectious diseases such as brucellosis, influenza, and pneumonia, and suPAR levels have been reported to increase in infectious diseases like pneumonia, brucellosis, pancreatitis, sepsis, tuberculosis, and malaria (22,26-30). In a previous study that reported a significant difference between CCHF patients and healthy individuals, a positive correlation was found between suPAR and the ALT, aPTT, AST, and LDH values in CCHF patients, as in our study (13). This result suggests that suPAR acts as another non-specific biomarker of infection and may be useful in diagnostic algorithms.

This study had some limitations, including the small number of enrolled patients, its design as a single-center study, and the lack of a healthy control group.

CONCLUSION

This study confirms the importance of isolating patients who come to the emergency service with clinical and laboratory findings resembling CCHF infection in terms of disease control and referring them to the relevant clinic. However, the use of suPAR as a biomarker appears to have no diagnostic value in differentiating patients with similar findings in clinical practice in the emergency department. Nevertheless, suPAR could be useful as a parameter in algorithm development due to its correlation with other non-specific biomarkers.

Ethics Committee Approval: The study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital (05.03.2018, 05-35).

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Effect of Supportive Positioning on COMFORT Scale Scores in Preterm Newborns

Destekleyici Konumlandırmanın Preterm Yenidoğanlarda COMFORT Ölçek Puanlarına Etkisi

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ABSTRACT

Aim: Premature babies are vulnerable to environmental stress factors mostly in the first weeks of life. During this time, supportive positioning, especially used all-around the baby, makes them feel better as if they are in utero. The aim of the study was to evaluate the effect of supportive positioning on weight gain, vital signs, feeding intolerance, duration of ventilation, duration of hospitalization and comfort scale scores of the premature babies in neonatal intensive care unit.

Material and Methods: A total of 50 premature infants were recruited into the study randomly, 25 in the supported group and 25 in the control group. The babies in the supported group were nested with soft blankets and pillows as position material. There was no nesting or swaddling in the control group. Demographic findings, comfort scale scores, heart rate, respiratory rate and oxygen saturation of infants were recorded and compared.

Results: Mean gestational weeks and birth weights of the supported and control groups were 32.9±2.5 (26-36) vs. 32.7±2.8 (26-36) weeks (p=0.791) and 1554±492 (680-2380) vs. 1772±439 (590-2375) g (p=0.105), respectively. Weight gain, ventilator days and days of hospitalization were similar in groups, however mean oxygen saturation and comfort scale scores showing deep sedation were higher in the supported group (p=0,024, p<0,001, respectively) after daily care.

Conclusion: Although supportive positioning does not have an effect on duration of hospitalization, ventilation and weight gain, it has a positive effect on mean oxygen saturation and comfort scale scores of premature infants and recommended in newborn care.

Keywords: Nursing care; intensive care units; neonatal; preterm birth; patient comfort.

ÖZ

Amaç: Prematüre bebekler, çevresel stres faktörlerine karşı özellikle yaşamın ilk haftalarında daha savunmasızdır. Bu süre zarfında, özellikle bebeklerin etrafında kullanılan destekleyici konumlandırma, kendilerini uterus içinde olduğu gibi daha iyi hissetmelerini sağlar. Bu çalışmanın amacı, yenidoğan yoğun bakım ünitesinde destekleyici konumlandırmanın; prematüre bebeklerin kilo alımı, yaşamsal belirtileri, beslenme intoleransları, ventilasyon süreleri, hastanede kalış süreleri ve konfor ölçeği puanları üzerine etkisini değerlendirmektir.

Gereç ve Yöntemler: Çalışmaya rastgele olarak seçilen, 25'i desteklenen grupta ve 25'i kontrol grubunda olmak üzere toplam 50 prematüre bebek dahil edildi. Desteklenen gruptaki bebekler pozisyon malzemesi olarak yumuşak örtüler ve yastıklarla yuvalandı. Kontrol grubunda yuvalama ya da kundaklama yoktu. Bebeklerin demografik özellikleri, konfor ölçeği skorları, kalp hızı, solunum hızı ve oksijen satürasyonu kaydedildi ve karşılaştırıldı.

Bulgular: Desteklenen grup ve kontrol grubunun sırasıyla ortalama gestasyonel haftaları 32,9±2,5 (26-36) ve 32,7±2,8 (26-36) hafta (p=0,791), ortalama doğum ağırlıkları ise 1554±492 (680-2380) ve 1772±439 (590-2375) gramdı (p=0,105). Kilo alımı, ventilatör günleri ve hastanede kalış günleri gruplar arasında benzerdi, ancak ortalama oksijen satürasyonu ve derin sedasyon gösteren konfor ölçeği skorları desteklenen grupta günlük bakım sonrası daha yüksekti (sırasıyla; p=0,024, p<0,001).

Sonuç: Destekleyici konumlandırmanın hastanede kalış süresi, ventilasyon ve kilo alımı üzerine etkisi olmamakla birlikte prematüre bebeklerin ortalama oksijen satürasyonu ve konfor ölçeği puanları üzerinde olumlu etkisi vardır ve yenidoğan bakımında önerilmektedir.

Anahtar kelimeler: Hemşirelik bakımı; yoğun bakım üniteleri; yenidoğan; erken doğum; hasta konforu.

INTRODUCTION

High level of comfort in premature babies include that they are exposed to less stress and means that they are more stable in terms of behavioral (agitation, alertness, crying, facial expression, and muscle tone) and physiological (heart ratio) situations (1). Especially in premature babies younger than 32 weeks, the organization of sleep and wakefulness is limited since the central nervous system is not yet mature. For this reason, it is very important to provide and maintain their comfort (2,3).

As premature babies are more hypotonic compared to term babies, they do not have adequate muscle strength and tone at birth. This often causes them to maintain their bodies in extended positions. Neonatal intensive care unit (NICU) professionals attempt to encourage flexed position using various methods. Care positioning such as swaddling the infant in a blanket, as well as using blankets and cloth rolls to create boundaries or a nest around the infant, facilitated tucking or regular changes in positioning have been shown to positively impact neuromuscular development, improve motor performance and postural development and improve movement across midline (4). Swaddling/tucking was found to be effective in reducing pain in premature infants (5).

In recent years, a series of observational tools have been developed to measure stress and pain (6,7). The comfort scale which is well-known multidimensional tool, is originally developed as a continuous measure of distress, sedation and pain in nonverbal pediatric patients aged from birth to 18 years (8,9).

In the present study we investigated the effect of supportive positions swaddling and nesting on weight gain, oxygen saturation, comfort scale scores, days of ventilation and days of hospitalization in preterm babies in NICU.

MATERIAL AND METHODS

This study was conducted in NICU of Çukurova University, Balcali Research and Training Hospital. The sample size per group to find two unit difference between groups was calculated based on information on the comfort scale scores of the newborns in the standard position found in the literature were found as 13.73±2.77 (10). At standard settings, α=0.05, β=0.20 (power=80%) and assuming the standard deviation=3, the sample size was calculated as 22 observations per group. We expected 20 percent loss of follow due to any reason and decided to randomize 54 infants. Small envelopes with information on group were placed in a box and randomization was done for every new infant, which met the inclusion criteria. At the end of study, we included one more infant to the supported group and closed our randomization (28 cases in supported group and 27 cases in control group). Premature babies (gestational age ≤36 weeks 6 days) who did not take analgesic, muscle relaxant or inotropic medications, did not have a serious neurological disease, had spontaneous breathing, were born in our hospital, and whose parents gave their consent to participate voluntarily were included in the study. Infants with TORCH infections, chromosomal abnormalities, major congenital abnormalities and cardiac defects, metabolic diseases, hydropic babies, outborn babies, and infants without parent consent were excluded. The babies in supportive group had nest around them. Soft blankets and pillows were used as position materials. These babies were also loosely swaddled with a blanket stimulating natural fetal position that facilitates flexion, hand-to-mouth positions, and containment of extremities. Body temperature was followed in incubators at baby mode in both groups. The control group had no nest or swaddling. All of the babies in this study had rolling pillow under shoulders to keep semi-extension of the head and were supported in left, right, prone or supine positions. Positions of the babies have been changed every 3 hour and the head side of the incubator was kept at 30°.

Demographic findings, surfactant use, ventilator use, oxygen use, comfort scale scores, weight gain, nosocomial infection rate, feeding intolerance, heart and respiratory rate and oxygen saturations were recorded. Comfort scale scores, heart rate, respiratory rate and oxygen saturation of infants were recorded half an hour after the routine daily care at 10.00 a.m. until discharge. Comfort scale score was not performed in infants who were getting sedation or analgesic drugs. 8-16 points indicate deep sedation, 17-26 points indicate adequate sedation, 27-40 points indicate inadequate sedation in comfort scale.

Postnatal first 10 days were neglected while calculating the daily weight gain as most of the preterm infants reach their birth weight approximately in the second week of life. Gastric residual ≥1/3 of each feeding was accepted as feeding intolerance. Ethical Approval was given by the Çukurova University Ethical Board Committee (14.02.2013 and 16/56). Information and explanation were provided to the parents, and written informed consent was obtained from those who agreed to participate in the study.

Statistical Analysis

The data were analyzed using SPSS v.21.0. In the comparison of groups in terms of categorical data, Pearson chi-square, Fisher's exact and Fisher-Freeman-Halton tests were used, as appropriate. In comparison of the continuous variables, normality assumption was tested with Kolmogorov-Smirnov test on a group basis. The t-test was used for variables with normal distribution, and the Mann-Whitney U test was used when the normality assumption could not be achieved. Mean±standard deviation and/or median (min-max) values were used in summarizing the continuous variables. Categorical variables were summarized using count and percentage. $p \leq 0.05$ was accepted as statistically significant.

RESULTS

There were 28 and 27 newborns in supported and control groups respectively, however 2 patients died in the first week of life and 3 parents refused to participate and were excluded. There were 25 infants in both groups. All infants survived and discharged from the unit. The gestational ages of the patients in the supported and control groups were 32.9±2.5 and 32.7±2.8 weeks, respectively; birth weights were 1554±492 g and 1772±439 g, respectively, and there was no statistically significant difference between the groups in these respects (p=0.791, p=0.105, respectively). Also, there were no statistically significant difference between groups in terms of gender, ventilator use, surfactant treatment, nosocomial infection rate, feeding intolerance and duration of hospitalization. Characteristics and comparisons of the patients according

to their groups are shown in Table 1. When the saturation value of all 25 patients (100%) in the supported group was 93% and above; the saturation value of 4 patients (16%) in the control group was below 93%, and when the mean oxygen saturation values were compared, a statistically significant difference was found between the two groups in this respect (p=0.024). The comfort scale scores of 17 (68%) patients in the supported group were between 8-16, while 13 (52%) patients in the control group had comfort scale scores between 17-26, and when the groups were compared in this respect, the comfort scale scores of the supported group were found to be statistically lower (p<0.001). The vital signs and comfort scale scores of both groups were shown in Table 2.

DISCUSSION

Preterm babies show signs of physiological or behavioral stress and pain symptoms. To manage these situations, non-pharmacological and pharmacological methods can be used. Appropriate supportive positioning method is considered an important non-pharmacological intervention for reducing pain responses (11). Nesting positioning is a key factor for the neonate to maintain the appropriate position, making the babies to feel safer and physiologically more stable as they often get used to this position in-utero. Also, they feel comfortable by sucking their fingers and grasping their hands together (12). In a meta-analysis, combined use of a postural support roll and support nappy was shown to improve hip and shoulder position in premature babies, reduce energy expenditures and conserve effort for maximum development and growth (13).

Minimizing energy expenditure while promoting a balance between flexion and extension of any infant are goals of the developmentally supportive care giving practices. In this study we aimed to evaluate the effect of nesting technique and swaddling on comfort scale score and physiological functions of premature infants. We obtained significantly higher oxygen saturation and lower comfort scale scores in nested and swaddled babies. As comfort scale scores increased, we would expect more weight gain in the supported group. Similarly Cole and Gavey (14) emphasized that the effect of the practice of nesting position helps to increase calm and comfort of babies so that they can maintain weight gain. However there were no differences in terms of ventilator use, weight gain and hospitalization days between the groups in the present study.

Developmentally supportive care procedures reduces especially the iatrogenic complications of newborn intensive care for infants and provide the infant's competence, the staff's role satisfaction and the parents' confidence. In addition, many studies reported that all available practices of various developmental supportive care procedures showed positive results for babies such as improved lung function, reduced hospital stay, feeding behavior and growth, improved neurobehavioral, neurophysiological and neurostructural functioning (15,16). El Nagger and Bayoumi (17) reported more normal heart rates in the nested group in supine positions; and more normal respiratory rate during supine, side-lying and prone positions. On the other hand, different studies on this subject have shown that positioning has no effect on the heart rate or respiratory rate in preterm babies (18-20). The results of the current study showed no statistically significant difference regarding the mean respiratory rate and heart rate between supported and control groups. As prone position enables better saturation, it would be better to compare respiratory rates in different positions.

The results of our study showed that premature infants in the supported group had better SpO_2 levels compared to the control group. These results were in agreement with the studies which showed positive effects for the newborns through improving lungs and neurophysiological functioning (15). In painful and stressful procedures, oxygen consumption increases and oxygen saturation decreases, also an increase in heart rate may be expected. While nesting positions increased the oxygenation, it did not affect heart rate and respiratory rate in our study similar to the studies (15).

Developmental care positioning provides normal musculoskeletal and postural development, protects patients' airway and supports their thermal regulation.

Table 1. The characteristics of the groups

	Supported (n=25)	Control (n=25)	
	Mean±SD	**Mean±SD**	**p**
Gestational age (weeks)	32.9±2.5	32.7±2.8	0.791[a]
Birth weight (g)	1554±492	1772±439	0.105[a]
Daily weight gain (g)	15.5±7.5	14.8±6.1	0.719[a]
Hospitalization (days), median (min-max)	20.6±13.9 14 (4-57)	15.5±11.9 12 (3-48)	0.216[b]
	n (%)	**n (%)**	**p**
Gender			
Male	10 (40)	11 (44)	0.774[c]
Female	15 (60)	14 (56)	
Surfactant use	5 (20)	6 (24)	0.733[c]
Ventilator use (invasive)	6 (24)	8 (32)	0.529[c]
NCPAP use	10 (40)	13 (52)	0.395[c]
Hood use	12 (48)	12 (48)	-
Nosocomial infection rate	10 (40)	7 (28)	0.370[c]
Feeding intolerance	12 (48)	8 (32)	0.248[c]

SD: standard deviation; [a]: t test, [b]: Mann-Whitney U test, [c]: Pearson's chi-square test

Table 2. Vital signs and comfort scale scores of groups

	Supported (n=25)	Control (n=25)	p
Heart rate			
100-160/min	24 (96)	23 (92)	0.999[a]
≥161/min	1 (4)	2 (8)	
Mean respiratory rate			
40-60/min	24 (96)	22 (88)	0.609[a]
≥61/min	1 (4)	3 (12)	
Mean SpO_2			
88-92%	0 (0)	4 (16)	
93-96%	7 (28)	11 (44)	0.024[b]
97-100%	18 (72)	10 (40)	
Comfort scale score*			
8-16 points	17 (68)	3 (12)	
17-26 points	7 (28)	13 (52)	<0.001[b]
27-40 points	1 (4)	9 (36)	

[a]: Fischer's exact test, [b]: Fischer-Freeman-Halton test, *: 8-16 points indicate deep sedation, 17-26 points indicate adequate sedation, 27-40 points indicate inadequate sedation

Premature infants who have developmentally positioning cry less, have less flailing of their extremities, have low pain scores and improved physiologic outcomes and sleep states (21). Swaddling is effective also in pain relief (5,22,23). Comaru and Miura (24) reported significantly less pain scores and less distress for babies nested compared to non-nested ones. Loose swaddling of newborns during interventional procedures was found to be effective on physiological and behavioral pain responses (25,26). In their study in 2020, Özdel and Sarı (27) found that the mean comfort scores and the mean distress scores were lower in the supported position (such as kangaroo care position) than in the prone position. We have also showed better comfort scale scores in the supported group.

CONCLUSION

In our study we reported that swaddling and nesting do not have any influence on weight gain, feeding tolerance and ventilator support duration. However applying nesting technique as a developmental care has a positive effect on oxygen saturation and comfort scale scores of premature infants. Supportive positions have benefits for providing sedation for the babies in neonatal intensive care units and are suggested for better oxygenation.

Ethics Committee Approval: The study was approved by the Ethics Committee of Çukurova University Faculty of Medicine (14.02.2013, 16/56).

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HIV Seropositivity in Patients Admitted to Our Hospital: Six Year Evaluation

Hastanemize Başvuran Hastalardaki HIV Seropozitifliği: Altı Yıllık Değerlendirme

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ABSTRACT

Aim: Detecting human immunodeficiency virus (HIV)-infected individuals is very important in terms of quality of patients' lives. However, it should be kept in mind that there may be false positives and tests must be confirmed. In this study, it was aimed to determine HIV seropositivity in our hospital for a period of six years and to contribute to the epidemiological data of the country.

Material and Methods: The samples that came to serology laboratory between January 2014 and December 2019 were analyzed retrospectively. Anti-HIV antibodies were screened in a total of 180,413 samples by the macro-ELISA method (Architect i2000sr, Abbott, USA). Patient samples were sent to the National HIV/AIDS Confirmation and Viral Hepatitis Reference Laboratory for verification of reagents results.

Results: Anti-HIV results of 178 (0.1%) of 180,413 patients whose blood serum samples were studied with the macro-ELISA method were evaluated as reactive. Confirmation test of 102 patients were negative while 76 were positive. Patients with positive confirmation result account for 0.04% of all patients. While 67 (88.2%) of the patients with positive confirmation were male and 9 (11.8%) were female. The ages of 26-35 and 36-45 years were equal with the highest number of new cases detected (n=20, 26.3%).

Conclusion: As a result of our retrospective evaluation, HIV seropositivity, gender and age distributions in our hospital were found to be compatible with the data of our country and other studies conducted in our country. The high false positivity rate of screening tests, again indicates the importance of confirmation tests.

Keywords: Anti-HIV; ELISA; HIV verification.

ÖZ

Amaç: İnsan bağışıklık yetmezliği virüsü (human immundeficiency virüs, HIV) ile infekte bireylerin tespit edilmesi yaşam kalitesi açısından oldukça önemlidir. Ancak yalancı pozitifliklerin olabileceği ve testlerin mutlaka doğrulanması gerçeği unutulmamalıdır. Bu çalışmada hastanemizdeki altı yıllık süre zarfında HIV seropozitifliğinin saptanması ve ülke epidemiyolojik verilerine katkı sağlanması amaçlanmıştır.

Gereç ve Yöntemler: Ocak 2014 ve Aralık 2019 tarihleri arasında seroloji laboratuvarına gelen örnekler geriye dönük olarak incelenmiştir. Toplamda 180,413 örnekte makro-ELISA yöntemi ile (Architect i2000sr, Abbott, USA) anti-HIV antikoru taranmıştır. Reaktif gelen sonuçların hasta örnekleri doğrulanmak üzere Ulusal HIV-AIDS Doğrulama ve Viral Hepatitler Referans Laboratuvarı'na gönderilmiştir.

Bulgular: Makro-ELISA yöntemi ile kan serum örnekleri çalışılan 180,413 hastadan 178 (%0,1)'inin anti-HIV sonucu reaktif olarak değerlendirilmiştir. 102 hastanın doğrulama testi negatif iken 76'sı pozitifti. Doğrulama testi sonucu pozitif olan hastalar tüm tetkik edilen hastaların %0,04'ünü oluşturmuştur. Doğrulama testi sonucu pozitif olan hastaların 67 (%88,2)'si erkek iken 9 (%11,8)'u ise kadındır. Yaş dağılımı incelendiğinde ise 26-35 yaş ve 36-45 yaş aralıkları eşit oranda olup en fazla (n=20, %26,3) yeni vaka tespit edilen yaş aralıkları olmuştur.

Sonuç: Yapılan geriye dönük bu değerlendirmemiz sonucunda, HIV seropozitifliği, cinsiyet ve yaş dağılımlarının ülkemiz verileri ve ülkemizde yapılan diğer çalışmaların verileri ile uyumlu bulunmuştur. Tarama testlerindeki yalancı pozitifliğin oldukça yüksek olması, doğrulama testlerinin ne denli önemli olduğunu bir kez daha hatırlatmıştır.

Anahtar kelimeler: Anti-HIV; ELISA; HIV doğrulama.

INTRODUCTION

All around the world and in our country, the number of individuals infected with Human Immunodeficiency Virus (HIV) tends to increase, and the rates are considered to be much higher than those tested. According to the Joint United Nations Program on HIV/AIDS (UNAIDS), it was reported that there were 37.9 million people infected with HIV to date, 1.8 million individuals had been infected with HIV and 770,000 individuals had died due to acquired immunodeficiency syndrome (AIDS) in 2018 (1). In our country, it was found that 19,748 individuals had been infected with HIV since the first case seen in 1985 until the end of 2018 and 1772 of them had progressed to AIDS (2). HIV targets immune system cells, mainly CD4 T lymphocytes. After the virus enters the human body, it leads to clinical manifestations of acute and chronic HIV infection. Unless the individual receives early diagnosis and treatment, AIDS occurs with various concomitant opportunistic infections and malignancies accompanying (3). Two different viral strains, whose nucleotide sequences show 40% similarity to each other and both of which can progress to the clinical manifestations of AIDS, were isolated all around the world. While HIV-1 is the most common type all over the world, HIV-2 is seen as endemic in West Africa (4).

The virus is transmitted through sexual contact, blood and blood products, vertical transmission from mother to baby, and tissue-organ transplantation. Transmission among healthcare workers can also occur due to their occupational risk. Those who have more than one sexual partner, particularly the male who have sex with male, injecting drug users, babies born from HIV-infected mothers play a major role in viral transmission (5). Recognizing the risk groups and receiving the early diagnosis and treatment are essential in order to ensure the quality of life of individuals and community. In this context, in order to control the HIV epidemic, implementing the 90-90-90 targets (Diagnosing 90% of HIV-infected people, initiating antiretroviral therapy in 90% of people diagnosed, providing viral suppression among 90% of patients who receive antiretroviral therapy) holds great importance for the UNAIDS organization (6).

For disease control, early, accurate and rapid diagnosis and initiation of treatment as soon as possible is the key point in terms of reducing the individual's mortality and morbidity as well as preventing transmission among the community (7).

The aim of this study was to evaluate the anti-HIV seropositivity and the results of confirmation tests in patients admitted to our hospital between 2014 and 2019 and so to contribute to Turkey's epidemiological data.

MATERIAL AND METHODS

Anti-HIV test results of 180,413 patients, who admitted to Recep Tayyip Erdoğan University Faculty of Medicine Training and Research Hospital and got examined for miscellaneous reasons between the years 2014 and 2019, were retrospectively analyzed. The patients' reagent samples, which were repeatedly detected, were excluded in the study. Regardless of age and sex, anti-HIV test samples from all clinics were included in the study.

The samples delivered to our laboratory were first centrifuged at 4000 rpm for 20 minutes and then serum was obtained. Subsequently, anti-HIV antibody was analyzed by macro-ELISA method using Architect i2000sr Chemiluminescence Microparticle Immunoassay (CMIA; Abbott, USA) System. The kit used, which detects HIV p24 antigen and HIV type 1 (HIV-1 group M and group O) and HIV type-2 antibodies, is a fourth generation. Depending on the manufacturer's recommendations, samples with values below 1 s/co were evaluated as 'non-reactive', whereas samples with values of 1 s/co and above were considered as reactive. A new sample was requested from the patients whose anti-HIV test results were reactive, and the test was repeated in both samples of the patients. Non-reactive results were reported as non-reactive. The samples which both tests were detected as reactive, were sent to the Republic of Turkey Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products, National HIV-AIDS Confirmation and Viral Hepatitis Reference Laboratory for confirmation tests.

Confirmation was performed with the LIA (INNO-LIA ™ HIV I/II Score; Innogenetics, Ghent, Belgium) method at the National HIV-AIDS Confirmation and Viral Hepatitis Reference Laboratory. Within this method, the strips in which HIV-1 -and HIV-2- specific recombinant proteins and synthetic peptides, and HIV-1 group O- specific synthetic peptides are replaced are being used. In case of the presence of antibodies against the protein and peptides in the strip of the sample tested, bands are seen.

At the reference center, after the year 2016, although the sample shows ELISA reactivity, in case of indeterminate or negative results taken with LIA, the sample sentare being examined for HIV-1 RNA PCR (artus HI virus-1 RG RT-PCR, Qiagen; Hilden, Germany) in terms of acute HIV infection.

Ethics committee approval for this study was obtained from the Non-Interventional Clinical Research Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (01.10.2020, 209).

Statistical Analysis

The data were summarized as number and percentage. SPSS v.21.0 statistical package was used for statistical data analysis.

RESULTS

The mean age of 180,413 patients evaluated in the study was 50 years, 93,598 (51.9%) patients were male and their mean age was 49 years. 86,815 (48.1%) patients were female and their mean age was 51 years. When we evaluated the distribution of the tests by years, it was found that the highest rate with 33,449 (18.5%) was in 2018. The distribution of the number of patients requested tests and test positivity by years were shown in Table 1. The majority of samples delivered to the laboratory, 21,610 (12%), was belonged to the gastroenterology clinics. The highest number of positive patients was in 2019 with 20 patients.

It was determined that 178 (0.1%) of the patients, whose Anti-HIV antibody was analyzed with macro-ELISA method, were reactive and 180,235 (99.9%) of them were non-reactive. 112 (62.9%) of the reactive results were of male patients. Infectious Diseases and Clinical Microbiology Outpatient Clinics had the highest number of reactive results with 37 (20.8%) patients.

Table 1. Distribution of HIV tests by years

Years	Number of patients	Anti-HIV reactive	Anti-HIV non-reactive	Positive Confirmation Test	Negative Confirmation Test
2014	27 727	23	27 704	10	13
2015	27 263	30	27 233	6	24
2016	30 213	23	30 190	10	13
2017	31 331	29	31 302	16	13
2018	33 449	38	33 411	14	24
2019	30 430	35	30 395	20	15
Total	180 413	178	180 235	76	102

For confirmation tests, 102 (57.3%) of the samples sent to the National HIV/AIDS Confirmation and Viral Hepatitis Reference Laboratory were negative, and 76 (42.7%) of them were positive. Patients with positive confirmation test results comprised 0.04% (n=76) of all patients (Table 2). As 67 (88.2%) of the positive patients were male, the majority of these individuals consisted of 28 (36.8%) patients followed at the Infectious Diseases and Clinical Microbiology Outpatient Clinics. The highest number of false reactive patients was detected to be at the Internal Medicine Clinic with 10 (9.8%) patients. The distribution of positive patients by clinics was shown in Figure 1. While the mean age of patients with positive confirmation tests was 41.5 years, the age range of the patients was between 18-81 years. When we analyzed the age distribution of infected individuals, it was found that the age groups of 26-35 years and 36-45 years had equal percentages and the highest number of newly detected cases with 26.3% (n=20) were belong to this ranges (Figure 2). As the results of macro-ELISA method were examined according to the confirmation tests, the lowest Anti-HIV value as true reactive was determined as 9.36 s/co, and the highest Anti-HIV value as false reactive was determined as 173.92 s/co.

Table 2. Confirmation test results of anti-HIV reactive samples

		Macro-ELISA Test Results	
		Reactive	**Non-reactive**
Confirmation Test Results	**Positive**	76 (0.04%)	-
	Negative	102 (0.05%)	180 235 (99.9%)
	Total	178 (0.1%)	180 235 (99.9%)

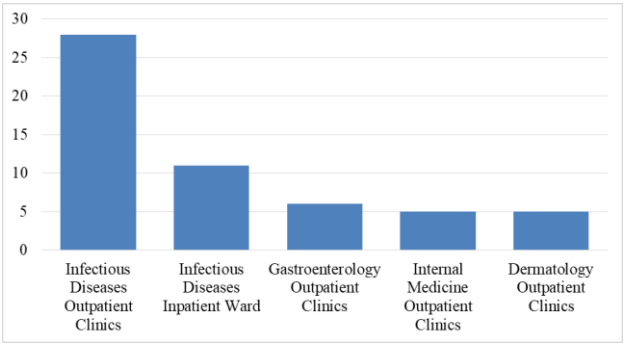

Figure 1. Distribution of HIV-positive patients by clinics

DISCUSSION

Despite all the progress made and all the measures taken, HIV infection and clinical presentation of AIDS continue to seem as a serious public health problem all over the world, particularly in developing countries. According to UNAIDS data, while 61% of the newly infected people with HIV were from Sub-Saharan African countries, African countries constitute the largest group with 25.7 million infected people worldwide in 2018 (1). It is very important to have information about the epidemiological data at country level in order to determine the shortcomings and to take the necessary measures. Additionally, early initiation of treatment by diagnosing individuals can increase the quality of life and contribute to the countries' level of development. In this context, our aim to make contribution to medical literature by presenting our own data.

Serological methods are used most frequently for the diagnosis of HIV infection, whereas ELISA method is used as screening test, and Indirect Immune Fluorescent Antibody Test (IFA), Line-Immunoassay (LIA), Western Blot, and HIV-1/2 antibody differentiating rapid confirmation tests are used as confirmation tests (4). In recent years, fourth generation combo tests, which detect the p24 antigen that emerge approximately one week before antibodies exist, have become routine as screening tests, besides HIV antibodies (7). Western Blot has been commonly performed as confirmation test, but it has been replaced by HIV-1/2 antibody differentiating rapid confirmation tests in the world and in our country due to its low sensitivity rate, excess of cross-reactions and demanding test properties.

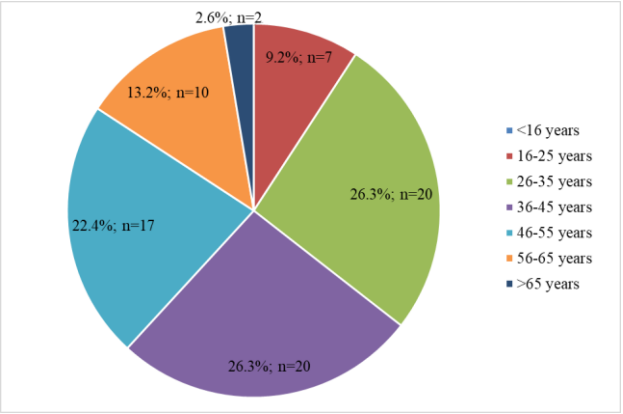

Figure 2. Distribution of HIV confirmed positive patients by age

Although the most common cause of HIV transmission worldwide is sexual transmission, the leading cause of transmission is homosexual contact in developed countries and heterosexual contact in underdeveloped countries (5). According to our country data, heterosexual sexual transmission ranks first among HIV-infected individuals (2). The study data were evaluated retrospectively, and the fact that transmission routes were not reached from epidemiological data is one of the limitations of this study. In some studies conducted in Turkey, as the distribution by gender are examined, it was found that HIV seropositive patients with rates varying between 75.5% and 84.7% mostly consisted of male patients (6,8-19). In our study, 62.9% of HIV seropositive patients were male in majority, as in other studies. It has been reported worldwide that there are 18.8 million female and 17.4 million male patients, making female patients proportionally higher. This may be due to social reasons since as our country has a male-dominated society.

As the studies conducted in Turkey are evaluated without selecting a particular group, HIV seropositivity rate was found to be 0.04% and 0.08%, respectively, in two different studies (8,17) conducted in Izmir. In two different studies (14,18) conducted in Ankara, HIV seropositivity rate was 0.087% and 0.006%, respectively, on the other hand, in a study conducted in Istanbul (19), it was found to be 0.068%. According to the studies conducted in our provinces of Artvin (20), Balıkesir (21), Isparta (22) and Kars (23), HIV seropositivity rate was reported as 0.05%, 0.004%, 0.01%, and 0.009%, respectively. We observed that similar results were obtained in other provinces with a HIV seropositivity rate of 0.04% in our study. Eventhough, the study is one of the first studies evaluating seropositivity in the province of Rize, it should be supported with the other hospitals' data in a way to draw the profile of the entire province.

It is an obvious fact that HIV transmission is higher in certain age groups. Different rates have been reported within studies conducted throughout the country and around the world (24-27). In the data from Ministry of Health, the age group of 25-34 years draws attention as the age group with the most cases (2). This circumstance is considered to be related to the regional location of Rize province. In particular, it suggests that partner transmission should be investigated and relevant measures should be taken.

Confirmation test results with ELISA show differences. In previous studies conducted in Izmir (8), Istanbul (19) and Sakarya (6), the true positivity percentages were determined as 41%, 74%, and 68%, respectively. In our study, true HIV positivity was found to be 42% in anti-HIV reactive patients. We suppose that this situation may have stemmed from the unnecessary test requests by the clinicians at our hospital. The fact that the healthcare workers had tests to feel safe, especially during the pre-invasive interventions, and the individuals thinking that they carry risk in terms of sexual transmission due to their location. Similarly, it can be explained by the difference between the kits used for analysis, compared to other hospitals. Toptan et al. (6) found the highest value of 13.3 s/co as false reactive and the lowest value as true reactive, on the other hand, was found to be 5.29 s/co. Considering that we detected the lowest value 9.36 s/co as true reactive, it was in parallel with the mentioned study. Nevertheless, the highest Anti-HIV value 173.92 s/co, which we found as false reactive differ significantly, compared to Toptan et al.'s (6) study. In this context, attention should be paid to ensuring the controls of laboratory data follow-up by reference centers. In our study, there are limitations such as inability to differentiate between HIV types, unavailability to determine the distribution of transmission routes, and lack of information about the clinical processes of the patients. All the information gives the key points that provide support in the disease control. We consider that more medical studies, in which laboratory data are evaluated in conjunction with clinical aspects, patients are followed up with epidemiological forms, and shortcomings are determined, seem essential.

CONCLUSION

Surveillance studies conducted are of great importance for disease control. The study is one of the very first studies in which patients were evaluated in relation to HIV infection in the province of Rize, and the study data are largely compatible with other studies carried out throughout our country. However, the study should be assisted by using data from which patients are also clinically evaluated in terms of HIV infection. For all that, we consider that it is critical to remind the correct use of diagnostic tests for the disease, the need to confirm the reactive samples within the screening tests and the fact that unnecessary tests shouldn't be requested.

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Bibliometric Analysis of COVID-19 Publications in the Field of Chest and Infectious Diseases

Göğüs ve Enfeksiyon Hastalıkları Alanındaki COVID-19 Yayınların Bibliyometrik Analizi

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ABSTRACT

Aim: At the context of the chest and infectious diseases, the main goal of this study is to make a bibliometric analysis of publications on coronavirus disease 2019 (COVID-19). Visualizing it with visible and scientific mapping techniques is the secondary goal.
Material and Methods: Raw data for 2020 have been downloaded from the Web of Science Core Collection database. A total of 787 articles were reviewed. Raw data were analyzed with Bibliometrix and VOSviewer. The articles about COVID-19, related with the respiratory system and infectious diseases were included. The perspectives of other disciplines were excluded with the analysis.
Results: A total of 787 articles were published in 108 different journals. The average number of citations per article is 10.17. There are four studies with over 300 citations. The top three authors with the highest H index are Raoult D, Colson P and Rolasin JM. The h, g and m indices of the authors were calculated and the core authors were determined according to Lotka's law. The top three countries that publish the most articles are China, America and Italy. Finally, according to the word mining analysis, it was determined that the studies can be classified under three clusters.
Conclusion: One of the tools that will accelerate the basic reading process in the face of the numerical increase rate of publications on COVID-19 is the bibliometric analysis results. The most up-to-date and basic information on treatment options can be found collectively in bibliometric studies.
Keywords: Bibliometric; COVID-19; infectious diseases; respiratory system.

ÖZ

Amaç: Bu çalışmanın temel amacı, göğüs hastalıkları ve enfeksiyon hastalıkları alanında, koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) ile ilgili yayınların bibliyometrik analizini yapmaktır. İkincil amaç ise yayınların analizini görsel ve bilimsel haritalama teknikleriyle görselleştirmektir.
Gereç ve Yöntemler: 2020 yılına ait ham veriler, Web of Science Core Collection veri tabanından indirilmiştir. Toplamda 787 makale gözden geçirilmiştir. Ham veriler Bibliometrix ve VOSviewer yazılımları ile analiz edilmiştir. COVID-19 ile ilgili makalelerin sadece solunum sistemi ve enfeksiyon hastalıkları ile ilgili olanları bu analize dahil edilmiştir. Diğer disiplinlere ait bakış açıları ise analize dahil edilmemiştir.
Bulgular: Toplam 787 makale 108 farklı dergide yayınlanmıştır. Makale başına düşen ortalama atıf sayısı 10,17'dir. 300'ün üzerinde atıf alan dört adet çalışma bulunmaktadır. H indeksi en yüksek olan ilk üç yazar Raoult D, Colson P ve Rolasin JM olarak tespit edilmiştir. Yazarların h, g ve m indeksleri hesaplanmış ve Lotka yasasına göre core yazarlar tespit edilmiştir. En fazla yayın yapan ülkeler için ilk üç sırada Çin, Amerika ve İtalya yer almaktadır. Son olarak kelime madenciliği analizine göre çalışmaların üç küme altında sınıflandırılabileceği tespit edilmiştir.
Sonuç: COVID-19 ile ilgili yayınların sayısal artış hızı karşısında temel okuma sürecini hızlandıracak araçlardan biri de bibliyometrik analiz sonuçlarıdır. Tedavi seçenekleriyle ilgili en güncel ve temel bilgiler topluca bibliyometrik çalışmalarda bulunabilir.
Anahtar kelimeler: Bibliyometri; COVID-19; enfeksiyon hastalıkları; solunum sistemi.

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INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection outbreak has been named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). COVID-19 spread rapidly too many countries and was officially declared a pandemic by the WHO on March 11, 2020, with more than 4,000 deaths. (1). COVID-19 is caused by a new coronavirus first identified in Wuhan, China, in December 2019. The disease is highly contagious and its main clinical symptoms are fever, dry cough, fatigue, muscle pain, and shortness of breath (1,2).

The diagnosis is made by clinical and laboratory methods. There is no proven specific treatment. Vaccine studies are ongoing (3). Physicians treating patients with COVID-19 disease; uses different antivirals and anti-inflammatory agents and tries to manage the disease based on expert opinions, case series and prospective and randomized studies reported from all over the world (4).

Bibliometry is a statistical method that can perform quantitative analysis of research articles on a particular subject in mathematical ways. It can also access the quality of studies, analyze key areas of research and predict the direction of future studies. Since the COVID-19 pandemic is not completely under control, its bibliometric analysis is a critical need (5).

Bibliometry is a branch of information science and bibliometric methods are effective tools developed for assessing the particular aspects of research or the values of a particular journal (6,7). With bibliometric methods, the evolution of a research direction can be revealed because bibliometry consists of the combination of linguistics, information and statistical sciences in a given field (8).

Bibliometry with science mapping and visual mapping tools (9) has been applied to many research areas such as engineering, road safety (10), assessment of social life cycle (9), financial performance (11) and the re-planning of higher education (12).

Thanks to bibliometry, a picture of the development process of a journal can be drawn. For example, the development process of a journal, effectivity, the total number of publications (TP), and total citations (TC), the average number of citations per publication (AC), and some generally accepted bibliometric indicators such as h index, g index and m index can be evaluated with some generally accepted bibliometric indicators (13,14).

Visualization is an important technique for bibliometric analysis. Academicians can visually analyze the structure and trend of a research area or journal with bibliometric tools (15). Free softwares such as Bibliometrix (16), VoSviewer (17), CiteSpace (18) and SciMAT (19) have become popular tools in the bibliometric analysis since they have a powerful user graphical interface and map visualization capability. Many studies use the above softwares separately: Bibliometrix has been used on topics such as political marketing (20), social responsibility of universities (21), health policy (22), and cyber behaviour (23). VoSviewer, CiteSpace and SciMAT have been used in many fields such as food chemistry (24), emergency medicine (25), information literacy assessment (26) and COVID-19 (27).

There are many bibliometric analysis studies on COVID-19; Zhou et al. (28) in 2020, conducted a study for the entire coronavirus family based on the last twenty years. As the key finding, they clarified the finding that studies increased after SARS and MERS outbreaks. In a study by Kaya et al. (29), all studies published in the first four months of 2020 were examined. They drew attention to international cooperation between the authors. Ram (30), in 2020, has studied the last 50 years of studies for the whole coronavirus family. He examined the performance of the publications in terms of the country, the university, and the most publishing magazine. In another study, Nasab et al. (31) examined the publications in the first three months of 2020 in terms of key performance indicators. In this study, the general search strategy was not set up and the issue of COVID-19 was examined in terms of respiratory diseases and infectious diseases. When the previous studies are examined, it is seen that the publications either point to the early period (first three and fourth months of 2020) or the last 20 and last 50 years of the whole coronavirus family have been examined. There is a need for a study that examines COVID-19 in terms of chest diseases and infectious science and narrowly examines the performance and intellectual structure of the publications here. This study demonstrates the potential to be one of the studies that focus on this need. At this context of the chest and infectious diseases, the main goal of this study is to make a bibliometric analysis of publications on coronavirus disease 2019 (COVID-19). Visualizing it with visible and scientific mapping techniques is the secondary goal.

MATERIAL AND METHODS

A search was made from the Web of Science (WoS) Core Collection database (32) on August 14, 2020, with the following search strategy and the following article number was reached. The studies were filtered. Articles and reviews were chosen. The raw data were downloaded as "plain.txt". In this study, all articles and reviews from the first days of 2020 until the date of the search were included in the study. The analysis performed within the scope of the study are as shown in Table 1.

> *"Search Strategy: TOPIC: (COVID-19) OR TITLE: (COVID-19 pneumonia) AND TOPIC: (treatment) AND TOPIC: (respiratory failure) AND TOPIC: (SARS CoV-2) Refined by: WEB OF SCIENCE CATEGORIES: (INFECTIOUS DISEASES OR RESPIRATORY SYSTEM) AND DOCUMENT TYPES: (ARTICLE OR REVIEW) AND LANGUAGES: (ENGLISH) Timespan: 2020-2020. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH".*

WoS is preferred because it is one of the most common database among academics and many journals are available. WoS provides detailed information about publications (33). Three software were used in this study. There are aspects in which each software is superior to other programs. Bibliometrix, VOSviewer and CiteSpace software were used separately in this study. Bibliometrix was used for performance analysis of publications on COVID-19, VOSviewer for co-occurrence network map analysis, and CiteSpace software for co-citation analysis (usually used to reveal the intellectual structure of a discipline). See Table 1 for details of performance analyzes.

The rest of this article is organized as follows: Part 2 focuses on the main findings. Part 3 will examine organizations such as Productivity and Collaboration Networks of Countries and Institutions. In Part 4, Major clusters, time maps and citation bursts will be examined. In Part 5, word analysis will be done by using data mining. The remaining parts are written as discussion, limitations and conclusion.

RESULTS

Main Findings on COVID-19

The COVID-19 pandemic has and continues to pose a major threat to all international societies. All research on COVID-19 disease is of great importance both for the control of the disease and for the treatment of patients. The main purpose of this study is to make a visual analysis of publications on COVID-19 in the light of science mapping techniques. In this section, general information about the type of publications between the first month of 2020 and August 21, 2020, and the most cited publications will be given.

Main Statistics on Data

A total of 787 articles and reviews were written over a period of 8 months. The number of authors is 5535 and the number of studies with a single author is 26. The number of citations per article is 10.17 and the total number of references is 10865. The number of authors per article is 7.03 and all data are given in Table 2 below.

Best Authors and Journals

The relationships between keywords, authors and journals of the main authors were visualized by using the Three-Fields Plot. In this analysis, it was inspired by the study of Janik et al. (34). In Three-Fields Plot, the relevant elements are represented in the diagram by rectangles of different colors. The height of the rectangle depends on the element represented by the rectangle (the author's keyword, author, and one of the elements in the source diagram) and the value of the sum of the relationships that arise between the diagrams of other items. The more relationships the item has, the higher the rectangle that represents it is depicted. Figure 1 represents the research scheme in the COVID-19 literature focusing on the relationships between the main authors' keywords, authors, and journals. The analysis showed in which journals published the most COVID-19 publication and which topics were discussed the most. Research topics were determined here as the keywords of the authors. Analysis result highlighted 3 authors (i.e. Husueh PR, Li Y and Wamg J) and 3 journals (i.e. International Journal of Infectious Disease, International Journal of Antimicrobial Agents, and Epidemiology and Infection). In COVID-19 literature main research topics were COVID-19, SARS-CoV-2 and coronavirus disease 2019. Other information indicated in Figure 1.

Among the top three most published journals, the International Journal of Infectious Diseases has 126 publications, Eurosurveillance has 47 and Epidemiology and Infection has 35 publications (Table 3).

Bradford's Law

For the determination of the most basic and Well-established journals, only five of the journals obtained with the Biblioshiny program were found to be the core sources (Figure 2). International Journal of Infectious Diseases, Eurosurveillance, Epidemiology and Infection, International Journal of Antimicrobial Agents, and Journal of Infection have been identified as the most important and basic journals.

H, G and M Indexes of Journals

The "Hirsch index" or "h-index" designed by Jorge Hirsch for micro-level application is a unique and simple performance index that includes both the quantity and

Table 1. Software and analyzes

Data Source	Analysis Categories
Bibliometrics Biblioshiny	Main Statistics
	Total number of publications, number of citations and average number of citations
	Productivity and Collaboration Networks of Countries and Institutions • Country productivity map • Country cooperation map • Most cited countries • University citation numbers • Corresponding author's Country
	Highly Contribute Authors Papers, Citations • Top authors production over time • Lotka law and Number of authors who wrote the most articles
VosViewer	Keyword co-occurrence network map
	Density visualization
Cite Space	Document co-citation analysis
	Time map of clusters

Table 2. Main statistics on COVID-19

Description	Results
Main Information About Data	
Timespan	2020:2020
Sources (journals, books, etc.)	108
Documents	787
Average years from publication	0
Average citations per documents	10.17
Average citations per year per doc	11.15
References	10865
Document Types	
Article	708
Article; early access	78
Article; proceedings paper	1
Document Contents	
Keywords Plus (ID)	554
Author's Keywords (DE)	1447
Authors	
Authors	5535
Author appearances	6907
Authors of single-authored documents	26
Authors of multi-authored documents	5509
Authors Collaboration	
Single-authored documents	26
Documents per author	0.142
Authors per document	7.03
Co-Authors per documents	8.78
Collaboration index	7.24

visibility of publications. It is an author-level metric that tries to measure the productivity and citation impact of the publications made by scientists. Since h-indexes are affected by the citation traditions and methods of each discipline, it is difficult to compare this index between disciplines (35). The g-index, developed by Leo Egghe in 2006, is an alternative to the h-index that does not average citation numbers to measure the global citation performance of a series of articles. Egghe thinks that the h-index has a disadvantage that it does not take into account the citation scores of the top articles. The index is calculated based on the distribution of citations received by a particular researcher's publications. While the g-index gives more weight to articles with high citations, the h-index is insensitive to it. It helps to make the difference between the author's related effects more distinct when calculating the performance of the author's most-read articles (36). The h-index is a less appropriate measure of academic achievement for young academics because it does not yet have enough time to cite articles. Especially in social science, it can take more than five years for an article to generate a significant number of citations. Young academics may prefer impact factor for final assessment. Dividing the h-index by the number of years the academy has been active can facilitate the comparison between academics with different lengths of academic careers. This index created by Hirsch is defined as the m-index (37). As a result, when the index scores of journals (Table 3) in terms of all three indicators (h-g-m index) were evaluated, there are two journals (International Journal of Infectious Diseases and Journal of Infection) with the highest scores in terms of h and m index. The journal with the highest score in terms of G index is International Journal of Antimicrobial Agents.

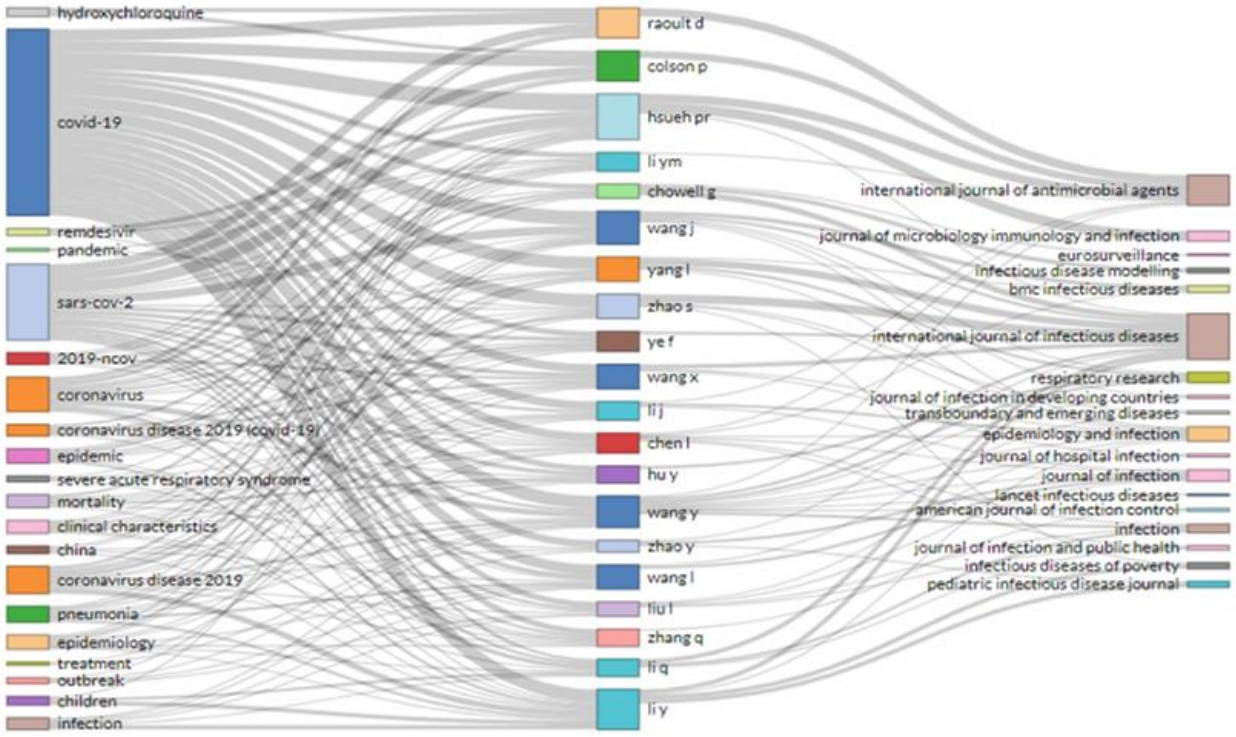

Figure 1. COVID 19 three area graph, keywords (left), authors (middle) and sources (right)

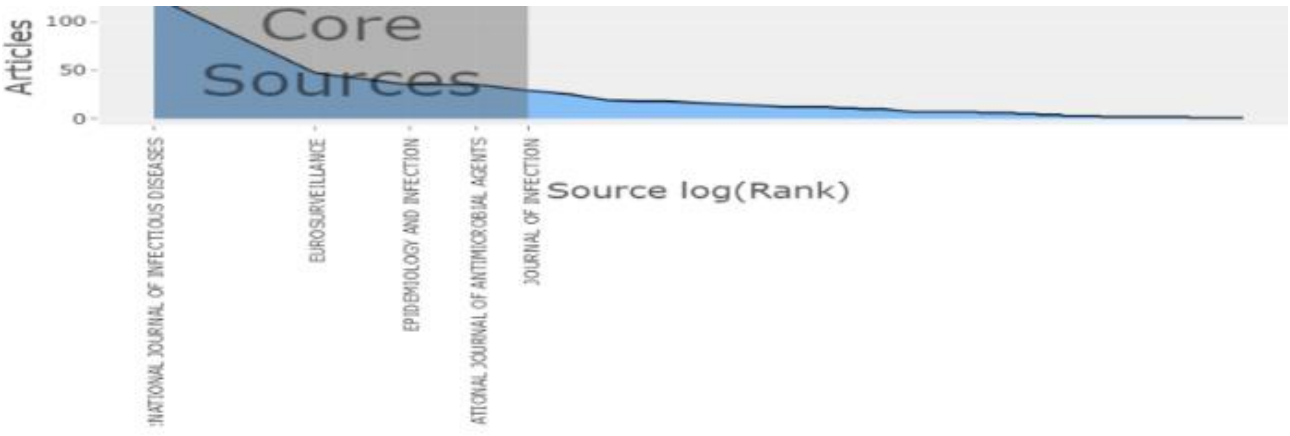

Figure 2. Bradford's law

Table 3. Most published journals, and h, g and m indexes (top 20 journals)

Source	TP	TC	h index	g index	m index
International Journal of Infectious Diseases	126	759	13	25	13
Eurosurveillance	47	411	8	19	8
Epidemiology and Infection	35	24	2	4	2
International Journal of Antimicrobial Agents	35	1488	12	35	12
Journal of Infection	29	702	13	26	13
Transplant Infectious Disease	25	13	1	3	
Infection	19	103	4	10	
Infectious Disease Modelling	18	103	5	10	5
Journal of Microbiology, Immunology and Infection	18	172	6	12	6
Journal of Infection and Public Health	17	27	2	5	2
Infectious Diseases of Poverty	16	41	2	6	2
Transboundary and Emerging Diseases	15	8	2	2	
Respiratory Research	14	20	2	4	2
BMC Infectious Diseases	13	13	2	3	2
American Journal of Infection Control	12	10	2	3	2
Journal of Hospital Infection	12	30	3	5	3
Journal of Infection in Developing Countries	12	81	4	9	4
Pediatric Infectious Disease Journal	12	75	3	8	3
Lancet Infectious Diseases	11	1224	9	11	9
Monaldi Archives for Chest Disease	11	32	2	5	2

TP: total number of publications, TC: total citations

Authors' h, g and m indexes

As a result, when the index scores of the authors (Table 4) are evaluated in terms of all three indicators (h-g-m index), the three authors with the highest scores in terms of h and m index are Raoult D, Colson P, and Rolain JM. The authors with the highest scores in terms of g index are Hsueh PR and Wang Y.

Productivity and Collaboration Networks of Countries and Institutions

When Figure 3 is examined, the countries marked as dark blue in the figures are the countries that produced more articles, made more international cooperation, and received the most citations. When the geographical maps are examined, it is seen that countries such as China, France, America and England are leading countries.

Total Publication Numbers of Universities

The most published universities are Huazhong Univ Sci and Technol, Wuhan Univ, Natl Taiwan Univ, Chinese Univ Hong Kong and Aix Marseille Univ (Table 5).

Looking at the graph of the number of publications of the universities, it is seen that the universities in the top five are universities of Chinese origin.

Table 4. Authors' h, g and m indexes (top 20 authors)

Author	TP	TC	h index	g index	m index
Raoult D	10	934	7	10	7
Colson P	9	934	7	9	7
Rolain JM	8	944	7	8	7
Hsueh PR	15	441	6	15	
Ye F	8	221	6	8	
Wang Y	14	124	5	11	
Zhao S	11	87	5	9	
Chowell G	9	296	5	9	5
Zhao Y	9	152	5	9	
Li YM	8	209	5	8	
Liu L	8	242	5	8	
Peng P	6	154	5	6	5
Huang L	5	849	5	5	5
Nicastri E	5	121	5	5	5
Wang L	9	123	4	9	
Wang X	8	99	4	8	4
Hu Y	8	144	4	8	4
Ko WC	7	379	4	7	4
Liu JY	6	147	4	6	4

TP: total number of publications, TC: total citations

Table 5. Publication numbers of universities

Affiliation	Article
Huazhong Univ Sci And Technol	98
Wuhan Univ	76
Natl Taiwan Univ	39
Chinese Univ Hong Kong	31
Aix Marseille Univ	28
Cent South Univ	28
Univ Hong Kong	26
Shanghai Jiao Tong Univ	25
Sun Yat Sen Univ	23
Capital Med Univ	22
Charite Univ Med Berlin	22
Guangzhou Med Univ	22
Xi An Jiao Tong Univ	22
Zhejiang Univ	20
Shahid Beheshti Univ Med Sci	18
Univ Oxford	18
Fudan Univ	16
Shandong Univ	16
Kyoto Univ	15
Southern Univ Sci And Technol	15

Figure 3. Productive countries, most cited countries and cooperation between countries

Corresponding Authors and Collaboration

Countries with an MCP rate of 50% and above are countries with high international cooperation in the field of COVID-19. Belgium, Australia and Canada are among the countries that cross this threshold. MCP rate in Turkey is 16.67% (Figure 4). SCP and MCP rates of the countries are shown in Table 6.

Lotka Law

Lotka law and the number of articles published by the authors are shown in Table 7. Lotka law predicts that 60% of the authors contribute with one article, 15% with 2 articles, and 7% with 3 articles (38). When articles and authors are examined within the framework of Lotka law, it was seen that 85% of the authors contributed with one article, 9.5% of the authors contributed with two articles, 2.5% of authors contributed with three articles, %0.8 of the authors contributed with four articles, %0.7 of the authors contributed with five articles. The reason why the distribution does not comply with Lotka law is that all relevant publications belong to 2020, and due to the examination of the publications in the eight-month period, the rapid publication process took place in a very short time. As the number of publishing years increases, the distribution is likely to change thanks to the newly published data. In addition, as a natural result of the work of numerous authors in COVID-19 articles, the findings may indicate excessive dispersal. It was understood that the author distribution of COVID-19 articles did not comply with Lotka's law. However, it should be accepted that authors with more than five publications have been deepened in the field of COVID-19 and should be considered as core authors (Figure 5).

A total of seven publications in 787 articles originated in Turkey. Only one of these publications is included in international collaboration. Others are studies based on research results that take place in Turkey. At the time of the study, one study received two citations, two studies received one citation, and the other studies received no citations. Because of this, Turkey data did not appear much in the visuals. One of these studies is review. The other six studies are original articles.

Table 6. SCP and MCP rates of countries

Country	Article	Freq	SCP	MCP	MCP Ratio
China	295	0.37532	246	49	0.1661
USA	99	0.12595	73	26	0.2626
Italy	56	0.07125	46	10	0.1786
France	36	0.0458	25	11	0.3056
United Kingdom	36	0.0458	20	16	0.4444
Germany	21	0.02672	16	5	0.2381
Canada	20	0.02545	10	10	0.5
Japan	19	0.02417	14	5	0.2632
Australia	17	0.02163	7	10	0.5882
Brazil	16	0.02036	13	3	0.1875
Korea	16	0.02036	15	1	0.0625
Iran	14	0.01781	12	2	0.1429
India	13	0.01654	12	1	0.0769
Spain	12	0.01527	7	5	0.4167
Saudi Arabia	9	0.01145	5	4	0.4444
Singapore	8	0.01018	6	2	0.25
Belgium	7	0.00891	2	5	0.7143
Poland	6	0.00763	5	1	0.1667
Turkey	6	0.00763	5	1	0.1667
Austria	5	0.00636	2	3	0.6

MCP: multiple countries publication, SCP: single country publication

Table 7. Lotka law and the number of articles by the authors

Number of Article	Number of Author	Author Rate
1	4748	0.8580
2	528	0.0950
3	136	0.0250
4	46	0.0080
5	39	0.0070
6	11	0.0020
7	3	0.0010
8	12	0.0020
9	5	0.0010
10	2	0.0000
11	1	0.0000
13	1	0.0000
14	1	0.0000
15	2	0.0000

Figure 4. Corresponding author's country

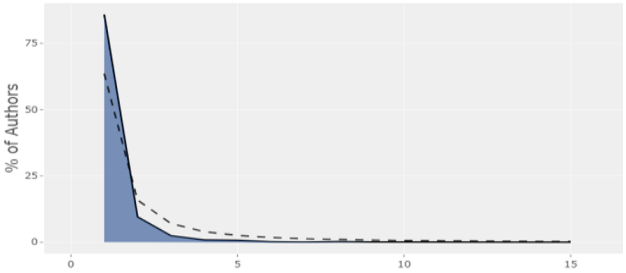

Figure 5. Lotka law

In a review written by Şenturk et al. (39), the authors mentioned the procedures necessary to provide general airway management in thoracic surgery and other anesthesia in COVID-19 patients. In the study of Kilic M et al. (40), they investigated the presence of SARS-CoV-2 in patients who only consult with sudden sensorineural hearing loss (SSHNL) during the COVID-19 pandemic. In the multi-center study of Kant et al. (41), it was aimed to demonstrate the diagnostic value of thoracic computed tomography (CT) imaging in terms of symptom duration and as a result, they stated that it should be done with chest CT imaging when RT-PCR test cannot be performed or gives negative result. In a multicenter study involving authors from Turkey, the aim of the study was to retrospectively investigate the epidemiological and clinical features, laboratory results, radiological findings and results of COVID-19 in patients with transfusion-induced thalassemia major (TM), -thalassemia intermedia (TI) and sickle cell disease (42). In the study of Pınar Senkalfa et al. (43), it was aimed to evaluate the anxiety associated with the COVID-19 pandemic in children with cystic fibrosis (CF) and their mothers. They stated that informing parents of children with CF about COVID-19 via teleconference can reduce anxiety (43). Ucpinar et al. (44) presented the first case of pneumothorax, which is one of the complications that may develop in patients with COVID-19. In the study of Dost et al. (45), they evaluated the knowledge of anesthesiologists and assistants in Turkey about COVID-19 and their attitude towards the strategies and methods of application to be used.

Major Clusters and Time Map

Major Clusters

The Citespace program identified nine significant clusters in terms of co-citation analysis technique. The largest of these clusters is the zero cluster and is named hydroxychloroquine. The silhouette value is used as a measure of whether clusters are identified in a meaningful way. The silhoutte threshold value required for the cluster to be meaningful is 0.60. Each cluster is named in three different ways. The name of the naming algorithm proposed by the inventor of the program is LLR (18). A Landscape View of the Citation Network (LRF=3, LBY=8 and e=2.0) The scientific view on the subject above has been produced based on publications for the first eight months of 2020. The network consisted of 787 articles and 10865 references were analyzed. The network has a modularity value of 0.6468, which is considered high. This shows that the specializations in the science map are clearly defined in terms of common resource sets. The modularity value is expected to be equal to or greater than 0.6. Mean silhoutte value is desired to be 0.7 and higher (18). A small mean score of 0.313 indicates the presence of many small clusters with very few members. The Citespace software is able to extract small clusters and display clusters with a meaningful dace silhoutte value. The cluster with the youngest clusters and the highest number of publications is the "hydroxychloroquine" cluster. Therefore, clusters that are very small in the relevant program have been removed from the landscape view and analyzed. The differently colored fields indicate when common reference links in these fields appeared for the first time. The program also includes coloring feature of clusters. Each set can be tagged with title terms, keywords, and terms derived from the summary. In the first image, all clusters are shown, and in the second image, small clusters with insignificant silhoutte values are removed from the analysis and visualized. The largest cluster is #0 hydroxychloroquine (program starts the largest cluster from scratch, (Figure 6).

Time Map

Looking at the time map, it is understood that all the clusters that are particularly meaningful are very lively and up-to-date. There is no cluster that has lost its vitality. We can agree with this interpretation with a cautious approach. We believe that it is an early comment since the publication period only covers eight months. A clearer interpretation can be made when a study is carried out over the years (Figure 7). There are no data in WoS regarding the publication speed of the articles. However, the full texts of the relevant publications have the date of arrival and the date of acceptance of the article. It has been observed that these periods are relatively brief.

Word Analysis (Lexical Analysis)

Word analysis was analyzed with both Bibliometrix and VosViewer software. Bibliometrix software analyzes words by using the title of the article, keywords, abstract and the bibliography of the article. The analysis obtained by using the bibliography of the article is called keyword plus (a feature not found in other software). The words with the highest frequency in the words obtained from the abstracts are COVID, patients, cases disease, SARS-CoV, coronavirus, clinical, infection, severe and respiratory. In other words, the authors mostly preferred these words in the title of the article (Figure 8).

When Figure 9 was examined, it was seen that the words of COVID-19 publications were combined in three main clusters. The most prominent words of the red cluster are pandemic, epidemic, country, spread, and region. The most prominent words of the green cluster are admission, fever, therapy, and year. In the blue set, words such as PCR and specificity are in the foreground (Figures 9 and 10).

DISCUSSION and CONCLUSION

Science mapping is becoming an important activity for academics working in all disciplines. As the number of publications increases and publications partially deepen, the task of accessing information, analyzing and transferring it to academic platforms becomes more complex. Conceptual structure is the basic themes that science speaks and follows. Intellectual structure is the work with which authors penetrate a scientific community. Social structure is how writers, institutions and countries affect each other. Determination of the conceptual structure, intellectual structure and the social structure have the potential to enable countries and universities to use their scarce resources more profitably. In addition, scientific research areas and resources can be arranged thanks to the motor themes obtained from the thematic development process. Results from science mapping, data visualization, and bibliometric software can also be one of the most rational inputs for policymaking.

First, the variety of topics and subheadings addressed by scientists regarding the COVID-19 crisis is increasing exponentially, indicating that the virus affects our current and future lifestyle on various fronts (46). The effect of COVID-19 on respiratory diseases and infection disciplines needs research that brings new perspectives to the research. Second, the key issues identified serve as a path for practitioners and academics seeking future research.

The biggest limitation of this study is that it examines the bibliometric analysis of COVID-19 publications only in terms of WoS database. Studies in Scopus and other databases were not included in the analysis. In addition, a bibliometric analysis of the first 100 articles with the most citations can be made. Contribution to the health of countries other than Continental Europe, America and China is also worth investigating.

Journals in Scopus and WoS databases are published and reviewed each year to ensure their high quality. This study only used WoS, and this is a limitation. Therefore, future studies need to cover more databases in order to collect more comprehensive data and avoid bias.

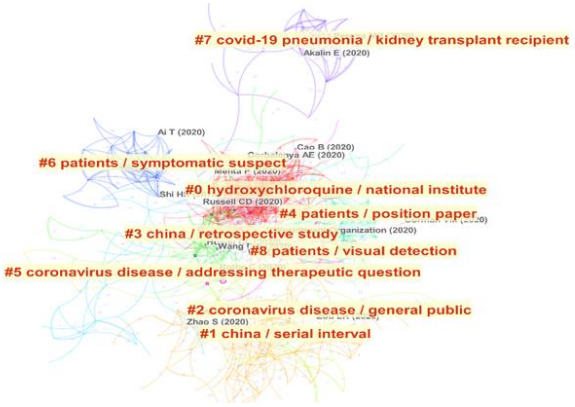

Figure 6. Document co-citation clusters visualization (based on citespace landscape analysis)

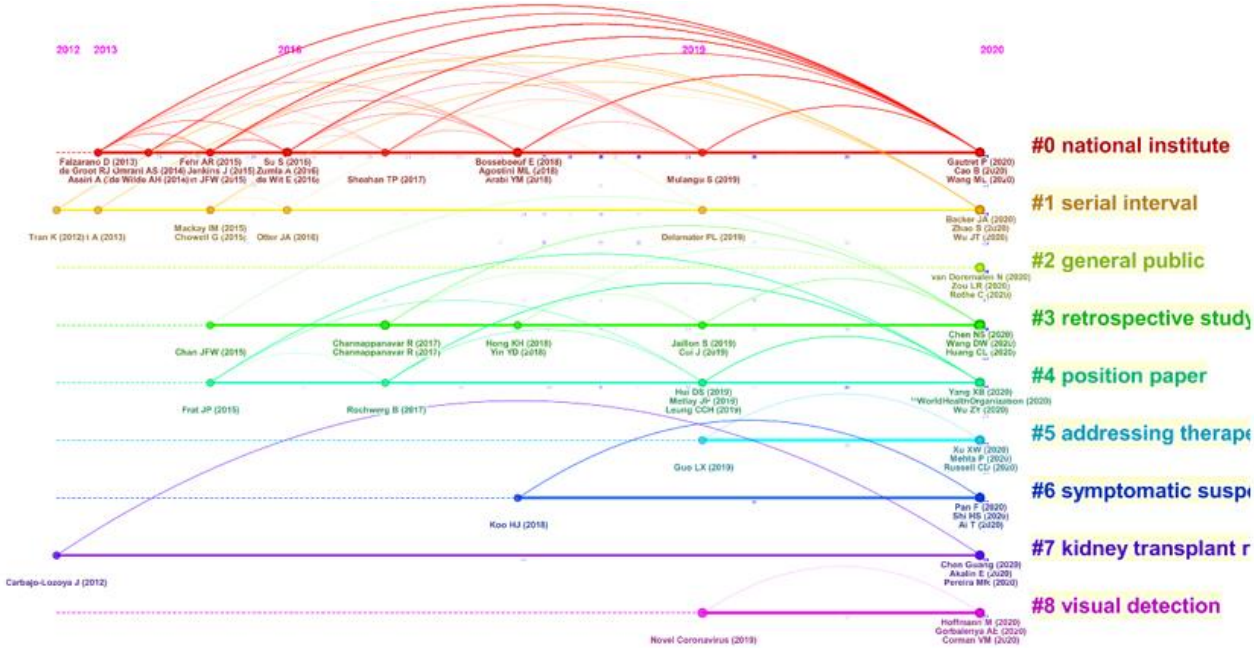

Figure 7. Time map (2020)

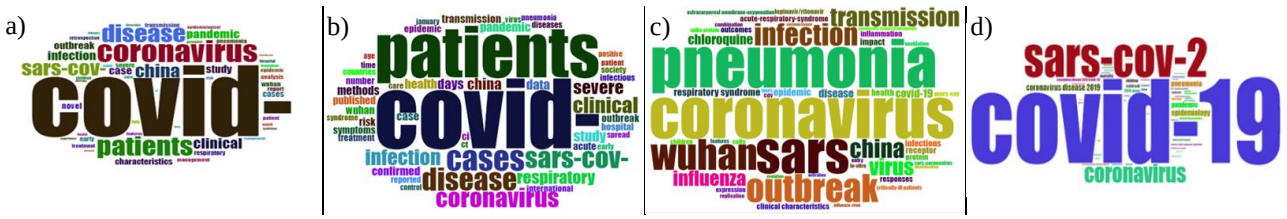

Figure 8. Word trees; **a)** by article title, **b)** by summary, **c)** by keyword plus, **d)** by keywords

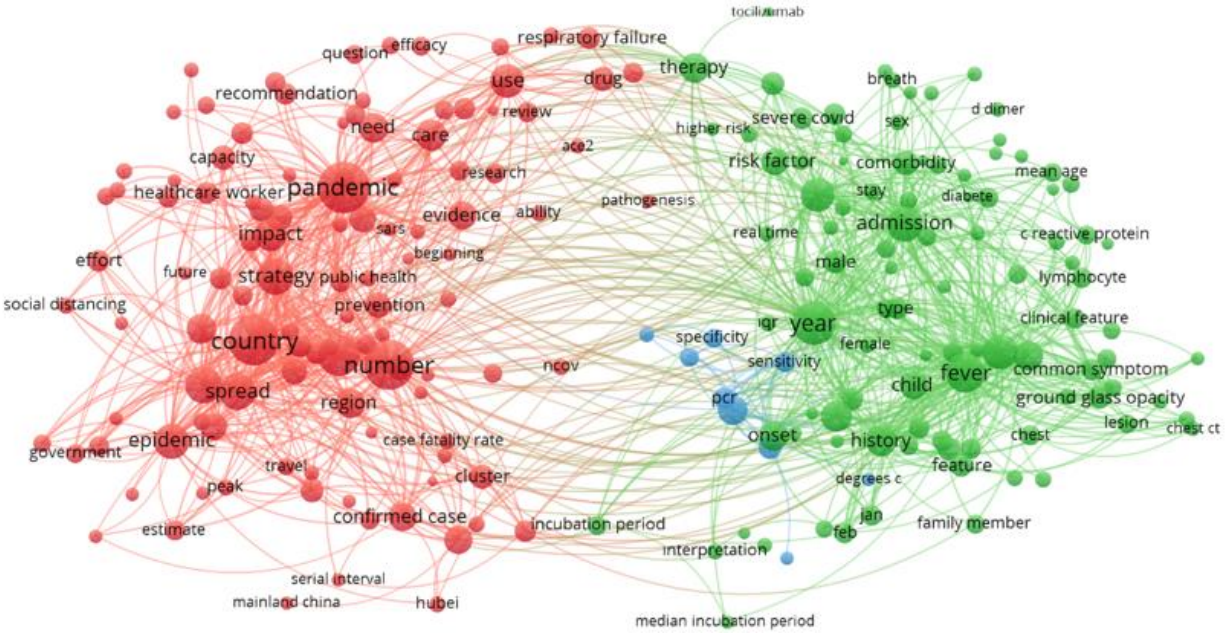

Figure 9. Word mining (VosViewer)

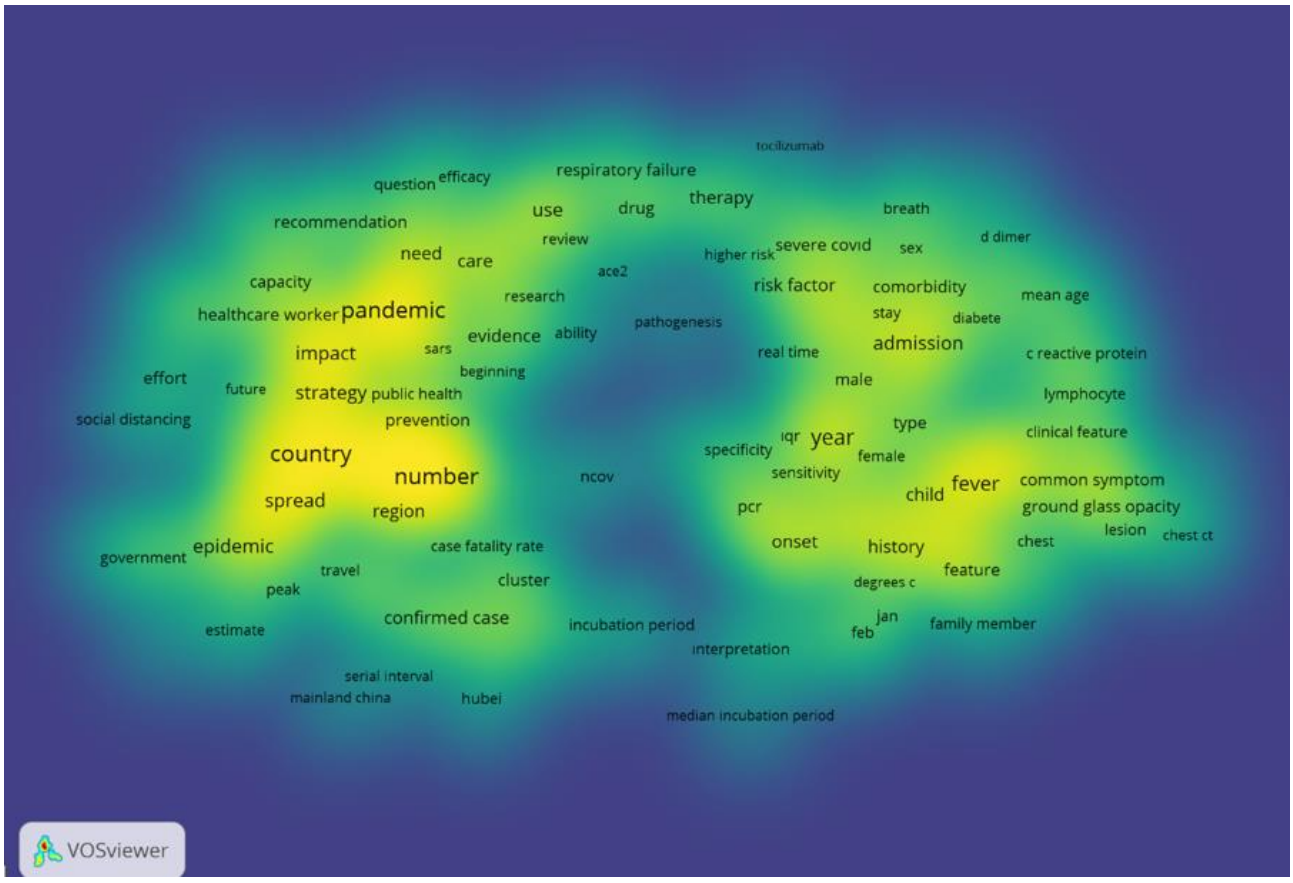

Figure 10. Keyword density map

Ethics Committee Approval: Since our study was not an experimental study including human or animal subject, ethics committee approval was not required.

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The Relationship of Lactate Levels with Carboxyhemoglobin Levels and Clinical Findings in Patients Admitted with Acute Carbon Monoxide Poisoning

Akut Karbon Monoksit Zehirlenmesiyle Başvuran Hastaların Laktat Düzeyinin Karboksihemoglobin ve Klinik Bulgularla İlişkisi

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ABSTRACT

Aim: Acute carbon monoxide (CO) poisoning is a potentially mortal, though preventable, condition. Mild poisoning presents with non-specific symptoms, such as fatigue, headache, nausea and vomiting, whereas severe exposure to CO can result in loss of consciousness, coma, and death. The aim of this study was to investigate the utility of lactate and carboxyhemoglobin (COHb) levels in the clinical presentation and treatment of patients with acute CO poisoning.
Material and Methods: Data were obtained from the hospital information system and patient files with ICD-10 code "T58: Toxic effects of CO". The blood parameters and vital signs of patients at admission, causes of poisoning, time to hospital, and Glasgow coma scores during admission were recorded. Within related and relevant complications of CO intoxication and data concerning treatment plans and hospitalization status were recorded.
Results: A statistically significant difference was found between COHb and lactate levels of patients who did and did not develop neurological and cardiac complications ($p<0.001$). Moreover, a statistically significant difference was found in COHb and lactate levels among patients who did or did not receive hyperbaric oxygen therapy, and who were hospitalized or not ($p<0.001$). As looking for biochemical profile, significant correlations was found between COHb and pH, base excess, and bicarbonate and lactate levels.
Conclusion: This study shows that COHb and lactate levels at admission to the emergency department are significant for prognosis, follow-up, and treatment of patients with CO poisoning. Elevated lactate and COHb levels may also found to be associated with neurological and cardiac complications.
Keywords: Poisoning; carbon monoxide; lactate; hyperbaric oxygenation; carboxyhemoglobin.

ÖZ

Amaç: Akut karbon monoksit (carbon monoxide, CO) zehirlenmesi, ölümle sonuçlanan önemli ve önlenebilir bir zehirlenme nedenidir. Hafif zehirlenmelerde yorgunluk, baş ağrısı, bulantı ve kusma gibi spesifik olmayan belirtiler görülürken şiddetli maruziyetlerde bilinç kaybı, koma ve ölüm ortaya çıkabilir. Bu çalışmanın amacı, laktat ve karboksihemoglobin (carboxyhemoglobin, COHb) düzeylerinin akut CO zehirlenmesi olan hastaların klinik ve tedavi sürecindeki yerini araştırmaktır.
Gereç ve Yöntemler: Veriler hastane otomasyon sistemi ve hasta dosyalarından, ICD-10 kodu "T58: Karbonmonoksitin Toksik Etkisi" olanlar seçilerek elde edildi. Hastaların başvuru sırasındaki kan parametreleri ve yaşamsal belirtileri, zehirlenme nedenleri, hastaneye geliş süreleri ve başvuru sırasındaki Glasgow koma skorları kayıt altına alınmıştır. Hastaların CO zehirlenmesine bağlı ve zehirlenme ile ilgili komplikasyonları ve aldıkları tedavi planı ve hastaneye yatış durumu ile ilgili verileri kaydedilmiştir.
Bulgular: Nörolojik ve kardiyak komplikasyon görülen ve görülmeyen hastalar arasında CO ve laktat değerlerinde istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0,001$). Ayrıca hiperbarik oksijen tedavisi alan ve almayan hastalar ile hastaneye yatışı olan ve olmayan hastalar arasında COHb ve laktat değerlerinde istatistiksel olarak anlamlı bir farklılık bulunmuştur ($p<0,001$). Biyokimyasal parametreler açısından bakıldığında, COHb ile pH, baz açığı, bikarbonat ve laktat düzeyleri arasında anlamlı korelasyon saptanmıştır.
Sonuç: Bu çalışmada, acil servise başvuru anındaki COHb ve laktat değerlerinin, CO zehirlenmesi olan hastaların prognozu, klinik seyrinin takip edilmesi ve tedavi sürecinde önemli olduğu gösterilmiştir. Ayrıca, yüksek laktat ve COHb değerlerinin nörolojik ve kardiyak komplikasyon gözlenmesiyle de ilişkili olabileceği düşünülmektedir.
Anahtar kelimeler: Zehirlenme; karbon monoksit; laktat; hiperbarik oksijen; karboksihemoglobin.

INTRODUCTION

Acute carbon monoxide (CO) poisoning is a prominent and potentially fatal, however preventable condition and is a leading cause of accidental poisoning. It is a serious public health problem in developing countries due to the widespread use of coal stoves and various other hazardous heating systems (1-7). Mild intoxications may present with non-specific symptoms such as fatigue, headache, nausea, and vomiting, whereas severe exposure to CO can result in loss of consciousness, coma, and death (2).

Carbon monoxide is released into the environment via the incomplete combustion of carbon-based compounds (8). It is a tasteless, odorless, and colorless gas, often causing patients to lose consciousness before realizing they are poisoned. Decreased oxygen supply results in hypoxemia (9). Due to their high metabolic requirement, the heart and brain are the chiefly sensitive to CO poisoning, and therefore death and neurological sequelae are the most common and devastating complications of this intoxication (10,11).

Lactate, a by-product of anaerobic glycolysis, is an important indicator of tissue hypoxia, and high lactate levels are increasingly utilized in the treatment of critical patients with CO poisoning (12-14). In addition, a study on adults found that the use of lactate levels recorded at admission, along with clinical symptoms and carboxyhemoglobin (COHb) levels, can be a useful predictor of intoxication severity (7).

In this study, the aim was to analyze the utility of blood lactate and COHb levels in the interpretation of clinical presentation and treatment of patients with acute CO intoxication.

MATERIALS AND METHODS

This was a retrospective cohort study and conducted in compliance with the principles of the World Medical Association Declaration of Helsinki and in accordance with research guidelines. This study was approved by the Ethics Committee of Ankara Dışkapı Training and Research Hospital (16.02.2015, 20/04). The inclusion criteria were: 1) being over 18 years of age and 2) having a diagnosis of acute CO poisoning. The exclusion criteria were: 1) pregnancy, and 2) being below 18 years of age. Data were collected in the tertiary education and research hospital emergency room between 2016 and 2017.

Data were recorded from the hospital information system and patient files with the ICD-10 code T58. The analyzed laboratory parameters included hemogram, blood biochemical profile, bleeding profile, and blood gas results. The blood results and vital signs of the patients at admission, their cause of poisoning, the time to hospital, Glasgow coma scores (GCS) at admission, relevant neurological (loss of consciousness, syncope, fainting) and cardiac complications (myocardial ischemia and/or ECG changes), and data concerning treatment and their status of hospitalization were recorded.

At the clinic, the criteria for receiving hyperbaric oxygen (HBO) therapy comprised of altered consciousness, ischemic ECG changes, focal neurological deficits, pregnant women with COHb >15%, and patients with headache and nausea together with a COHb level >25%.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics v.25.0 (IBM Corp., Armonk, NY, USA) and MedCalc v.15.8 (MedCalc Software bvba, Ostend, Belgium) package. The data were summarized using descriptive statistics (frequency, percentage, mean, standard deviation, median and min-max), and the qualitative data were evaluated using the Pearson chi-square test. The conformity of data to normal distribution was evaluated with the Kolmogorov-Smirnov test. Quantitative data showing normal distribution was evaluated using the Independent Samples t test, and the Mann-Whitney U test was used for numerical data that did not conform to the normal distribution. Correlations between the variables were evaluated using Pearson's and Spearman's correlation coefficient. The receiver operating characteristic (ROC) curve method was used to determine the diagnostic significance of the tested parameters. Cut-off values for the test were set according to Youden's index. Statistical significance level was considered as 0.05.

RESULTS

The study included 292 patients comprising 189 (64.7%) females and 103 (35.3%) males. The mean age was 42.5±16.9 (range, 2-94) years. The most common cause of CO poisoning was coal stoves (44.2%). The mean lactate level was 2.4±1.8 mmol/L (Table 1).

Table 1. Characteristic of patients included in the study

	n	**%**
Gender		
Female	189	64.7
Male	103	35.3
Cause of poisoning		
Coal stove	129	44.2
Water heater	40	13.7
Boiler	123	42.1
	Mean±SD	**Median (min-max)**
Age (years)	42.5±16.9	39.5 (2.0-94.0)
pH	7.4±0.1	7.4 (6.9-7.6)
COHb (%)	20.9±10.9	21.2 (0.4-48.8)
Lactate (mmol/L)	2.4±1.8	2.0 (0.2-13.7)
BE (mmol/L)	-0.7±3.6	-0.4 (-25.7-6.5)
HCO_3 (mmol/L)	23.4±3.1	23.6 (7.0-30.6)
TROP (µg/L)	0.088±0.803	0.003 (0.0-12.0)
CK (u/L)	129.2±152.8	88.5 (0.6-1400.0)
CKMB (IU/L)	20.5±19.7	15.5 (0.3-202.0)
HB (g/dL)	13.7±1.9	13.8 (2.5-19.8)
WBC (/µL)	9.2±4.9	8.4 (2.6-65.9)
PLT (/µL)	247.7±79.3	248.5 (17.0-513.0)
Time to admission (minutes)	339.8±253.9	250.0 (20.0-1159.0)
SAP (mmHg)	124.6±22.9	120.0 (80.0-220.0)
DAP (mmHg)	72.8±12.4	70.0 (50.0-117.0)
Respiratory rate (breaths/min)	17.5±3.2	18.0 (10.0-26.0)
Temperature (^{0}C)	36.4±0.4	36.4 (35.0-38.1)
Heart rate (bpm)	88.0±16.1	84.0 (54.0-157.0)
sPO_2 (%)	96.1±4.0	97.0 (60.0-100.0)
GCS	14.9±0.5	15.0 (9.0-15.0)

SD: standard deviation, COHb: carboxyhemoglobin, BE: base excess, HCO3: bicarbonate, TROP: Troponin, CK: creatine kinase, CKMB: creatine kinase myocardial band, HB: hemoglobin, WBC: white blood cell count, PLT: platelet count, SAP: systolic arterial pressure, DAP: diastolic arterial pressure, sPO2: oxygen saturation, GCS: Glasgow coma scale

Comparing the neurological complications with the obtained blood parameters, a difference of statistical significance was found between the patients with and without neurological complications in COHb (p<0.001), lactate levels (p=0.003), base excess (p=0.009), troponin (p=0.002), white blood cell (WBC) count (p=0.028), systolic blood pressure (p=0.031), and respiratory rate (p=0.041). No difference of significance was observed in terms of remaining parameters such as the cause of poisoning and gender between the patients with and without neurological complications (Table 2).

Patients with elevated lactate levels were significantly more likely to have cardiac complications and a difference of statistical significance was found between the patients with and without cardiac complications in terms of lactate levels (p=0.040).

Furthermore, a statistically significant difference was found between patients who did and did not receive HBO therapy regarding their COHb (p=0.004), lactate levels (p=0.005), base excess (p=0.034), and systolic blood pressure (p=0.031, Table 3).

Analyzing the biochemical blood parameters of patients admitted with CO intoxication, a correlation was found between COHb and pH, base excess, bicarbonate, hemoglobin (HB), and lactate levels. Also, lactate was found to be correlated with all parameters, but more significantly correlated with pH (r=-0.367, p<0.001), base excess (r=-0.554, p<0.001), bicarbonate (r=-0.544, p<0.001), troponin (r=0.218, p<0.001), and WBC levels (r=0.219, p<0.001, Table 4).

A ROC analysis regarding neurological complications seen in CO intoxication revealed an area under curve (AUC) of 0.815 (95% CI, 0.766-0.858) with a cut-off value of 21.2 (sensitivity of 88.7%, specificity of 56.1%) for COHb, and an AUC of 0.629 (95% CI, 0.571-0.685) with a cut-off value of 2.48 (sensitivity of 50.9%, specificity of 75.4%) for lactate (Table 5).

Another ROC analysis performed regarding hyperbaric therapy showed an AUC of 0.682 (95% CI, 0.625-0.735) and cut-off value of 21.8 for COHb (sensitivity of 75.0%, specificity of 55.6%), while for lactate the AUC was 0.710 (95% CI, 0.654-0.762) and cut-off value was 2.54 (sensitivity of 62.5%, specificity of 74.7%, Table 6).

It was also found that patients who had elevated lactate levels due to carbon monoxide intoxication were significantly more likely to require hospitalization.

Table 2. Comparison of patient characteristics according to neurological complications

	Without NC (n=239)	**With NC (n=53)**	**p**
Gender			
Female	154 (64.4%)	35 (66.0%)	0.951[a]
Male	85 (35.6%)	18 (34.0%)	
Cause of poisoning			
Coal stove	99 (41.4%)	30 (56.6%)	
Water heater	34 (14.2%)	6 (11.3%)	0.130[a]
Boiler	106 (44.4%)	17 (32.1%)	
Age (years)	42.3±16.6	43.8±18.3	0.557[b]
pH	7.4 (7.4-7.4) [7.2-7.6]	7.4 (7.3-7.4) [6.9-7.5]	0.105[c]
COHb (%)	19.7±10.5	25.9±11.1	**<0.001**[b]
Lactate (mmol/L)	1.9 (1.4-2.5) [0.2-11.0]	2.5 (1.7-4.1) [0.7-13.7]	**0.003**[c]
BE (mmol/L)	-0.26±2.35	-2.75±6.62	**0.009**[b]
HCO_3 (mmol/L)	23.7±2.5	22.1±4.9	**0.021**[b]
TROP (µg/L)	0.0 (0.0-0.0) [0.0-0.5]	0.0 (0.0-0.1) [0.0-12.0]	**0.002**[c]
CK (u/L)	88.0 (60.3-135.8) [0.6-1.400.0]	95.0 (73.3-140.0) [32.0-1.314.0]	0.400[c]
CKMB (IU/L)	15.0 (12.0-20.0) [0.4-202.0]	16.0 (12.0-31.0) [0.3-88.0]	0.772[c]
HB (g/dL)	13.7±1.9	13.8±2.3	0.725[b]
WBC (/µL)	8.2 (6.5-10.2) [2.6-31.9]	9.2 (7.3-12.0) [4.4-65.9]	**0.028**[c]
PLT (/µL)	248.5 (203.8-287.0) [17.0-513.0]	248.5 (208.5-281.8) [31.0-394.0]	0.987[c]
Time to admission (minutes)	250.0 (150.0-480.0) [20.0-1.159.0]	180.0 (60.0-350.0) [60.0-880.0]	**0.003**[c]
SAP (mmHg)	120.0 (110.0-132.3) [80.0-220.0]	110.0 (100.0-130.0) [90.0-200.0]	**0.031**[c]
DAP (mmHg)	70.0 (60.0-80.0) [50.0-117.0]	60.0 (60.0-80.0) [60.0-100.0]	0.107[c]
Respiratory rate (breaths/min)	17.0 (14.0-20.0) [10.0-26.0]	18.0 (16.0-20.0) [14.0-26.0]	**0.041**[c]
Temperature (^{0}C)	36.4 (36.1-36.6) [35.5-38.1]	36.4 (36.0-36.6) [35.0-38.0]	0.705[c]
Heart rate (bpm)	84.0 (78.0-94.0) [54.0-150.0]	84.0 (78.0-100.0) [60.0-157.0]	0.375[c]
sPO_2 (%)	97.0 (95.0-99.0) [80.0-100.0]	97.0 (93.0-99.0) [60.0-100.0]	0.672[c]
GCS	15.0 (15.0-15.0) [15.0-15.0]	15.0 (15.0-15.0) [9.0-15.0]	**<0.001**[c]

NC: neurological complication, [a]: Chi-square test, [b]: Independent samples t test, [c]: Mann-Whitney U test, descriptive statistics are given as n (%), mean±standard deviation, or median (interquartile range, Q1-Q3) [min-max], as appropriate, COHb: carboxyhemoglobin, BE: base excess, HCO3: bicarbonate, TROP: Troponin, CK: creatine kinase, CKMB: creatine kinase myocardial band, HB: hemoglobin, WBC: white blood cell count, PLT: platelet count, SAP: systolic arterial pressure, DAP: diastolic arterial pressure, sPO2: oxygen saturation, GCS: Glasgow coma scale

Table 3. Comparison of the patient characteristics according to hyperbaric oxygen therapy status

	Without HBO (n=276)	With HBO (n=16)	p
Gender			
Female	179 (64.9%)	10 (62.5%)	0.999[a]
Male	97 (35.1%)	6 (37.5%)	
Cause of poisoning			
Coal stove	118 (42.8%)	11 (68.8%)	
Water heater	40 (14.5%)	0 (0.0%)	0.078[a]
Boiler	118 (42.8%)	5 (31.3%)	
Age (years)	42.4±16.8 39.0 (30.0-53.0)	45.0±18.0 46.5 (32.8-57.8)	0.550[b]
pH	7.4 (7.4-7.4) [7.1-7.6]	7.4 (7.2-7.4) [6.9-7.5]	0.056[c]
COHb (%)	20.4±10.7	28.4±12.0	**0.004**[b]
Lactate (mmol/L)	1.9 (1.4-2.6) [0.2-13.7]	2.7 (1.8-5.4) [0.7-12.3]	**0.005**[c]
BE (mmol/L)	-0.42±2.80	-5.78±9.19	**0.034**[b]
HCO_3 (mmol/L)	23.6±2.6	19.7±6.6	**0.032**[b]
TROP (µg/L)	0.0 (0.0-0.0) [0.0-12.0]	0.1 (0.0-0.2) [0.0-4.7]	0.121[c]
CK (u/L)	89.0 (61.0-138.0) [0.6-1400.0]	80.0 (70.2-122.0) [65.0-1314.0]	0.828[c]
CKMB (IU/L)	15.0 (12.0-20.0) [0.3-202.0]	22.0 (17.0-32.0) [2.3-88.0]	**0.040**[c]
HB (g/dL)	13.7±1.9	14.6±2.5	0.125[b]
WBC (/µL)	8.4 (6.7-10.4) [2.6-65.9]	10.4 (6.5-13.5) [5.5-35.0]	0.314[c]
PLT (/µL)	248.5 (206.3-285.3) [17.0-513.0]	253.5 (185.8-344.8) [138.0-371.0]	0.612[c]
Time to admission (minutes)	250.0 (150.0-480.0) [20.0-1159.0]	120.0 (60.0-240.0) [60.0-850.0]	**0.003**[c]
SAP (mmHg)	120.0 (110.0-133.5) [80.0-220.0]	120.0 (100.0-130.0) [90.0-180.0]	0.312[c]
DAP (mmHg)	70.0 (60.0-80.0) [50.0-117.0]	60.0 (60.0-75.0) [60.0-90.0]	0.051[c]
Respiratory rate (breaths/min)	18.0 (14.0-20.0) [10.0-26.0]	20.0 (16.0-20.0) [14.0-26.0]	0.120[c]
Temperature (^{0}C)	36.4 (36.1-36.6) [35.5-38.1]	36.2 (36.0-36.6) [35.0-37.0]	0.185[c]
Heart rate (bpm)	84.0 (78.0-95.0) [54.0-152.0]	88.0 (79.0-100.0) [60.0-157.0]	0.498[c]
sPO_2 (%)	97.0 (95.0-99.0) [80.0-100.0]	96.0 (92.0-98.0) [60.0-99.0]	0.156[c]
GCS	15.0 (15.0-15.0) [9.0-15.0]	15.0 (15.0-15.0) [11.0-15.0]	**0.003**[c]

HBO: hyperbaric oxygen, [a]: Chi-square test, [b]: Independent samples t test, [c]: Mann-Whitney U test, descriptive statistics are given as n (%), mean±standard deviation, or median (interquartile range, Q1-Q3) [min-max], as appropriate, COHb: carboxyhemoglobin, BE: base excess, HCO3: bicarbonate, TROP: Troponin, CK: creatine kinase, CKMB: creatine kinase myocardial band, HB: hemoglobin, WBC: white blood cell count, PLT: platelet count, SAP: systolic arterial pressure, DAP: diastolic arterial pressure, sPO2: oxygen saturation, GCS: Glasgow coma scale

Table 4. Correlations between the biochemical parameters

	COHb (%)		Lactate (mmol/L)		TROP (µg/L)	
	r	p	r	p	r	p
pH	-0.164	**0.005**[a]	-0.367	**<0.001**[a]	-0.031	0.621[a]
BE (mmol/L)	-0.234	**<0.001**[a]	-0.554	**<0.001**[a]	-0.154	**0.014**[a]
HCO_3 (mmol/L)	-0.275	**<0.001**[a]	-0.544	**<0.001**[a]	-0.138	**0.027**[a]
CK (u/L)	0.000	0.996[b]	0.149	**0.027**[a]	0.179	**0.008**[a]
CKMB (IU/L)	0.034	0.605[b]	0.145	**0.029**[a]	0.273	**<0.001**[a]
HB (g/dL)	0.151	**0.014**[a]	0.153	**0.013**[a]	-0.003	0.960[a]
WBC (/µL)	0.039	0.523[b]	0.219	**<0.001**[a]	0.133	**0.034**[a]
PLT (/µL)	-0.006	0.926[b]	0.153	**0.013**[a]	0.123	**0.049**[a]
COHb (%)			0.369	**<0.001**[a]	0.086	0.170[a]
Lactate (mmol/L)	0.369	**<0.001**[a]			0.218	**<0.001**[a]
TROP (µg/L)	0.086	0.170[b]	0.218	**<0.001**[a]		

[a]: Spearman's rho correlation test, [b]: Pearson correlation test, COHb: carboxyhemoglobin, BE: base excess, HCO3: bicarbonate, TROP: Troponin, CK: creatine kinase, CKMB: creatine kinase myocardial band, HB: hemoglobin, WBC: white blood cell count, PLT: platelet count

Table 5. ROC curve analysis of neurological complications

	AUC	Cut-Off	Sensitivity	Specificity	95% CI	Youden's J	p
COHb (%)	0.815	>21.2	88.7	56.1	0.766 - 0.858	0.447	**0.001**
Lactate (mmol/L)	0.629	>2.48	50.9	75.4	0.571 - 0.685	0.264	**0.006**

ROC: receiver operating characteristic, AUC: area under curve, CI: confidence interval, COHb: carboxyhemoglobin

Table 6. ROC curve analysis of hyperbaric oxygen therapy

	AUC	Cut-Off	Sensitivity	Specificity	95% CI	Youden's J	p
COHb (%)	0.682	>21.8	75.0	55.6	0.625 - 0.735	0.306	**0.014**
Lactate (mmol/L)	0.710	>2.54	62.5	74.7	0.654 - 0.762	0.372	**0.004**

ROC: receiver operating characteristic, AUC: area under curve, CI: confidence interval, COHb: carboxyhemoglobin

DISCUSSION

The primary aim of this study was to compare and analyze blood lactate and COHb levels in patients with CO intoxication so as to identify their use in the interpretation of clinical presentation, the treatment of patients, and their prognoses. A statistically significant difference was found between the blood CO and lactate levels of intoxicated patients who did and did not develop neurological and cardiac complications. Moreover, a statistically significant difference was found in COHb and lactate levels among patients who did or did not receive HBO therapy and who were hospitalized or not. Looking at the biochemical profile, a significant correlation was found between COHb and pH, base excess, and bicarbonate and lactate levels.

The rapid binding of CO to hemoglobin results in reduced oxygen-carrying capacity of blood, leading subsequently to tissue hypoxia. Due to their high oxygen demand, the brain, heart, and kidneys are the most susceptible organs to the hypoxic effects of CO. Central nervous system involvement is responsible for most symptoms of CO poisoning. The basis for treatment of CO intoxication is to administer oxygen which will competitively bind and remove CO from hemoglobin. This can be achieved by increasing the oxygen concentration or the pressure of inspired air.

Concerning the treatment of CO intoxication, normobaric and HBO therapies have an important role in its management. However, there is still debate over their efficacy and applications in different patient groups (15-18). That said, specific objective criteria regarding the administration of HBO therapy for CO poisoning are yet to be defined. Decision to treat is usually based on the clinical findings of patients. Loss of consciousness, signs of neurological involvement, evidence of cardiac ischemia, and severe acidosis are considered indications for HBO therapy (19).

Some studies have reported that the average age of adults admitted to the emergency department with CO poisoning ranged from 27 to 41 years, and that most patients are female (6,20). The results of this study showed similar findings, where the majority of CO poisoning patients were female, and the mean age was 42.5±16.9 years.

In this study, COHb and lactate levels were significantly higher in patients presenting with neurological symptoms. Solkal et al. (25) reported that patients with severe CO poisoning had significantly higher lactate levels when compared to patients with mild poisoning. Özkan et al. (21) indicated that lactate and COHb were significantly elevated in patients with CO poisoning. A study by Aslan et al. (22) similarly associated elevated COHb levels with an increased likelihood of developing neurological symptoms.

In a small series, Inoue et al. (13) demonstrated that blood lactate levels were a useful predictor of prognosis among patients who sustained severe CO poisoning due to attempted suicide. Furthermore, Moon et al. (12) studied 80 patients who presented to the emergency department with CO poisoning and found that their initial blood lactate level was an independent predictor of severe complications and intensive care unit admission. It was found herein that lactate levels could predict hospital admissions and cardiac and neurological complications. These results were consistent with the literature.

In the present study, a strong and significant correlation was found between COHb and pH, base excess, bicarbonate, and lactate levels. Moreover, lactate levels were strongly and significantly correlated with COHb, troponin, pH, base excess, and bicarbonate levels. Besli et al. (24) reported a positive correlation between lactate and COHb levels, as well as elevated lactate levels in patients who developed neurological symptoms. A recent study by Dogan et al. (7) found that the initial lactate levels of 74 patients with severe CO poisoning were positively correlated with COHb levels.

The results of this study showed that patients with elevated COHb and lactate levels were significantly more likely to develop neurological complications. Benaissa et al. (14) found that lactate levels were found to be significantly higher in intoxicated patients who developed neurological symptoms, but concluded that the clinical significance of this finding was questionable since the increase in lactate levels were minimal.

Also a result of this study, lactate levels of patients with cardiac findings were significantly higher. Similarly, Cervellin et al. (26) and Marchewka et al. (27) found that lactate levels were a useful predictor in determining the severity of CO intoxication, and were also well correlated with troponin levels.

Repplinger et al. (23) argued that lactate could be used as an indicator for HBO therapy. It was similarly found herein that COHb and lactate levels increased in correlation and also that patients with high lactate levels were more likely to be hospitalized. However, since lactate levels are influenced by multiple factors, further studies are needed for more conclusive results.

CONCLUSION

This study shows that COHb and lactate levels acquired at admission are significant in the prognosis, follow-up, and treatment of patients admitted to the emergency department with CO poisoning. Elevated lactate and CO levels may be associated with neurological and cardiac complications.

Ethics Committee Approval: The study was approved by the Ethics Committee of Dışkapı Yıldırım Beyazıt, Training and Research Hospital (16.02.2015, 20/04).

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Prediction of Difficult Tracheal Intubation by Artificial Intelligence: A Prospective Observational Study

Yapay Zekâ ile Zor Trakeal Entübasyon Tahmini: Prospektif Gözlemsel Bir Çalışma

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ABSTRACT

Aim: Many predictive clinical tests are used together for preoperative detection of patients with difficult airway risk. In this study, we aimed to predict difficult intubation with different artificial intelligence algorithms using various clinical tests and anthropometric measurements, besides, to evaluate the accuracy performance of Cormack and Lehane (C-L) classification with artificial intelligence.

Material and Methods: This study was conducted as a single-blind prospective observational study between 2016 and 2019. A total of 1486 patients with American Society of Anesthesiologists physical status I-III, scheduled to undergo elective surgery and requiring endotracheal intubation, were included. Demographic variables, clinical tests and anthropometric measurements of the patients were recorded. Difficult intubation was evaluated using the 4-grade C-L system according to the easy and difficult intubation criteria. Difficult intubation was tried to predict using 16 different artificial intelligence algorithms.

Results: The highest success rate among artificial intelligence algorithms was obtained by the RandomForest method. With this method, difficult intubation was predicted with 92.85% sensitivity, 96.94% specificity, 93.69% positive predictive value and 96.52% negative predictive value. C-L classification accuracy performance also determined as 95.60%.

Conclusion: Artificial intelligence has been considerably successful in predicting difficult intubation. Besides, C-L classifications of easy and difficult intubated patients were successfully predicted with artificial intelligence algorithms. Using a 6-grade modified C-L classification for laryngeal view may provide stronger difficult intubation prediction. A safer and more potent prediction in training artificial intelligence can be achieved by adding individual differences and clinical features that support the definition of difficult intubation.

Keywords: Tracheal intubation prediction; difficult intubation; artificial intelligence; Cormack-Lehane; intubation, anesthesia.

ÖZ

Amaç: Zor hava yolu riski olan hastaların preoperatif tespiti için birçok prediktif klinik test birlikte kullanılmaktadır. Bu çalışmada, çeşitli klinik testler ve antropometrik ölçümler kullanarak farklı yapay zekâ algoritmaları ile zor entübasyonun tahmin edilmesi, ayrıca Cormack ve Lehane (C-L) sınıflandırmasının doğruluk performansının yapay zekâ ile değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma, 2016 ve 2019 yılları arasında tek kör prospektif gözlemsel bir çalışma olarak gerçekleştirildi. Elektif cerrahi planlanan ve endotrakeal entübasyon gerektiren, Amerikan Anesteziyologlar Derneği fiziksel durumu I-III olan toplam 1486 hasta dahil edildi. Hastaların demografik değişkenleri, klinik testleri ve antropometrik ölçümleri kaydedildi. Zor entübasyon 4 dereceli C-L sistemi ile kolay ve zor entübasyon kriterlerine göre değerlendirildi. Zor entübasyon, 16 farklı yapay zekâ algoritması kullanılarak tahmin edilmeye çalışıldı.

Bulgular: Yapay zekâ algoritmaları arasında en yüksek başarı oranı RandomForest yöntemi ile elde edilmiştir. Bu yöntemle zor entübasyon %92,85 duyarlılık, %96,94 özgüllük, %93,69 pozitif öngörü değeri ve%96,52 negatif öngörü değeri ile tahmin edildi. C-L sınıflandırması doğruluk performansı ise %95,60 olarak belirlendi.

Sonuç: Yapay zekâ, zor entübasyonu tahmin etmede oldukça başarılı olmuştur. Ayrıca yapay zekâ algoritmaları ile kolay ve zor entübe hastaların C-L sınıflandırmaları başarıyla tahmin edilmiştir. Laringeal görünüm için 6 dereceli modifiye C-L sınıflandırması kullanmak, daha güçlü zor entübasyon tahmini sağlayabilir. Yapay zekâ eğitiminde daha güvenli ve daha güçlü bir tahmin, zor entübasyon tanımını destekleyen bireysel farklılıklar ve klinik özellikler eklenerek elde edilebilir.

Anahtar kelimeler: Trakeal entübasyon tahmini; zor entübasyon; yapay zekâ; Cormack-Lehane, entübasyon, anestezi.

INTRODUCTION

Airway management is the most important clinical skill that anesthesiologists must append. Although major complications are rare during airway management, they are among the most life-threatening causes in medicine (1). This status may lead to major financial medical cases accompanied by catastrophic sequelae such as irreversible brain injury and death (1-4). There is a lack of reliable information about the frequency and nature of major adverse events related to airway management (3). In the UK, airway and respiratory complications have been reported to account for 12% of anesthesia-related claims but, these were described to account for 53% of deaths, 27% of the cost, and 10 of the 50 most costly claims (2).

Successful intubation is not always possible due to the patient's anatomical features and systemic diseases. Although the incidence of difficult intubation varies between 1-13%, severe intubation difficulty is generally encountered in 2-3% of patients. Preoperative evaluation can help to identify the difficulty of intubation and take the necessary precautions to deal with the problem. The real danger and risk are that the intubation difficulty is unpredictable (5).

The unpredictable difficult airway is still a serious cause of concern (6). Published algorithms for unexpected difficult or failed tracheal intubation management, devices such as gum elastic bougie, videolaryngoscope and fiberoptic bronchoscope are widely used (7,8). However, the unpredictable difficult airway can lead to significant complications and up to 30% of anesthesia-related deaths (6). Several clinical tests and anthropometric features have been identified during preoperative evaluation to find safe airway management strategies (e.g. Modified Mallampati test [MMT], sternomental distance [SMD], thyromental distance [TMD] and neck circumference). However, the accuracy of difficult intubation prediction is not possible with the evaluation of a single parameter but can be improved by evaluating many other parameters (5,6,9-15). Artificial intelligence is currently used in many fields of medicine to create programs that can perform clinical diagnostic procedures and offer treatment recommendations (16,17). For the literature review, we asked the question "Can artificial intelligence help us predict difficult intubation?". In fact, there was insufficient published literature to provide this answer.

The main aim of our study is to predict difficult intubation with different artificial intelligence algorithms using various clinical tests and anthropometric measurements. Our second aim is to evaluate the accuracy performance of Cormack and Lehane (C-L) classification with artificial intelligence.

MATERIALS AND METHODS

Study Design and Ethical Considerations

This single-blind prospective observational study was conducted after the approval of the Ethics Committee of Kırşehir Ahi Evran University (21.09.2016, 10/02; 11.12.2019, 01/01). We planned our research according to current Helsinki guidelines. Before starting the study, we informed the volunteers about the research and received their signed informed consent.

Patients, Inclusion and Exclusion Criteria

This study included 1486 patients with American Anesthesiologists Association (ASA) physical status I-III, aged 18-70 years who were scheduled to undergo elective surgery and requiring endotracheal intubation between 2016 and 2019 at Kırşehir Ahi Evran University Training and Research Hospital.

We defined two groups as easy intubation group and difficult intubation group. Intubation was considered as easy when it was performed at the first intubation attempt without the use of any additional intubation aid. Intubation was considered as difficult when there was a need for a gum elastic bougie and/or three or more attempts and/or any additional intubation aid in patients with C-L grades 3 or 4. If the trachea could not be intubated after three attempts at least, it was considered as a failed intubation. Failed intubation patients were included in the difficult intubation group. It was aimed to strengthen artificial intelligence learning. The selection of patients was described in the flow diagram (Figure 1).

Patients with significant head and neck anomalies, history of cervical spine surgeries, cardiac surgery, facial surgery, no incisors, neuromuscular diseases, and uncooperative patients were excluded from the study. Patients with C-L grade 1 and 2 laryngeal views were excluded from the easy intubation group if there was a rigid stylet, cricoid pressure, and blade replacement during intubation. Because, according to our definition of easy intubation, it is accepted as easy intubation when performed at the first intubation attempt without using any additional intubation assistance.

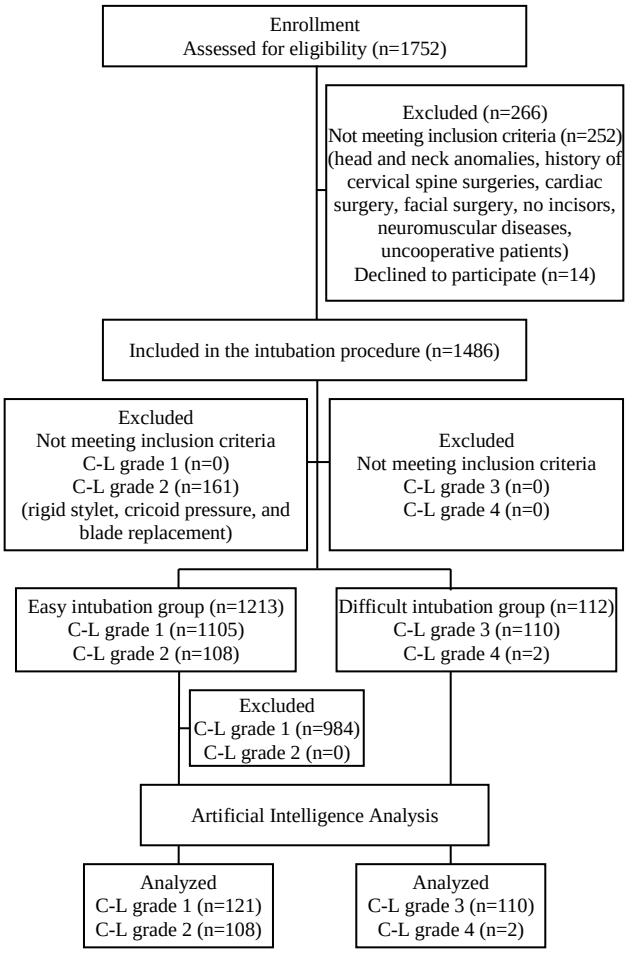

Figure 1. Flow diagram of study

Age, sex, height, weight, body mass index, ASA physical status, pregnancy and diabetes mellitus data of patients were recorded during their preoperative visits. Clinical tests and anthropometric measurements (MMT, upper-lip bite test, mouth opening, TMD, SMD, neck circumference, head circumference, neck length, atlanto-occipital joint movement, horizontal mandible length and bigonial width) were recorded by the investigator in charge before the operation.

The patients were evaluated as easy and difficult intubation. Then, the data obtained were taken to the stage of analysis with artificial intelligence. For artificial intelligence learning, analysis of a similar number of patients from C-L grade 1,2,3,4 groups is required. Due to the nature of the study, the number of patients with C-L grade 1 was high. Therefore, patients in the easy intubation group were excluded using a computer-generated random number list. It was paid attention that the number of patients in the C-L grade 1 group was not less than the number of the patients in the C-L grade 1 and 2 groups. Because the number of individuals with C-L grade 1 in the natural distribution is higher in the population.

Predictive Clinical Tests and Anthropometric Measurements

For anthropometric measurements, depth gauge (0-6 inch, 0-150 mm, ASIMETO® Electronic Depth Gauge) with a standard error of 0.01, standard 12-inch plastic goniometer and flexible tape measure were used.

Airway assessment tests and anthropometric measurements for each patient were defined as follows (Figure 2):

Modified Mallampati Test: It is used to determine difficult intubation (18). When the patient was in the sitting position, he was asked to open his mouth as wide as possible and to protrude his tongue as much as possible without phonation. The observer sitting at eye level examined the pharyngeal structures with a light source. According to the examination they were evaluated in four degrees as follows (5,19).

- *Class I:* Uvula, soft palate, tonsils, anterior and posterior pleats are easily visible
- *Class II:* Uvula and soft palate are visible
- *Class III:* Soft palate and uvula base are visible
- *Class IV:* Uvula is completely covered by tongue root, soft palate cannot be seen, the hard palate is visible

Sternomental Distance: It was measured as the distance between the upper limit of the manubrium stern and the mental protuberance in the supine position when the head is in full extension and the mouth is closed.

Thyromental Distance: The distance between the superior thyroid notch and mental protuberance was measured in the supine position, while the head is in full extension and the mouth is closed (5,20).

Mouth Opening: The patient was asked to open his mouth as much as possible with the head in the neutral position while sitting and the distance between the upper and lower incisors was measured (15).

Upper-Lip Bite Test: The patient's ability to bite his upper-lip with his lower incisors was evaluated in three degrees while he was in the sitting position (21).

- *Class I:* The vermilion line of the upper-lip is not completely visible when the lower incisors bite the upper-lip.
- *Class II:* With the same biting maneuver, the upper-lip mucosa appears partially.
- *Class III:* The lower incisors cannot bite the upper-lip.

Atlanto-Occipital Joint Movement: The line passing through the tragus and the mouth corner was determined. In the supine patient, the angle between this line and the horizontal line was measured when the head was in full extension (5).

Neck Circumference: The neck circumference was measured at the level of the cricoid cartilage while the head was in the neutral position (22).

Horizontal Mandible Length: Measured by taking the distance between gonion and mental protuberance.

Bigonial Width: Measured by taking the distance between two gonions.

Neck Length: Distance between the processus mastoideus and upper medial point of the manubrium stern was measured while the patient was in the supine position, the head at full extension and the mouth closed (13).

Head Circumference: The head circumference was measured by encircling the protuberantia occipitalis externa and the superciliary arches with tape measure (20).

Anesthesia Management

After the acceptance of the patient to the operation room, patients were monitored with end-tidal carbon dioxide, peripheral oxygen saturation, electrocardiogram, and non-invasive arterial blood pressure. After 5 minutes of preoxygenation, induction was performed with intravenous propofol 2 mg kg^{-1}, rocuronium bromide 0.6 mg kg^{-1}, fentanyl citrate 2 µg kg^{-1}. Later, the patient was ventilated using a standard face mask for 90 seconds. The physician who performed the intubation procedure was blinded to all preoperative measurement information. After evaluating the C-L classification and intubation difficulty, the physician performing the intubation was asked to inform the responsible investigator verbally. Intubation was performed by the same experienced anesthesiologist using the standard Macintosh 4 or 5 blades (HEINE Classic® Macintosh Fiber Optic Blades, Germany) to achieve standardization. The laryngeal view was evaluated according to the C-L scale without applying cricoid pressure while the patient was in sniffing position (23):

- *Grade 1:* Glottis is fully visible
- *Grade 2:* Glottis is partially visible, anterior commissure of the glottis is not visible
- *Grade 3:* No part of the glottis is visible. Only epiglottis is visible
- *Grade 4:* Epiglottis is not visible

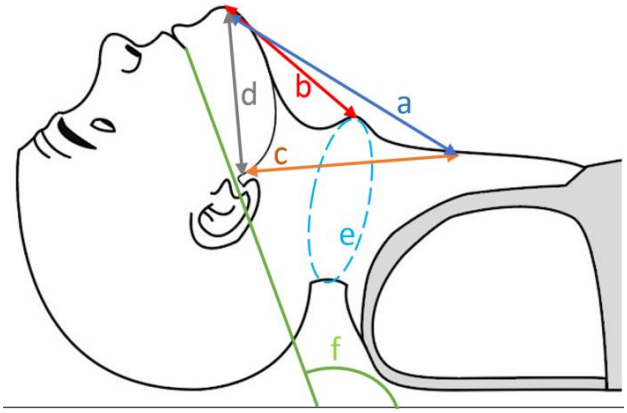

Figure 2. Anthropometric measurements. **a.** sternomental distance, **b.** thyromental distance, **c.** neck length, **d.** horizontal mandible length, **e.** neck circumference, **f.** atlanto-occipital joint movement

Artificial Intelligence

Many different programs are used for artificial intelligence analysis. In this study, the Waikato Environment for Knowledge Analysis (WEKA) Version 3.8 program was used to evaluate the data. WEKA includes several classifier algorithms that can be accessed on the internet developed at the University of Waikato in New Zealand (24). In this study, RandomForest, REPTree, RandomTree, LMT, J48, HoeffdingTree, DecisionStump, JRip, DecisionTable, LWL, KStar, IBk, SMO, SimpleLogistic, MultiLayerPerception, Logistic algorithms were used in WEKA program. Data were separated as training and test data using the 10-fold cross validation method. Correctly Classified Instances, F-Measure and ROC area values were used for the success of the methods. The formulas used to calculate these values are as follows:

$$Sensitivity = \frac{tp}{tp+fn}$$

$$Specificity = \frac{tn}{tn+fp}$$

$$F - Measure = 2\frac{pr}{p+r}$$

$$Correctly\ Classified\ Instances = \frac{tp+tn}{fp+fn+fp+fn}$$

where, tp represents the true positive, whose actual value is predicted to be positive and positive; fn represents the false negative, whose actual value is positive but predicted as negative, fp represents the false positive, predicted to be positive, but the actual value is negative, and tn represents the true negative, whose actual value is predicted to be negative and negative.

The reported incidence of difficult intubation ranges from 1 to 13% (5). In artificial intelligence learning, a similar number of patients is expected to be taken for each class. Therefore, it was planned to include a similar number of patients from each C-L grade to be able to evaluate the accuracy performance of the C-L classification. In determining this number, the number of difficult intubated patients with low incidence was mainly considered. Thus, a similar number of patient data from each C-L class was analyzed with artificial intelligence. A computer-generated random numbers list was used to select patients with C-L grade 1 from the easy intubation group for artificial intelligence analysis.

RESULTS

The number of difficult intubated patients was low by the nature of the study. Therefore, only 112 of 1752 patients were considered as difficult intubation in three years. Among 1752 patients whose eligibility for the study was evaluated, 252 patients were excluded due to head and neck anomalies, history of cervical spine surgery, cardiac surgery, facial surgery, no incisors, neuromuscular diseases, and uncooperative. Among the 1752 patients, 14 refused to participate in the study.

One hundred and sixty-one patients with C-L grade 2 laryngeal views were excluded from the easy intubation group due to the application of a rigid stylet, cricoid pressure, and blade replacement during intubation.

For artificial intelligence learning, analysis of a similar number of patients from C-L grade 1,2,3,4 groups is necessary. The number of C-L grade 1 patients was high. Therefore, in the easy intubation group, 121 patients with a total of 1105 C-L grade 1 were selected using a computer-generated random number list, and 984 patients were excluded. A total of 229 patients were recorded as easy intubation (Figure 1). Two patients with C-L grade 4 in the difficult intubation class were intubated with flexible fiberoptic bronchoscopy. All 341 patients were successfully intubated. Eventually, data obtained from 341 patients (212, 62.17% male and 129, 37.83% female, aged 18-70 years) were included in the study. The categorical demographic and clinical features of the participants are shown in Table 1. The numerical characteristics of the demographic and anthropometric measurements of the patients are shown in Table 2 as mean, standard deviation and minimum-maximum values.

10-fold cross validation method was used in the separation of data as training and test. Accordingly, the data was divided into 10 parts, the first of which was used for testing and the remaining nine for training. In the second application, the second part was used for testing and the remaining nine parts were used for training. This continued with the application of all parts. Thus, all data were used for both training and testing. This results in more realistic outcomes than randomly allocating data for training and testing. After analyzing the data with artificial intelligence algorithms, the highest classification success was obtained with the RandomForest algorithm. Table 3 shows the C-L classification success of different algorithms.

Table 1. Categorical data of demographic and clinical features of the patients, n (%)

	Difficult Intubation (n=112)	Easy Intubation (n=229)	Total (n=341)
Sex			
Male	88 (25.81%)	124 (36.36%)	212 (62.17%)
Female	24 (7.04%)	105 (30.79%)	129 (37.83%)
ASA			
I	20 (5.87%)	102 (29.91%)	122 (35.78%)
II	72 (21.11%)	95 (27.86%)	167 (48.97%)
III	20 (5.87%)	32 (9.38%)	52 (15.25%)
Pregnancy			
Yes	9 (2.64%)	22 (6.45%)	31 (9.09%)
No	103 (30.21%)	207 (60.7%)	310 (90.91%)
DM			
Yes	15 (4.4%)	8 (2.35%)	23 (6.74%)
No	97 (28.45%)	221 (64.81%)	318 (93.26%)
MMT			
I	0 (0.00%)	58 (17.01%)	58 (17.01%)
II	3 (0.88%)	78 (22.87%)	81 (23.75%)
III	52 (15.25%)	68 (19.94%)	120 (35.19%)
IV	57 (16.72%)	25 (7.33%)	82 (24.05%)
ULBT			
I	42 (12.32%)	88 (25.81%)	130 (38.12%)
II	63 (18.48%)	124 (36.36%)	187 (54.84%)
III	7 (2.05%)	17 (4.99%)	24 (7.04%)
C-L			
1	110 (32.26%)	-	112 (32.84%)
2	2 (0.59%)	-	
3	-	121 (35.48%)	229 (67.16%)
4	-	108 (31.67%)	

ASA: American Society of Anesthesiologist physical status, DM: Diabetes Mellitus, MMT: Modified Mallampati Test, ULBT: Upper-Lip Bite Test, C-L: Cormack and Lehane classification

Table 2. Descriptive statistics for numerical data of the demographic and anthropometric measurements of the patients

	Difficult Intubation (n=112)		Easy Intubation (n=229)		Total (n=341)	
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**
Age (y)	46.04±12.00	22-69	40.58±14.42	18-70	42.37±13.91	18-70
Weight (kg)	88.04±11.08	63-123	81.11±14.54	50-140	83.39±13.89	50-140
Height (cm)	169.73±6.87	155-185	168.79±8.93	150-193	169.10±8.32	150-193
BMI (kg/m^2)	30.61±3.93	21.80-48.05	28.57±5.28	18.71-46.88	29.24±4.97	18.71-48.05
TMD (mm)	65.12±5.23	50.70-75.95	74.94±8.52	50.50-89.56	71.72±8.89	50.50-89.56
SMD (cm)	15.35±1.16	12-18	17.36±1.77	12-21.5	16.70±1.85	12-21.5
Mouth opening (mm)	43.87±5.67	30.25-59.20	46.28±5.14	25.20-59.22	45.49±5.44	25.20-59.22
Neck circumference (cm)	44.21±3.33	38-49.9	43.40±4.01	31-49	43.66±3.82	31-49.9
Neck length (cm)	15.62±1.29	12-18.5	16.66±1.25	12-19	16.32±1.35	12-19
Head circumference (cm)	58.98±1.76	55.5-62	58.79±1.79	56-65	58.85±1.78	55.5-65
AOJM (degree)	88.42±4.23	78-99	101.90±6.85	86-119	97.47±8.80	78-119
HML (mm)	106.04±5.27	92.18-113.89	104.13±6.81	80.25-116.39	104.76±6.41	80.25-116.39
Bigonial Width (mm)	122.32±4.02	110.03-129.90	116.90±5.52	100.92-129.40	118.68±5.68	100.92-129.90

SD: Standard deviation, Min-Max: Minimum-maximum, BMI: Body mass index (weight/(height)^2), TMD: Thyromental distance, SMD: Sternomental distance, AOJM: Atlanto-occipital joint movement, HML: Horizontal mandible length

When Table 3 is examined, it is seen that Logistic, LMT and SimpleLogistc algorithms have similar classification success to RandomForest algorithm. The results obtained with the RandomForest algorithm, which has the highest success rate, are given in Table 4. The F-Measure value was 0.967 for the classification success rate of the patients grouped as easy intubate classification. According to this, the classification success rate is 96.7% in the patients evaluated as easy intubation. Difficult intubation classification success is 93.3%. The average success rate of C-L classification was 95.6%.

The Confusion Matrix table of the RandomForest algorithm with the highest success rate is shown in Table 5. Two hundred and twenty-two of the 229 patients in the easy intubation group were classified as easy intubation and correctly recognized. However, 7 easy intubations were misclassified as difficult intubation. One hundred four of the 112 patients in the difficult intubation group were classified as difficult intubation and correctly recognized. However, 8 difficult intubations were misclassified as easy intubation. It is demonstrated in the confusion matrix that difficult intubation was predicted with 92.86% sensitivity, 96.94% specificity, 93.69% positive predictive value, and 96.52% negative predictive value. Overall accuracy rate was 95.60%.

Table 3. C-L classification success with different artificial intelligence algorithms

Algorithm	**Classification Success (%)**
Trees	
RandomForest	95.60
REPTree	91.49
RandomTree	91.78
LMT	94.13
J48	91.49
Hoeffding Tree	92.66
Decision Stump	86.80
Rules	
JRip	91.49
DecisonTable	88.86
Lazy	
LWL	87.68
KStar	90.32
IBk	88.56
Functions	
SMO	93.84
Simple Logistic	94.42
MultiLayer Perceptron	94.42
Logistic	94.13

Table 5. The confusion matrix of intubation prediction

		True Value		
		Difficult Intubation	Easy Intubation	
Predicted Value	Difficult Intubation	104	7	93.69%
	Easy Intubation	8	222	96.52%
		92.86%	96.94%	95.60%

Table 4. Easy and difficult intubation classification results for RandomForest algorithm

Class	**TP Rate**	**FP Rate**	**Precision**	**Recall**	**F-Measure**	**MCC**	**ROC Area**	**PRC Area**
Difficult Intubation	0.929	0.031	0.937	0.929	0.933	0.900	0.986	0.968
Easy Intubation	0.969	0.071	0.965	0.969	0.967	0.900	0.986	0.994
Weighted Average	0.956	0.058	0.956	0.956	0.956	0.900	0.986	0.985

DISCUSSION

Our study demonstrates that difficult intubation can be considerably predicted (92.86% sensitivity) with different artificial intelligence algorithms using various clinical tests and anthropometric measurements. Besides, in this study, C-L classification with artificial intelligence was calculated with a high accuracy rate (95.60%).

A limited number of studies have reported predictions of difficult intubation through artificial intelligence (25-27). The results of our study are consistent with these studies. Yan et al. (25) evaluated the C-L classification prediction only with a multi-layer perceptron network-based medical decision support system. The database was created to train and test the system using 13 features of 824 patients. While they reported C-L classification accuracy as 91.9%, we found 94.42% for the same algorithm. In another study with 13 physical features of 264 patients by Yan et al. (26), C-L classification accuracy was determined as 90.53% with the support vector machine (SVM) based decision support system. In another study with 10 features of 1200 patients by Lazouni et al. (27), the C-L classification success rate was reported as 97.26% with the SVM algorithm. In the previous three studies, a single artificial intelligence algorithm was evaluated. In our study, data of 19 features and 341 patients were assessed by the WEKA program in 16 different artificial intelligence algorithms.

The success rates of these studies seem to be high. On the other hand, the selection of patients, measurement of data and standardization of methods were not clearly explained in these three studies. However, the most considerable aspect of our study is it being a single-blind clinical trial. Standardization of our study was achieved by specifying the criteria for inclusion and exclusion of patients, performing intubation by the same experienced physician who was unaware of the patient's clinical test and anthropometric measurement information. Anesthesia induction and tracheal intubation were also standardized. Thus, the average success rate of C-L classification accuracy of easy and difficult intubations, which is our second goal in the study, seems to be stronger than previous studies, despite the possibility of 95.60%.

There is no accepted universal definition for difficult intubation. It is known that definitions vary widely. (e.g. C-L grade 3 and 4 classifications, the need for changing the equipment and the physician who is performing the intubation, number of intubation attempts [more than two or three attempts], duration of intubation lasting more than 10 minutes and the presence of failed intubation; 14,28). Difficult laryngoscopy does not mean difficult intubation. In previous studies, difficult laryngoscopy was predicted according to the laryngeal view. Generally, C-L grade 2 is considered as easy laryngoscopy, but the endotracheal tube may not be inserted into the trachea without the use of any intubation aid. A relative difficulty can be mentioned in patients with some C-L grades 2. For this reason, not all patients classified as C-L grade 2 can be considered easy intubation. In our study, rigid stylet, cricoid pressure, blade replacement was used for 161 patients evaluated as C-L grade 2, so they could not be included in the easy intubation group (Figure 1). Since these patients did not meet our diagnostic criteria for difficult intubation, they could not be identified in the difficult intubation group. In the light of these definitions, not each difficult intubation will be of equal difficulty. It will also vary if the artificial intelligence recognizes and distinguishes the difficulty spectrum of the intubation. Artificial intelligence predicted difficult intubation with a sensitivity of 92.86%, specificity of 96.94%, positive predictive value of 93.69% and negative predictive value of 96.52% according to our easy and difficult intubation criteria. When we classify the intubation difficulty as easy, difficult and very difficult, we can provide significant improvements for artificial intelligence learning. This classification we recommend can makes a distinction beyond easy or difficult classification. On the other hand, tracheal intubation is generally considered to be "easy" or "difficult" in practice. Currently, there are no intubation definitions that classify difficulty as easy, difficult and very difficult. Also, recognizing and distinguishing difficulty in intubation can be improved by adding some adding individual differences and clinical features. These features may include questioning the snoring and sleep apnea history, rigid stylet or gum elastic bougie use, blade replacement, presence of cricoid pressure, requirement for changing the physician performing intubation, intubation times, etc.

Laryngeal view of easy and difficult intubated patients was evaluated with 4-grade C-L classification, which is widely accepted. Recently modified C-L grades are offered to achieve more sensitive classifications. Yentis et al. (29) modified the C-L classification to 5 grades by separating C-L grade 2 into two subclasses: 2a (glottis partial visible) and 2b (posterior part of vocal cords or only arytenoids are visible). Cook TM (28) defined the laryngeal view with a 6-grade modified C-L classification. C-L grade 2 and grade 3 were divided into two subclasses: C-L grade 2a (posterior part of the vocal cords is visible) and 2b (only arytenoids are visible), C-L grade 3a (epiglottis can be seen and lifted) and 3b (epiglottis adherent to pharynx). According to this classification, C-L grade 1 was defined as easy laryngeal view, grade 2a and 3a as restricted, grade 3b and 4 as difficult. The laryngeal view is defined as C-L grade 1 of easy, grade 2a and 3a of restricted, grade 3b and 4 of difficult in this classification. Thus, the use of modified C-L classifications will provide more sensitive data for artificial intelligence algorithms (e.g. artificial intelligence can better distinguish the degree of difficulty between C-L grade 3a and grade 3b in a patient difficult intubated). Although 4-grade C-L classification was used in our study, the accuracy performance of C-L classification was achieved with a substantial rate of 95.60%.

One of the limitations of the present study is that it was a single-center study. If the study can be a multi-center study and carried out in a specific population likely to include a high proportion of difficult intubation, there would be higher estimation success. We are planning a multi-center study for this purpose in the future. Another limitation of the study was the low sample size. There were only two patients in the C-L grade 4 class. Therefore, C-L grade 4 classification accuracy performance could not be assessed, it was evaluated within the C-L grade 3 class.

CONCLUSIONS

In conclusion, artificial intelligence provided a remarkable distinctive prediction about predicting difficult intubation. Besides, C-L classifications of easy and difficult intubated

patients were successfully predicted with artificial intelligence algorithms. A safer and more potent prediction in training artificial intelligence can be achieved by adding individual differences and clinical features that support the definition of difficult intubation. In further studies, modified 6-grade C-L classifications (2a, 2b, 3a, and 3b) can be used to predict difficult intubation. This will provide us with more detailed and powerful defined patient data. Future, we believe that artificial intelligence can safely achieve difficult intubation prediction and management with an anesthetist's perspective.

Ethics Committee Approval: The study was approved by the Ethics Committee of Kırşehir Ahi Evran University (21.09.2016, 10/02; 11.12.2019, 01/01).

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Infection Rate of Tunneled Hemodialysis Catheters

Tünelli Hemodiyaliz Kateterlerinin Enfeksiyon Oranları

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ABSTRACT

Aim: Tunneled hemodialysis catheters are frequently used for hemodialysis patients and provide temporary venous access. However, it causes complications such as catheter-related infection, pneumothorax and hematoma. In this study, we aimed to evaluate the infections, complications and catheter patency rates that developed after the use of tunnel catheters connected to three different access routes.
Material and Methods: A total of 145 patients who underwent hemodialysis due to chronic renal failure and who were placed on permanent hemodialysis catheter were included. In this study, jugular vein route was used as the first choice for the dialysis access route, when other access routes were needed due to complications and infection, femoral vein route was the second choice, while subclavian vein route was the third choice.
Results: The femoral vein group had the highest infection rate and the lowest patency rate (both $p<0.001$). The infection rate at the end of one year was 65.3%, 95.6%, and 64.0% for the jugular vein, femoral vein and subclavian vein, respectively. At the end of one year, patency rates for the jugular vein, subclavian vein and femoral vein were 57.3%, 6.7%, and 32.0%, respectively.
Conclusion: Although the jugular vein is the first choice for venous entry in hemodialysis patients, femoral and subclavian veins are also used. In this study, the jugular vein was the best option in terms of patency rate and infection. The femoral vein, on the other hand, had the worst patency rate and was also the access route with the highest infection rate.
Keywords: Hemodialysis; infection; catheter.

ÖZ

Amaç: Tünelli hemodiyaliz kateterleri hemodiyaliz hastaları için sıklıkla kullanılır ve hastalara hemodializ için geçici venöz erişim sağlar. Ancak kateter ile ilişkili enfeksiyon, pnomotoraks ve hematom gibi komplikasyonlara neden olur. Bu çalışmada, üç farklı giriş yoluna bağlı tünelli kateterlerin enfeksiyon, komplikasyon ve açıklık oranlarının değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntemler: Çalışmaya kronik böbrek yetmezliği nedeniyle hemodiyaliz yapılan ve kalıcı hemodiyaliz kateteri takılan toplam 145 hasta dahil edildi. Bu çalışmada diyaliz giriş yolu olarak juguler ven yolu ilk tercih olarak kullanıldı, komplikasyonlar ve enfeksiyona bağlı olarak diğer giriş yollarına ihtiyaç duyulduğunda femoral ven yolu ikinci tercih olurken subklavian ven yol ise üçüncü tercih oldu.
Bulgular: Femoral ven grubu en yüksek enfeksiyon oranına ve en düşük açıklık oranına sahipti (her iki $p<0.001$). Birinci yılın sonunda enfeksiyon oranı juguler ven, femoral ven ve subklavyen vende sırasıyla %65,3; %95,6 ve %64,0 idi. Birinci yıl sonunda juguler ven, subklavyen ven ve femoral ven açıklık oranları ise sırasıyla %57,3; %6,7 ve %32,0 idi.
Sonuç: Hemodiyaliz hastalarında juguler ven venöz giriş için ilk seçenek olmasına rağmen femoral ve subklavyen venler de kullanılmaktadır. Bu çalışmada, juguler ven açıklık oranı ve enfeksiyon açısından en iyi seçenek idi. Diğer taraftan, femoral ven ise en kötü açıklık oranına sahipti ve aynı zamanda enfeksiyon oranı en yüksek olan giriş yolu idi.
Anahtar kelimeler: Hemodiyaliz; enfeksiyon; kateter.

INTRODUCTION

The number of patients in need of hemodialysis due to chronic kidney failure increases with the development of technology and the health system (1). For chronic hemodialysis patients, the best route of entry for hemodialysis is primarily arteriovenous fistula (AVF), as indicated in more than one guideline (2,3). However, during AVF maturation process or in cases where there is no suitable vessel, alternative access ways are preferred. Tunneled hemodialysis catheter (THC) is a good alternative in this group of patients for hemodialysis. It is previously shown that less than 10% of hemodialysis patients begin hemodialysis with an indwelling catheter, as recommended by guidelines (2,3).

THC are usually placed in the rib cage through a central vein. Mostly, the internal jugular vein is used. Less frequently, and the subclavian or femoral vein is used if it's thought that jugular vein is hard to access. The femoral vein is used when bilateral occlusion occurs in the jugular and subclavian veins due to prolonged use in upper extremity veins (4). THC provide temporary venous intervention for hemodialysis patients until the AVF or polyfluoroethylene graft is ready after maturation, but it should always be in mind that catheter may cause infection complications. Infections are serious complications for THC. Catheter-related infection is on average 0.2-0.4% patient/day and the equivalent of 0.7-1.5 catheter per year in a prior study (5). Catheter-related bacteremia can result in endocarditis, osteomyelitis, epidural abscess, septic arthritis, and death (6).

In this study, we aimed to evaluate the infection and catheter-related complications, and patency rates for the three different ways of THC.

MATERIAL AND METHODS

Our study, which was taken with the approval of the Ethics Committee of Selçuk University Faculty of Medicine (07.01.2014, 1/16), was designed as a retrospective study. A total of 145 patients who underwent hemodialysis due to chronic renal failure and who were placed on permanent hemodialysis catheter were included in the study.

The internal jugular vein was the primarily venous access route chosen for the patients. The femoral vein was preferred as the second choice when a new access route was needed for certain reasons such as catheter thrombosis and infection. The subclavian vein was our third choice for this catheter.

The procedures were carried out by cardiovascular surgeons, using standard surgical area cleaning, with the guide of ultrasonographic visualization. 24 cm 12-14 French size permanent hemodialysis catheter was used for internal jugular vein and subclavian vein, while 36-42 cm catheter was used for femoral route. We routinely confirmed place of each catheter by direct radiography or ultrasonography with the inserted internal jugular catheter was in the right atrium and the femoral catheter in the proximal inferior cava. Catheters were immediately flushed with heparinized saline before use.

In the follow-up of the patients, when there were signs of fever and chills, blood and catheter cultures were taken for bacteriological analysis from the patients. Antibiotic treatment and catheter replacement were considered according to the laboratory results.

Statistical Analysis

The normality assumption was examined with Kolmogorov-Simirnov test. Comparisons between groups were made using One-Way ANOVA followed by LSD post hoc test or Kruskal-Wallis followed by Dunn's post hoc test. Pearson chi-square or Fisher-Freeman-Halton test was used to analyze categorical variables. Numerical variables were summarized as mean±standard deviation or median, interquartile range, minimum-maximum, while categorical variables were summarized with frequency and percentage. Infection free survival and patency rates were analyzed by using life tables and Kaplan-Meier survival analysis, and the Log-rank test was used to compare groups. Statistical analyses were performed with SPSS v.22 statistical package and 0.05 was considered as the statistical significance level.

RESULTS

Of the patients 76 (52.4%) were male and 69 (47.6%) were female. The mean age of the patients was 52.48±9.83 years. There were 75 (51.7%) patients in internal jugular vein group, while 45 (31.0%) patients in subclavian vein and 25 (17.2%) patients in femoral vein groups. There was no significant difference in terms of gender, age, hypertension, diabetes mellitus, and congestive heart failure between the jugular vein access, the subclavian vein and femoral vein access patients (Table 1). Patients with internal jugular vein catheters underwent hemodialysis for a median of 1 year, while patients who were placed hemodialysis catheters through femoral and subclavian veins were on hemodialysis for median of 4 and 3 years, respectively ($p<0.001$). When the patients were examined in terms of postoperative complications, pneumothorax developed in 1 (1.3%) patient in the internal jugular vein group and 2 (8.0%) in the subclavian vein group after the insertion of central hemodialysis catheter (Table 2). There was no significant difference between the groups in terms of pneumothorax ($p=0.105$). Hemothorax developed only in 1 (4.0%) patient in the subclavian catheter group. Hematoma development was seen in 7 patients, 4 (5.3%) in the internal jugular vein group and 3 (6.7%) in the femoral vein group. However, there was no significant difference between the groups in terms of both hemothorax and hematoma formation ($p=0.172$ and $p=0.524$, respectively). There were 6 patients with misdirection in total, whom 4 (5.3%) were in internal jugular vein group while 2 (8.0%) of them in subclavian vein group. Interestingly, all internal jugular misdirected catheters were inserted via the left jugular vein. But there was no significant difference between groups ($p=0.252$).

Considering the infection rates of the groups, the possibility of developing an infection at the end of 1 year was 65.3% (n=49), 95.6% (n=43), and 64.0% (n=16) in the jugular vein, femoral vein and subclavian vein, respectively. Infection development rate at the end of first year was highest in the femoral vein group and it was statistically significantly higher than other two groups ($p<0.001$). Infection free survival in femoral vein group was significantly lower in comparison to internal jugular vein and subclavian vein groups (Table 3, Figure 1). On the other hand, although the jugular and subclavian groups were close in terms of the risk of developing an infection, the jugular group was slightly better in freedom from infection. At the end of one year, patency rates of the

jugular vein, subclavian vein and femoral vein were 57.3% (n=43), 6.7% (n=3), and 32.0% (n=8), respectively. There was a statistically significant difference between the groups in terms of patency (p<0.001). The lowest patency rate was in the femoral vein group, at the end of first year, with 6.7%. On the other hand, the jugular vein group had the best patency with 57.3%. Accordingly, patency survival in femoral vein group was found significantly lower than internal jugular vein and subclavian vein groups (Table 3, Figure 2). Bacteriological cultures revealed, methicillin-resistant *Staphylococcus aureus*, *Enterococcus* and *Pseudomonas* species, and patients were treated with cephalosporin (e.g., ceftazidime, cefepime) or aminoglycoside according to our antibiotic regime.

Table1. Preoperative patient characteristics

	Internal Jugular Vein (n=75)	Femoral Vein (n=45)	Subclavian Vein (n=25)	p
Age (years), mean±SD	51.61±10.02	52.51±10.55	55.00±7.53	0.331
Male, n (%)	39 (52.0)	23 (51.1)	14 (56.0)	0.921
DM, n (%)	25 (33.3)	13 (28.9)	7 (28.0)	0.823
HT, n (%)	19 (25.3)	10 (22.2)	7 (28.0)	0.857
CAD, n (%)	13 (17.3)[a]	0 (0.0)[b]	0 (0.0)[b]	**0.002**
Obesite, n (%)	11 (14.7)	6 (13.3)	3 (12.0)	0.940
CHF, n (%)	6 (8.0)	3 (6.7)	2 (8.0)	0.116
Years on HD (years), median (IQR) [min-max]	1 (1) [1-3][a]	4 (2) [2-7][b]	3 (1) [2-4][c]	**<0.001**

DM: diabetes mellitus, HT: hypertension, CAD: coronary artery disease, CHF: congestive heart failure, SD: standard deviation, IQR: interquartile range, [a,b,c]: different superscript letters denote significant difference between groups according to the post hoc test result

Table 2. Postoperative complications

	Internal Jugular Vein (n=75)	Femoral Vein (n=45)	Subclavian Vein (n=25)	p
Pneumothorax, n (%)	1 (1.3)	0 (0.0)	2 (8.0)	0.105
Hemothorax, n (%)	0 (0.0)	0 (0.0)	1 (4.0)	0.172
Hematoma, n (%)	4 (5.3)	3 (6.7)	0 (0.0)	0.524
Misdirection, n (%)	4 (5.3)	0 (0.0)	2 (8.0)	0.252

Table 3. Patency and infection free survival in groups (day)

	Internal Jugular Vein (n=75)	Femoral Vein (n=45)	Subclavian Vein (n=25)	p
Infection Free Survival				
Median±SE (95% CI)	250.0±25.9 (199.1 - 300.9)	70.0±23.5 (23.9 - 116.0)	240.0±24.9 (191.0 - 288.9)	**<0.001**
Mean±SE (95% CI)	232.4±13.9 (205.0 - 259.8)	111.5±13.8 (84.3- 138.7)	216.8±26.4 (165.1 - 268.5)	
Patency				
Median±SE (95% CI)	-	70.0±23.5 (23.9 - 116.0)	240.0±33.3 (174.7 - 305.3)	**<0.001**
Mean±SE (95% CI)	262.8±14.9 (233.5 - 292.1)	118.7±15.4 (88.5- 149.0)	217.6±25.8 (167.0 - 268.1)	

SE: standard error, CI: confidence interval

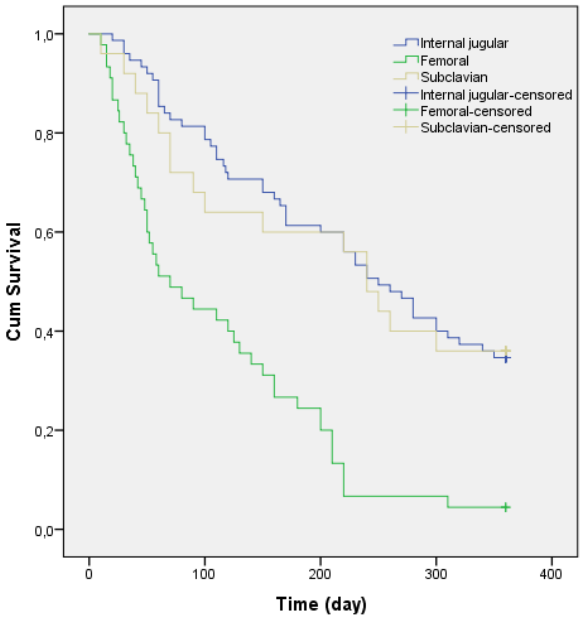

Figure 1. Infection free survival in groups

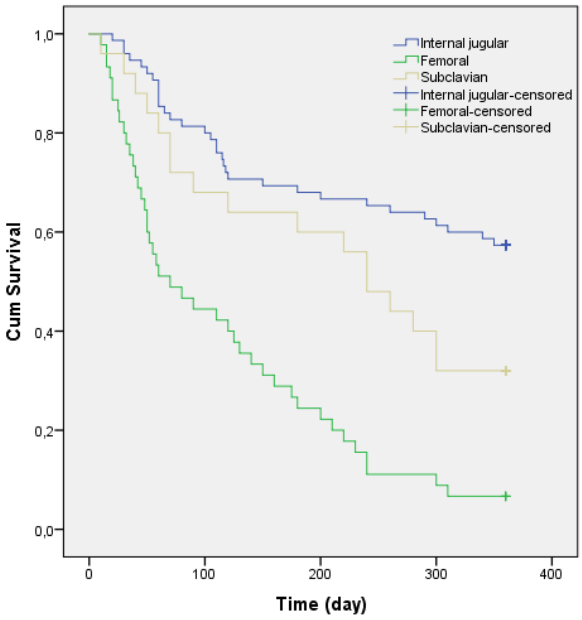

Figure 2. Patency rates in groups

DISCUSSION

Dialysis access is the most important problem in patients with chronic kidney failure. An AVF is still the most valid access route, but this is not always possible in practice. THC is required in patients who are on dialysis for a long time or expected maturation of the fistula. The Kidney Disease Outcomes Quality Initiative (K/DOQI) practice guidelines mandate standards for dialysis access and maintenance (2-3). The current K/DOQI guidelines recommend that 50% of all new accesses for hemodialysis be natural vein fistulas, with a goal prevalence of 4% fistulas throughout the dialysis population in the United States (2-3). THC remains an integral tool both as a bridge for fistula maturation and as the sole venous access to achieve this goal in a select population of patients.

A sudden deterioration in the general condition of the patient after a central venous catheter should suggest a complication (7). Complications related to tunneled dialysis catheters may cause various problems. These complications are also important in terms of patients and treatment compliance in complications related to the preferred access routes. These are pneumothorax, hemathorax, hematoma, infections, catheter dysfunction, arterial puncture, arterial-venous fistula, nerve injury, thoracic duct injury (left side only), venous air embolism, intraluminal dissection, and puncture of the aorta and central vein stenosis (8,9). Diagnostic procedures, including a bedside ultrasound, transesophageal echocardiography, or vascular computed tomography, and the execution of fast and necessary management procedures can save the patient's life (10). Serious complications play an important role in mortality and morbidity. Complications also cause a significant increase in treatment cost.

Pneumothorax is one of the common complications after THC (9). The incidence of pneumothorax after central catheter insertion varies between 1 and 6% (11). Chest radiography and ultrasonography can be used in the diagnosis of pneumothorax (9). In our study, pneumothorax was seen in only 3 patients and there was no significant difference between the groups. Subclavian vein insertion has been reported to have a higher pneumothorax incidence of than other veins insertion (12). In our patients, pneumothorax was more common in the subclavian vein, but there was no significant difference with the jugular vein. When a pneumothorax develops, the size, symptoms, spontaneous use of breathing or mechanical ventilation, and clinical diagnosis of tension pneumothorax affects the treatment strategy. Treatment options include: observation, outpatient insertion of a Heimlich valve, and inpatient tube thoracostomy (13). We were content with just observing our patients.

Hemothorax occurs as a result of vascular injury. Arterial puncture, venous perforation/laceration, myocardial injury, and associated hemorrhage and hematoma (hemopericardium, hemothorax, hemomediastinum) also occur due to vascular injury (14). Hematoma formation has been reported in variable manner, about 0% to 4.7% of all catheter placements (15,16). Hematorax occurred in only 1 patient, however, hematoma appeared in a total of 7 patients in our study. Catheter placement sites may leak for variable periods, although there is a much higher risk for patients with hematological malignancies, coagulopathies, thrombocytopenia, or heparin use.

A lesser known but important complication of central venous catheter insertion is the misplacement of the tip of the central venous catheter in a vein other than the central vein. Misdirection has been described in the literature in approximately 7% of thoracic central venous catheter insertion cases and can lead to serious complications indeed (17). Misdirection of subclavian vein catheters increases the risks of catheter dysfunction, catheter wedging, local venous thrombosis, erosion or perforation of vessel walls, and cranial retrograde injection (18). A higher incidence of malpositioning in the left thoracic venous system compared to the right side has been reported. As stated by Liberek at al. (19), incorrect catheter orientation can also be seen in patients with permanent left superior vena cava.

The right side of the circulation should be considered the first choice for central venous catheter insertion, unless the right side insertion sites are contraindicated. Chest pain may be associated with infusion via a central venous catheter that is misplaced into small branches of major central vessels. Retrosternal pain radiating to the back by infusion of fluid into the left internal mammary vein has been reported in numerous case series (20). In our study, only 6 patients had catheter malposition, 4 of them were inserted into the jugular vein, 2 of them were placed in the subclavian vein. In most cases, if a catheter is positioned incorrectly, the priority should be to reposition, replace, or remove it as soon as possible (21,22). In our patients, a new catheter was inserted after partial retraction and redirection of the wire guide, which could correct the mispositioning.

Catheter related infections are the most common and actually the most frightening complications. The incidence of catheter-related bacteremia has been reported in most studies from 2.5 to 5.5 episodes/1000 catheter days (23,24) Factors for catheter related infections include previous bacteremia attacks, advanced age, diabetes, malnutrition, iron overload, longer catheter use, and peripheral atherosclerosis (25). In addition, other factors specific to hemodialysis related catheter infections include the frequent use of catheters, contamination of dialysis solutions, and colonization with bacteria (26). Good catheter and exit site care with body material isolation and prophylactic topical antibiotics reduce hemodialysis related catheter infections (27,28). We observed that anatomical site hygiene was more important in this region. This is especially in overweight patients, specifically as the duration of catheter use was increased, catheter-related infection rates were higher, especially irrespective of the clinic status, due to the high infection rate in the femoral vein due to thrombosis because of the catheter. We thought it facilitated the formation of infection. In our study, we found that the rates of infection were higher in patient groups in whom femoral veins were preferred compared to other access routes and were statistically significant. While removal of an infected catheter has long been considered the only way to eliminate bloodstream infection, the lack of vascular access sites in hemodialysis patients has encouraged catheter salvage and preservation of the vascular access site. Treatment is initiated with empirical antibiotics usually based on the prevalence of organisms in the dialysis unit or healthcare facility. It has been

mentioned in several publications that early replacement of the catheter with a guide reduces the progression of infection in catheter-related infections (29,30).

In our study, catheter-related infection was mostly seen in the femoral region and it was statistically significant. We ensured the continuity of their treatment by providing a new dialysis method with catheter replacement under antibiotic treatment in patients with high acute phase reactants and patients with positive blood culture. In addition, patency rates were best in the jugular, subclavian, and femoral region catheters, respectively.

CONCLUSION

In patients with chronic renal failure who need hemodialysis, jugular vein catheterization seems to be the first choice for tunneled hemodialysis catheter choice. When infection rates and patency rates were taken into consideration, other venous routes should be preferred in the presence of infection and catheter incompatibility at jugular site.

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Comparison of Sympathetic Activity by Use of Skin Conductance Monitor in Patients with and without Complex Regional Pain Syndrome

Kompleks Bölgesel Ağrı Sendromu Olan ve Olmayan Hastalarda Cilt İletkenlik Monitörü Kullanılarak Sempatik Aktivitenin Karşılaştırılması

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ABSTRACT

Aim: Sympathetic system dysfunction has been described among the theories involved in the pathophysiology of complex regional pain syndrome. Objective measurement of the sympathetic activity of the affected extremity in patients with complex regional pain syndrome has not been compared to normal subjects in the literature. The skin conductance monitor is a non-invasive monitor that can objectively measure sympathetic skin activity of the extremity. In this study, we aimed to compare the differences in sympathetic activity of extremities in subjects with and without complex regional pain syndrome by using a skin conductance monitor.
Material and Methods: Sympathetic skin responses were evaluated in 63 subjects, including 25 measurements on the affected extremities in 13 patients with lower extremity complex regional pain syndrome type 1 and 50 measurements in 50 normal subjects in the control group. Among the skin conductance indices, the "peaks per second (0.02)" index was used as the main sympathetic skin response index. Descriptive criteria and the mean values of sympathetic skin response measurements were compared.
Results: When sympathetic skin responses were compared with a skin conductance monitor using the "peaks per second (0.02)" index, no statistically significant difference was found between the complex regional pain syndrome and control groups in terms of sympathetic activity in the extremities (p=0.837).
Conclusion: In this study, skin sympathetic nerve activity in the affected limb of patients with complex regional pain syndrome was similar to normal subjects. Further studies are required to assess the changes in sympathetic activity in complex regional pain syndrome.
Keywords: complex regional pain syndrome; reflex sympathetic dystrophy; skin conductance; sympathetic system; causalgia.

ÖZ

Amaç: Sempatik sinir sistem disfonksiyonu, kompleks bölgesel ağrı sendromu patofizyolojisinde yer alan teoriler arasında tanımlanmıştır. Kompleks bölgesel ağrı sendromu olan hastalarda etkilenen ekstremitenin sempatik aktivitesinin objektif ölçümü, literatürde normal denekler ile karşılaştırılmamıştır. Cilt iletkenlik monitörü, invazif olmayan ve ekstremitenin cilt sempatik aktivitesini objektif olarak ölçebilen bir monitördür. Bu çalışmada, kompleks bölgesel ağrı sendromu olan ve olmayan deneklerde ekstremitelerin sempatik aktivitesindeki farklılıkların bir cilt iletkenlik monitörü kullanılarak karşılaştırılması amaçlanmıştır.
Gereç ve Yöntemler: Sempatik cilt yanıtları, alt ekstremite kompleks bölgesel ağrı sendromu tip 1 olan 13 hastada etkilenen ekstremitelerden alınan 25 ölçüm ve kontrol grubundaki 50 normal denekten alınan 50 ölçüm olmak üzere 63 denekte değerlendirildi. Cilt iletkenlik endeksleri arasında "saniyede tepe sayısı (0,02)" endeksi, ana sempatik cilt yanıt endeksi olarak kullanıldı. Tanımlayıcı kriterler ve sempatik cilt yanıt ölçümlerinin ortalama değerleri karşılaştırıldı.
Bulgular: Cilt iletkenlik monitörü ile "saniyede tepe sayısı (0,02)" endeksi kullanılarak sempatik cilt yanıtları karşılaştırıldığında, kompleks bölgesel ağrı sendromu olan grup ile kompleks bölgesel ağrı sendromu tanısı olmayan kontrol grubu arasında ekstremitelerde sempatik aktivite açısından istatistiksel olarak anlamlı bir fark bulunmadı (p=0,837).
Sonuç: Bu çalışmada, kompleks bölgesel ağrı sendromu olan hastaların etkilenen ekstremitelerindeki cilt sempatik sinir aktivitesi, kompleks bölgesel ağrı sendromu tanısı olmayan normal denekler ile benzerdi. Kompleks bölgesel ağrı sendromunda sempatik aktivitedeki değişiklikleri değerlendirmek için daha ileri çalışmalara ihtiyaç vardır.
Anahtar kelimeler: kompleks bölgesel ağrı sendromu; refleks sempatik distrofi; cilt iletkenliği; sempatik sistem, kozalji.

INTRODUCTION

The definitive pathophysiology of complex regional pain syndrome (CRPS) is unknown, but various concepts have been proposed (1). Dysfunction of the sympathetic system is among the proposed pathophysiological mechanisms (2) and is also included in the diagnostic criteria of CRPS (3). CRPS is classified into two subtypes (3): CRPS Type 1 and CRPS Type 2 (Table 1). CRPS patients may present as a warm-CRPS, usually in the early stages, or cold-CRPS, usually in the later stages (4). It is proposed that the sympathetic dysfunction might be responsible for clinical changes in the affected limb, such as under activity of the sympathetic nervous system in the warm-CRPS subtype and over activity of the sympathetic nervous system in the cold-CRPS subtype (2, Table 2). The current evaluations of proposed sympathetic changes in the affected limbs with CRPS are the indirect measurements of sympathetic activity. There is inadequate evidence in the scientific literature about the direct and objective measurement of the sympathetic activity of the affected limb in patients with CRPS. A skin conductance monitor (SCM) is a non-invasive monitor that can objectively measure the sympathetic activity in the limbs (5).

Skin conductance responses (SCR) can be measured in the limbs to reflect the variability of sympathetic nervous system activity. SCM is based on the concept that sympathetic nerves, when stimulated, act on muscarinic receptors in the skin to stimulate the sweat glands. When stimulated, the sweat glands secrete a mixture of sodium and other electrolytes, which will increase the electrical conductance and decrease the electrical resistance on the skin surface (6). This phenomenon can be monitored via SCM on palm and plantar skin using a computer program. The objective of this study was to compare the direct sympathetic activity by the use of sympathetic skin responses (SSR) in the limbs of subjects with and without a diagnosis of CRPS Type 1.

MATERIAL AND METHODS

After institutional review board (IRB) approval (Hospital for Special Surgery, 06.10.2020, 1039), subjects were recruited for this study, and written informed consent was obtained from each subject.

Table 1. CRPS, general subtypes (3)

CRPS Type 1 (RSD)
Absence of clinical signs of major peripheral nerve involvement
CRPS Type 2 (Causalgia)
Presence of clinical signs of major peripheral nerve involvement

CRPS: complex regional pain syndrome

Table 2. CRPS, warm vs cold subtypes (1)

Warm-subtype
In the early and acute stages
Inflammatory characteristics are dominant
Associated with a warm, red, and edematous extremity
Cold-subtype
In the late and chronic stages
Autonomic features dominate
Associated with a cold, dusky, sweaty extremity

CRPS: complex regional pain syndrome

The inclusion criteria include:

- Patients aged 18-99.
- Patients who are meeting the 2012 International Association for the Study of Pain (IASP) clinical criteria (i.e., the Budapest Criteria, Table 3) for CRPS (3) and with a clinically cold-CRPS subtype were recruited for the CRPS arm of this study.
- Normal subjects without the diagnosis of CRPS were recruited for the control arm.

The exclusion criteria include:

- patients having pacemakers, cardiac defibrillators, and spinal cord stimulators
- patients having dermatological conditions in the plantar aspect of the foot where SCM electrodes are going to be attached
- History of an allergic reaction to adhesive tape
- patients with the diagnosis of dysautonomia or sympathetic dysfunction (such as Raynaud disease or Buerger disease)
- patients with disorders of sweating (such as acquired idiopathic generalized anhidrosis)
- patients who use vasoactive drugs with the mechanism of action that directly influences vascular tone

The standard data obtained from both groups were as follows: Descriptive data including age, gender, body mass index (BMI). Skin conductance measurements for the "peaks per second (0.02)" index [peaks/sec (0.02) in microSiemens] were recorded for 5 minutes in each subject.

We hypothesized that in patients with cold-CRPS, SSRs would be higher in the affected extremity than normal subjects.

The SCM is a device that can measure changes in skin conductance in real-time to assess sympathetic activity in

Table 3. Budapest clinical diagnostic criteria for CRPS (7)

1. Continuing pain, which is disproportionate to any inciting event
2. Must report at least one symptom in three of the four following categories • *Sensory:* reports of hyperesthesia and/or allodynia • *Vasomotor:* reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry • *Sudomotor/edema:* reports of edema and/or sweating changes and/or sweating asymmetry • *Motor/trophic:* reports of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)
3. Must display at least one sign at time of evaluation in two or more of the following categories • *Sensory:* evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch and/or deep somatic pressure and/or joint movement) • *Vasomotor:* evidence of temperature asymmetry and/or skin color changes and/or asymmetry • *Sudomotor/edema:* evidence of edema and/or sweating changes and/or sweating asymmetry • *Motor/trophic:* evidence of a decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)
4. There is no other diagnosis that better explains the signs and symptoms

CRPS: complex regional pain syndrome

the limbs (Med-Storm Innovation version 2005, MedStorm, Oslo, Norway). Measurements can be obtained by use of three self-adhesive non-invasive electrodes denoted C (Current), R (Reference), and M (Measurement), and these electrodes can be attached to the palmar or plantar side of the skin (8-10). The definition of a skin conductance response is the sequence of the minimum followed by a maximum in conductance values in micro siemens (μS). The SCR can be calculated in real-time, is typically analyzed in a sliding 15 seconds window, and updated each second (10). The measurements were recorded for 5 minutes in each subject.

The measurement unit uses the C and R electrodes in a feedback configuration to apply an exact and constant alternating voltage between the R and M electrodes. The return current from the M-electrode is recorded, as its value provides direct information on the skin conductance. The recorded alternating current signal is subjected to advanced filtering, removing noise and interference before the signal is sent on to the display computer (8-10). The system can measure conductance values in the range 1-200 μS, with a noise level below 0.002 μS. The measuring unit also has error detection that provides a warning for events caused by a loose electrode and external interference (8-10). The threshold for SCR recordings in this study was 0.02 μS.

Electrodes containing AgCl are used, and the measuring area under the M-electrode is critical because the SCR reflects the number of sweat glands below the electrode. The density of sweat glands on the palmar and plantar surface of hand/foot is consistent in normal subjects. The M-electrode is suited for the indices in the SCM (9,10).

The "peaks per second (0.02)" is a skin conductance monitor index that measures the frequency of sympathetic discharges at the skin and was reported to be the most reliable index for measurement of the skin sympathetic activity (9,11).

Sympathetic skin responses were evaluated in 63 subjects, including 13 patients with lower extremity CRPS Type 1 (cold subtype) and a healthy control group including 50 individuals. SSRs were recorded on the affected extremities of patients with CRPS and normal extremities of the control group. A total of 25 SSR measurements were recorded on different days in 13 patients with CRPS. If more than one SSR measurement was obtained in a patient with CRPS, the mean value was used. In the control group, a total of 50 SSR measurements were recorded in 50 healthy individuals. The "peaks per second (0.02)" index, which is reported to be the most reliable index for measuring sympathetic skin activity, was used as the main SSR index in this study. Descriptive criteria and the mean value of SSR measurements were compared in both groups.

A clinically meaningful difference in time to determine SSR between the CRPS group and the normal group was taken to be five minutes (300 seconds). Assuming a standard deviation of 180 seconds, 20 measurements would provide 80% power to detect a 300-second difference in time to determine SSR between the CRPS and normal groups. Besides, skin conductance activity has shown a statistically significant increase during painful events in several studies (9,10,12-19). In these studies, the number of patients to obtain statistically significant changes was between 20 and 75. Therefore, in this study, we estimated that 25 measurements in the CRPS group and 50 measurements in the normal (without CRPS) group would be appropriate to study how the SCR correlates with sympathetic activity between each group.

The study design made it impossible to blind the participants.

Statistical Analysis

Demographics were presented descriptively and compared between the CRPS and the control group. Continuous variables are summarized as means with standard deviations. Categorical variables are summarized as counts and percentages. For our primary outcome of interest, mean SSR recordings using the "peaks per second (0.02)" index was compared between the CRPS and the control group using two-sample t-test. We tested normality of the SSR before conduction t-test, Shapiro-Wilk test for normality, p=0.173. The data was normal; therefore, we used a t-test for comparison. All statistical hypothesis tests were two-sided, with p values of less than 0.05 defined as statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). The data were compared statistically by a biostatistician who was not involved with the data collection process and was also blinded to this study's hypotheses and data groups.

RESULTS

Descriptive statistics are shown in Table 4. There was no statistically significant difference in regards to gender between the two groups (p=0.536). There were statistically significant differences in the age and BMI between the two groups (p=0.016 and p=0.001, respectively).

When SSR were compared with a SCM using the "peaks per second (0.02)" index, no statistically significant difference was found between the CRPS and control groups in terms of sympathetic activity in the extremities (p=0.837, Table 5).

DISCUSSION

In this study, we used SSR via SCM to reflect the variability of the sympathetic nervous system on the limbs in patients with CRPS. We compared it to similar data obtained from healthy subjects. The definitive pathophysiology of CRPS

Table 4. Comparison of demographic characteristics

	Control (n=50)	CRPS (n=13)	p
Gender, n (%)			
Female	26 (52.0)	5 (38.5)	0.536
Male	24 (48.0)	8 (61.5)	
Age, mean (SD)	64.11 (8.71)	53.46 (13.34)	**0.016**
BMI, mean (SD)	30.47 (6.25)	26.29 (2.74)	**0.001**

CRPS: complex regional pain syndrome, SD: standard deviation, BMI: body mass index

Table 5. Comparison of sympathetic skin responses

	Control (n=50)	CRPS (n=25)	p
Mean (SD) [Min-Max]	0.06 (0.09) [0-0.31]	0.07 (0.07) [0-0.22]	0.837

CRPS: complex regional pain syndrome, SD: standard deviation

is unknown, but various concepts have been proposed (1). Dysfunction of the sympathetic system is among the proposed pathophysiological mechanisms (2) and is also included in the diagnostic criteria of CRPS (3). CRPS is classified into two subtypes: CRPS Type 1 and Type 2. (3). CRPS patients may present as a warm-CRPS, usually in the early stages, or cold-CRPS, usually in the later stages (4). It is proposed that the sympathetic dysfunction might be responsible for clinical changes in the affected limb, such as under activity of the sympathetic nervous system in the warm-CRPS subtype and over activity of the sympathetic nervous system in the cold-CRPS subtype (2). The current evaluation of proposed sympathetic changes in the affected limbs with CRPS is an indirect measurement of sympathetic activity. There is inadequate evidence in the medical literature about the direct and objective measurement of the sympathetic activity of the affected limb in patients with CRPS. A skin conductance monitor (SCM) is a non-invasive monitor that can objectively measure the sympathetic activity in the limbs (5).

The evaluation of sympathetic activity in current practice is based on observation of clinical signs such as color changes due to increased blood flow, dilated cutaneous blood vessels, edema, and skin temperature monitoring. These clinical signs are often unpredictable, and various confounding factors like ambient temperature may influence the outcome. Hence, we propose using an objective evaluation method of sympathetic nerve activity via a SCM (MedStorm, Oslo, Norway). SCM is based on the concept that sympathetic nerves, when stimulated, act on muscarinic receptors in the skin to stimulate the sweat glands. When stimulated, the sweat glands secrete a mixture of sodium and other electrolytes, which will increase the electrical conductance and decrease the electric resistance on the skin surface (6). This phenomenon can be monitored via SCM on palm and plantar skin using a computer program. Various types of indices may be utilized that were incorporated into the software of SCM. Among these indices, an index, known as "peaks per second (0.02)", is a better indicator of the frequency of sympathetic discharges at the skin level (11). Therefore, we elected to use the "peaks per second (0.02)" index among SCM indices to compare the groups with and without a diagnosis of CRPS.

There was no difference in regards to gender between the two groups. There was a statistically significant difference in age and BMI, indicating the older age and higher BMI for the control group. However, it is unlikely that age and BMI interfere with skin conductance measurements (9).

This study did not demonstrate a significant difference between baseline sympathetic activity, measured by SCM by utilizing "peaks per second (0.02)" index, in the limbs of patients with and without CRPS. SCM has been shown to measure sympathetic activity in normal skin (9). Clinically, skin trophic changes are commonly observed in the advanced stages of CRPS, such as in the cold-CRPS subtype (1). Moreover, microscopic pathological changes have been documented in the skin biopsies taken from patients with CRPS (20,21). It is also possible that these pathological changes may reduce the sensitivity of the SCM device and may not reflect the actual variations in the sympathetic activity of the limb that could have been otherwise detected in normal subjects. Further studies are needed to explore the sensitivity of other skin conductance indices and the potential contribution of other factors such as skin trophic changes to the skin sympathetic activity measurements via SCM.

The study design made it impossible to blind the participants and the data collectors. However, the biostatistician was blinded to study groups, the hypotheses of this study and was not involved in the data collection process. The other limitation of this study was that the number of subjects was different in each group. CRPS is a rare condition in the general population and often goes unrecognized by the medical community. The incidence of CRPS is approximately 5% in the orthopedic patient population after trauma or surgery (1). Among the patients with a diagnosis of CRPS, about 20% becomes chronic and progresses to a cold-CRPS subtype that clinically presents with the increased sympathetic activity such as the cold and clammy extremities (1). Therefore, the number of patients recruited for the cold-CRPS arm was limited to 13 patients during the study period, in whom a total of 25 SSR measurements were obtained.

CONCLUSION

According to this study, skin sympathetic nerve activity in the affected limb of patients with CRPS was similar to normal subjects without the diagnosis of CRPS. Further studies are required to assess the changes in sympathetic activity in CRPS.

Ethics Committee Approval: The study was approved by the Ethics Committee of Hospital for Special Surgery, Weill Medical College of Cornell University (06.10.2020, 1039).

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Assessment of the Immunogenicity and Protective Aspects of a DNA Vaccine Targeting Crimean Congo Hemorrhagic Fever Virus Glycoprotein Gc

Kırım Kongo Kanamalı Ateşi Virüsü Glikoprotein Gc'yi Hedef Alan Bir DNA Aşısının Bağışıklık ve Koruyuculuk Sağlama Özelliklerinin Değerlendirilmesi

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ABSTRACT

Aim: Crimean Congo Hemorrhagic Fever (CCHF) is a lethal, endemic infectious disease in human. For the preventive measures of the disease, there is currently no safe and efficient vaccine, widely for human use. Vaccine development for CCHF virus is an actively researched subject. In this study, we aimed to investigate the immunizing and protective potentials of the CCHF virus surface glycoprotein Gc that is delivered as a single antigen via a DNA based vaccine vector.
Material and Methods: A DNA based vaccine targeting the immunogenic envelope glycoprotein Gc of a CCHF virus isolate with Turkey origin (Ank2) was generated and its immunogenicity and protective capability against lethal challenge in IFNα/βR-/- receptor knock out mice was assessed.
Results: The developed vaccine candidate (pGc) elicited a considerable amount of neutralizing antibody responses in the vaccinated mice. The vaccine candidate significantly induced both antiviral Th1 and B cell activating Th2 immune responses deduced from the cytokine production profiles in the vaccinated mice. However, despite the immune responses elicited post-immunization, the vaccine failed to confer protection against lethal CCHF virus infection.
Conclusion: To the best of our knowledge, this is the first report of a DNA vaccine candidate generated against CCHF virus based on the glycoprotein Gc. The pGc vaccine candidate exhibited antigen-specific immunity in IFN/α/βR-/- mice, but was unable to produce a protection upon lethal challenge with the homologous CCHF virus. Once we comprehensively understand the immune correlates of protection, we will be more eligible to significantly improve the efficacy of vaccines.
Keywords: Crimean Congo hemorrhagic fever virus; DNA vaccine; immune responses; lethal challenge.

ÖZ

Amaç: Kırım Kongo Kanamalı Ateşi (KKKA), insanda ölümcül, endemik bir enfeksiyon hastalığıdır. Hastalığın önleyici tedbirleri için şu anda insanlarda yaygın olarak kullanılmak üzere güvenli ve etkili bir aşı bulunmamaktadır. KKKA virüsü için aşı geliştirilmesi, aktif olarak araştırılan bir konudur. Bu çalışmada, DNA esaslı bir aşı vektörü ile tek bir antijen olarak verilen KKKA virüsü yüzey glikoproteini Gc'nin bağışıklık kazandırıcı ve koruyucu potansiyellerinin araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Türkiye menşeli (Ank2) bir KKKA virüs izolatının immünojenik özellikteki zarf glikoproteini Gc'yi hedefleyen DNA esaslı bir aşı oluşturulmuş ve bu aşı adayının immünojenisitesi ve aşılanmış IFNα/βR-/- farelerde öldürücü doza karşı koruyucu yeteneği değerlendirilmiştir.
Bulgular: Geliştirilen aşı adayı (pGc), aşılanmış farelerde önemli miktarda nötralize edici antikor yanıtı ortaya çıkardı. Aşı adayı, aşılanmış farelerde hem antiviral Th1, hem de B hücresini aktive eden Th2 bağışıklık tepkilerini önemli ölçüde uyardı. Bununla birlikte, aşılama sonrasında ortaya çıkan bağışıklık yanıtlarına rağmen, aşı, ölümcül KKKA virüsü enfeksiyonuna karşı koruma sağlayamadı.
Sonuç: Bildiğimiz kadarıyla bu çalışma, glikoprotein Gc'yi hedef alan KKKA virüsüne karşı oluşturulan bir DNA aşı adayının ilk raporudur. PGc aşı adayı, IFNα/βR-/- farelerde antijene özgü bağışıklık yanıtı oluşturdu, ancak ölümcül dozdaki homolog KKKA virüsüne karşı bir koruma üretemedi. Aşı aracılı korunmanın bağışıklık ile olan ilişkilerini daha detaylı olarak anladığımızda, aşıların etkinliğini önemli ölçüde iyileştirme kabiliyetine sahip olacağız.
Anahtar kelimeler: Kırım Kongo kanamalı ateşi virüsü; DNA aşısı; immün yanıt; ölümcül sınama.

Part of this study was presented as an oral presentation at the 2nd International Conference on Crimean Congo Hemorrhagic Fever (September 10-12, 2017; Thessaloniki, Greece)

INTRODUCTION

Crimean Congo hemorrhagic fever virus (CCHFV) is a negative sense, single-stranded RNA virus belonging to the *Nairoviridae* family of the order *Bunyavirales*. The virus has a tripartite genome consisting of small (S) segment encoding for nucleocapsid protein (NC), medium (M) segment encoding for a polyprotein which is proteolytically processed into two structural glycoproteins (Gn and Gc) and other nonstructural proteins, and the large (L) segment encoding for the RNA dependent RNA polymerase (RdRp) (1). With being the causative agent of the most widely distributed tick-borne viral zoonosis, infection with CCHFV leads to severe hemorrhagic fever syndrome in humans with the case fatality rates ranging from 5% to 80% (2). Although CCHFV infections can be detected in various vertebrates including livestock animals, they do not cause any sign of disease in these organisms and hence humans appear to be the only host for CCHFV in which pathogeny takes place. CCHFV transmission to humans may occur via tick bite or tick crushing by hand and through direct contact with body fluids of an infected animal or patient (2-4). The disease occurs widely in western Asia, southern Russia, the Middle East, much of Africa and parts of Eastern Europe (2,3). The geographical distribution and prevalence of Crimean Congo hemorrhagic fever (CCHF) disease appear to gradually increase in Europe since the last two decades (5,6). Between 2002 and 2017, more than 10000 patients with CCHF were reported in Turkey involving the mortality rate of around 5% (7).

Research on developing preventive measures for CCHF have been significantly hindered due to the requirement of laboratories with adequate biosafety containment levels for handling the virus and the absence of proper animal models of the disease. Recently, two lethal mouse models which reproduce at least some of the disease symptoms due to having deficiencies in their interferon pathways were generated (8,9). The generation of these two CCHF model, a type I interferon α/β receptor knockout (IFN/α/βR-/-) mice and STAT1 knockout mice paved the way for testing the efficacy of newly developed CCHF vaccine candidates.

To date, the only available vaccine used to immunize human is a suckling mouse brain-derived, chloroform and heat inactivated virus subsequently formulated with aluminum hydroxide adjuvant (10). This inactivated virus vaccine has been used in Bulgaria to vaccinate people from risk groups since 1974 (10). Due to its crude preparation which causes concerns for safety, along with the absence of controlled human studies and the laboratory assessments of the efficacy of this vaccine, it is unlikely to gain approval by the international regulatory bodies. Therefore, there is currently no internationally approved, safe and effective vaccine against CCHFV available for widespread human use.

In regards to the CCHFV vaccine research, there are some recently reported studies on the development of vaccine candidates against CCHFV exhibiting protective efficacy in the IFN/α/βR-/- mice (11-18). These vaccine research studies mostly focus on recombinant virus based and DNA based vaccines development approaches (11-13,16-18). Recent approaches used on the development of vaccines against CCHFV were comprehensively reviewed elsewhere (19).

Here, we aimed to examine the immunizing properties and the protective abilities of a DNA based vaccine targeting the glycoprotein Gc antigen of a CCHFV isolate with Turkey origin (Ank2) by means of measuring the humoral and cell-mediated immune responses and conducting a challenge assay in IFN/α/βR-/- mice. To our knowledge, this is the first study reporting on the immunogenic characteristics and the disease protective properties of a CCHFV Gc antigen expressed via a DNA based vaccine candidate.

MATERIAL AND METHODS

Cells and Virus

Baby hamster kidney (BHK-21-C13) and Scott and White No. 13 (SW-13) cells were used in this study. Both cells were cultured in their suitable mediums supplemented with relevant concentrations of fetal bovine serum and antibiotics and maintained at 37°C in a humidified incubator containing 5% CO_2 as described previously (20). CCHFV clinical isolate Ank2 from the virus collection of Ankara University, Faculty of Veterinary Medicine, Department of Virology was used for all experiments. The third passage of this virus propagated in SW-13 cells was used in this study. All experiments related to CCHFV was carried out in the biosafety level 3 plus (BSL3+) and animal biosafety level 3 plus (ABSL3+) facilities of the Virology Department at Veterinary Faculty of Ankara University.

Animals

Five to nine weeks old, female, inbreed BALB/c mice and IFN/α/βR-/- mice with AG129 background were used in this study. Mice housing and handling procedures were followed according to the ethical rules of The Republic of Turkey Ministry of Food, Agriculture, and Livestock. All the *in vivo* experimental protocols were carried out under The Ankara University Ethical Committee's approval and in ABSL3+ animal facility at Virology Department of the Faculty of Veterinary Medicine. The study was approved by the Local Ethics Committee for Animal Experiments of Ankara University (17.12.2014, 23/155).

Construction of DNA Vaccine

pVAX1 (Thermo Fischer Scientific, USA) and pEGFP-C1 (Clontech, USA) plasmid vectors were used for the phylogenetic analysis of the cloned Gc ORF of CCHFV Isolate Ank2 and the construction of DNA vaccine vector, respectively. Plasmid isolations were carried out using the GeneJET Plasmid Maxiprep Kit (Thermo Scientific, USA). SLiCE (Seamless Ligation Cloning Extract) cloning method, which works through homologous recombination was used for all cloning experiments (21). Bacterial transformation and selection of the right clones were carried out according to the standard protocols (22). In the cloning of Gc ORF into pVAX1, viral RNA was extracted from the original stock of CCHFV Isolate Ank2 using the standard procedure of QIAamp Cador Pathogen Mini Kit (QIAGEN, Germany) and reverse transcribed with random hexamers using the standard procedure of Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The Gc ORF was then PCR amplified from the full-length M segment cDNA using the standard protocol of MyTaq HS DNA Polymerase (BIOLINE, UK) with the oligonucleotide primer couple: SliceF1:

5'ACGACTCACTATAGGGAGACCCAAGCTGGCTA GCGTTTAAACTTAGCCACCATGTTCTTGGA CAGTATAGTTAAAGG3' and SliceR1: 5'AATTCCACCACACTGGACTAGTGGATCCGAGCT CGGTACCAAGCTTTAGCCAATGTGTGTTTTTGTG G3' and cloned into pVAX1 plasmid vector linearized with HindIII restriction enzyme (New England Biolabs, UK) and blunt-ended with Phusion DNA polymerase (Thermo Scientific, USA) beforehand using the SLiCE cloning method. This construct was named as pVAX1-Gc. pEGFP-Gc and pGc plasmid vectors were constructed using the pEGFP-C1 vector backbone and used for the *in vitro* expression and *in vivo* immunization experiments respectively. For the generation of pEGFP-Gc vector, the Gc ORF of isolate Ank2 was PCR amplified from the pVAX1-Gc vector using the standard protocol of MyTaq HS DNA Polymerase (BIOLINE, UK) with the oligonucleotide primer couple: SliceF2: 5'GAGCTGTACAAGTCCGGACTCAGATCTCGAGCT ATGTTCTTGGACAGTATAGTTAAAGG3' and SliceR2: 5'GTATGGCTGATTATGATCAGTTATCTAGATCCG GTTTAGCCAATGTGTGTTTTTGTGGAGAAC3' and cloned into pEGFP-C1 vector linearized with BamHI and HindIII restriction enzymes (New England Biolabs, UK) before, using the SLiCE cloning method. By this way, in the pEGFP-Gc vector, the cloned Gc ORF was designed to be expressed in-frame with EGFP as a C terminal fusion product under the control of the Cytomegalovirus (CMV) promoter. pGc vector was derived from the pEGFP-Gc vector by replacing the EGFP ORF with 8X Histidine Tag. For this purpose, the pEGFP-Gc vector backbone was PCR amplified by excluding the EGFP ORF using the standard protocol of MyTaq HS DNA Polymerase (BIOLINE, UK) with the oligonucleotide primer couple: EGFP RemovalF:5'CACCATCATCACCACCACCATCACTT CTTGGACAGTATAGTTAAAGGTATGAAAAATTTG C3' and EGFP RemovalR: 5'GTGATGGTGGTGGTGATGATGGTGCATGGTGG CGACCGGTAGCGC3' and then the amplicon was circularized using SLiCE. Thus, in the pGc vector, the Gc ORF was planned to be expressed in-frame with N-terminal 8X His Tag under the control of the CMV promoter. For the negative control experiments, an empty vector was generated from the pEGFP-C1 vector by excision of the CMV promoter from the vector backbone. For this purpose, the CMV promotor site was cut out from the vector using the NdeI and NheI restriction enzymes (New England Biolabs, UK) and the remaining vector backbone was circularized simply by the "alternative end joining" mechanism of *E. coli* (9). The vector map images were created using the SnapGene Viewer 4.1.6 Software (GSL Biotech LLC). The nucleotide sequences of each vector were confirmed with both next-generation sequencing (NGS) (Ion Torrent Platform) and Sanger dideoxy chain termination DNA sequencing methods prior to their use. In the latter method, the following sequencing primers were used:
CMV Forward: 5'CGCAAATGGGCGGTAGGCGTG3'
GcSeq1: 5'CCGACAACCACTACCTGAGCAC3',
GcSeq2: 5'GTGGTTGCACATCATCAACCTGC3',
GcSeq3: 5'GGATGTCCTGGGATGGTTGTGAC3' and
GcSeq4: 5'AAACTTGAGCAGCCACAGAGC3'.

Phylogenetic Analysis

Phylogenetic analysis of the Gc ORF of CCHFV Isolate Ank2 was performed based on its amino acid sequence using the MEGA7 software (23). Thirteen representative CCHFV mature Gc sequences of different strains from various geographical regions including Turkey were obtained from the NCBI GenBank database and used for the phylogenetic analysis. The phylogenetic tree was constructed based on the Neighbor-Joining algorithm (24). The bootstrap values higher than 50 were indicated for each branch based on 1000 replicates (25).

Transient and Stable Transfections of BHK-21-C13 Cells

pEGFP-Gc vector was used for the *in vitro* validation of Gc expression in the transfected BHK-21-C13 cells. For all transient and stable transfection experiments, standard protocol of Lipofectamine 3000 (Thermo Scientific, USA) transfection reagent was used. In the transient transfection experiments, the circular form of the pEGFP-Gc vector was used to transfect BHK-21-C13 cells. Transfected cells were then subjected to downstream analyses at the 48-h post-transfection time point. In the stable transfection experiments, both vectors were linearized with AlwNI restriction endonuclease enzyme (New England Biolabs, UK) before transfection and starting from the 48 h post-transfection, cells were treated with 800 µg of geneticin (G418, Thermo Scientific Fisher, USA) per ml of media during three weeks for the selection of stably transfected BHK-21-C13 cells.

Fluorescence Microscopy

Expression of the EGFP-Gc fusion product was demonstrated by viewing the EGFP-Gc expressing, transiently transfected BHK-21-C13 cells under an inverted fluorescence microscope (Zeiss Axio Vert.A1, Germany) using the blue light (488 nm) as the excitation source.

Western Blot

Following the three-week long geneticin selection process, the stably transfected BHK-21 cells were lysed with Pro-Prep Protein Extraction Solution (İNtRON Biotechnology). Cell lysates were mixed 1:1 with 2X Laemmli's sample buffer (Sigma-Aldrich) and heated at 95°C for 5 minutes. Proteins were separated by SDS-PAGE in Mini-PROTEAN TGX Stain-Free Precast Gels (BioRad, USA) and transferred to Trans-Blot Turbo Mini PVDF membranes (BioRad, USA). The membranes were blocked with 1x Tris Buffered Saline (1x TBS) (Sigma) supplemented with 0.1% Tween 20 (BioRad, USA) (TBST) and 5% BSA bovine serum albumin (Sigma, USA). Blocked membranes were probed for EGFP with 1/1000 GFP tag mouse monoclonal antibody (GF28R) (Invitrogen) prepared in 1x TBST and incubated at +4°C overnight. The membranes were washed 5 times with 1x TBST and incubated with horseradish peroxidase-conjugated anti-mouse secondary antibodies (Advansta, USA) diluted in 1x TBST at room temperature for 1 hour. The membranes were washed 5 additional times with 1x TBST and imaged with enhanced chemiluminescence (ECL) assay using the ChemiDoc XRS+ imaging system (BioRad, USA).

RT-PCR

For the demonstration of Gc expression at the transcription level, total RNA of BHK-21-C13 cells stably transfected with pEGFP-Gc vector was isolated using the Trizol reagent (26) (Thermo Scientific, USA) and following a DNase I (New England Biolabs, UK) treatment, the

isolated RNA was purified with the standard protocol of sodium acetate/ethanol precipitation followed by washing of the RNA pellet with 70% ethanol. The mRNA content of the purified total RNA was reverse transcribed with oligo(dt) 18 primer using the standard procedure of Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, USA) and the cDNA of Gc transcripts were then PCR amplified using MyTaq HS DNA Polymerase (BIOLINE, UK) with the primer couple: GcSeq4: 5'AAACTTGAGCAGCCACAGAGC3' and SLICER2: 5'GTATGGCTGATTATGATCAGTTATCTAGATCCG GTTTAGCCAATGTGTGTTTTTGTGGAGAAC3' targeting the downstream site of the Gc transcript.

Vaccination Protocol

Two groups of four Balb/c and four IFN/α/βR-/- mice were vaccinated in the medial thigh muscle with 100 µg of either the pGc vaccine vector or the empty vector dissolved in 50 µL of non-pyrogenic physiological saline solution using the 25-gauge syringes (BD, USA). All mice were vaccinated two times with two weeks intervals. Blood samples were collected via tail vein bleeds prior to each vaccination on days 0, 14 and 28.

Virus Neutralization Assay (VNA)

The presence and titer of the anti-CCHFV neutralizing antibodies in the sera of mice received two consecutive pGc DNA vaccinations were determined by micro virus neutralization assay (MVNA). The serum samples were initially heat inactivated at 56°C for 30 minutes. Two-fold serial dilutions were prepared starting from 1/8 to 1/532 and mixed with equal volumes of CCHFV Ank2 isolate at a 100TCID50 doses and incubated at 37°C for 1 hour. After the incubation, each reaction mixture was added to the 50,000 SW-13 cells adhered on a 96-well plate with four replicates and incubated for 1.5 hours at 37°C in a humidified cell culture incubator containing 5% CO2. The reaction mixtures were later replaced with fresh Leibovitz's l-15 medium (Gibco, USA) supplemented with 2% heat-inactivated FBS (Gibco, USA) and 1% Penicillin/streptomycin (Gibco, USA) and cells were incubated for one week under the same culture conditions described above. Cells were later fixed with 3.7% formaldehyde (Sigma, USA) and stained with 1% crystal violet (Sigma, USA) solution prepared in 20% ethanol (Sigma, USA) before being visualized by naked eye and under a simple light inverted microscope (Olympus, Japan).

Detection of Cytokines in the Sera of Vaccinated Mice

To examine the Th1 and Th2 type immune responses, cytokine production levels were determined in the serum samples of vaccinated mice using the LEGENDplex Mouse Th1/Th2 Panel (8-plex, Bead assay by flow cytometry) kit (BioLegend, USA, https://www.biolegend.com/en-us/legendplex). All the bead-based cytokine assays were conducted according to the manual of the manufacturer. The reactions were evaluated after reading FacsCanto II FlowCytometer (BD Bioscience, USA) using the LEGENDplexTM Data Analysis Software.

Challenge Assay of IFNα/βR-/- Mice

The CCHFV Ank2 strain, which was previously demonstrated as lethal for IFNα/βR-/- mice (11), was used in the intra-peritoneal challenge assay. Two weeks after the final vaccination, all mice (4 mice per group) were intraperitoneally inoculated with the virus (third passage in SW-13 cell) at a dose of 100LD50 (1000TCID50) prepared in 300µL of Leibovitz's l-15 medium (Gibco, USA). The negative control group consisted of four mice received only sterile physiological saline solution. The assay was continued for 13 days. Daily observations of the clinical signs of the disease including the appearance change on fur such as erectile hairs, weight loss, nasal or ocular discharge, depression, and death were noted.

Statistical Analysis

After performing the Shapiro-Wilk test for normality assumption, the cytokine assay data obtained from BALB/c and IFN/α/βR-/- mice were analyzed using the Mann-Whitney U test. The p values less than 0.05 were regarded as statistically significant. All analyses were performed using the SPSS software v.22.0.

RESULTS

Maps of the pEGFP-Gc, and pGc Vectors

Vector maps depicting the features of pEGFP-Gc and pGc plasmids including the regulatory sequences and protein coding regions were shown in Figure 1a and 1b respectively. While the pVAX1-Gc vector was used for the phylogenetic analysis of the cloned CCHFV Gc ORF and aided to the generation of pEGFP-Gc and pGc vectors, the pEGFP-Gc vector was used for the *in vitro* expression studies. Finally, the pGc vector was used for the mouse immunization experiments.

Phylogenetic Analysis of the CCHFV (Ank2) Glycoprotein Gc

The nucleotide sequence of cloned CCHFV Ank2 Gc ORF was determined using the NGS approach (Ion Torrent platform) and deposited to the NCBI GenBank under the accession number: MG969426. The amino acid sequence of the virus glycoprotein Gc was phylogenetically analyzed using the Gc sequences of a total of thirteen representative CCHFV strains from different geographical areas including Turkey. The phylogenetic tree was constructed based on the Neighbor-Joining algorithm using the MEGA7 software and presented in Figure 2. The CCHFV Ank2 was found to be closely related to the CCHFV strains (e.g. Kelkit06 and Turkey200310849) reported previously from Turkey in terms of Gc sequences (Figure 2).

***In Vitro* Expression of CCHFV (Ank2) Gc**

In order to confirm *in vitro* expression of CCHFV (Isolate Ank2) Gc, BHK-21 cells were transiently transfected with pEGFP-Gc vector and analyzed with fluorescence microscopy at 48 h post-transfection for the detection of EGFP-Gc fusion product. A considerable number of live cells emitting green fluorescent light were apparent as shown in Figure 3b. The expression of EGFP-Gc fusion product was later demonstrated with western blot analysis using the anti-GFP monoclonal antibody in the protein extracts of BHK-21 cells stably transfected with pEGFP-Gc vector (Figure 3d). Additionally, the expression of EGFP-Gc ORF was confirmed at the transcript level with RT-PCR analysis in the mRNA pool of BHK-21 cells stably transfected with pEGFP-Gc vector (Figure 3c).

Neutralizing Antibody Responses of Vaccinated Mice

Anti-CCHFV neutralizing antibody responses were evaluated in the serially diluted serum samples of both immunocompetent BALB/c and IFN/α/βR-/- mice received two consecutive pGc DNA vaccinations using the

micro virus neutralization assay (MVNA). Briefly, the SW-13 cells were subjected to 100 TCID50 of CCHFV (Isolate Ank2) pretreated with various dilutions of sera collected from the vaccinated mice and later, cells were checked for the occurrence of cytopathic effects (CPE) over a one-week period. The apparent formation of CPE in SW-13 cells infected with CCHFV is a well-known phenomenon (30). As a result, all four vaccinated mice in each mouse models responded with considerable amount of neutralizing antibody production with the mean titers of 6.8 and 7.5 Log2 for IFN/α/βR-/- and BALB/c mice respectively (Figure 4).

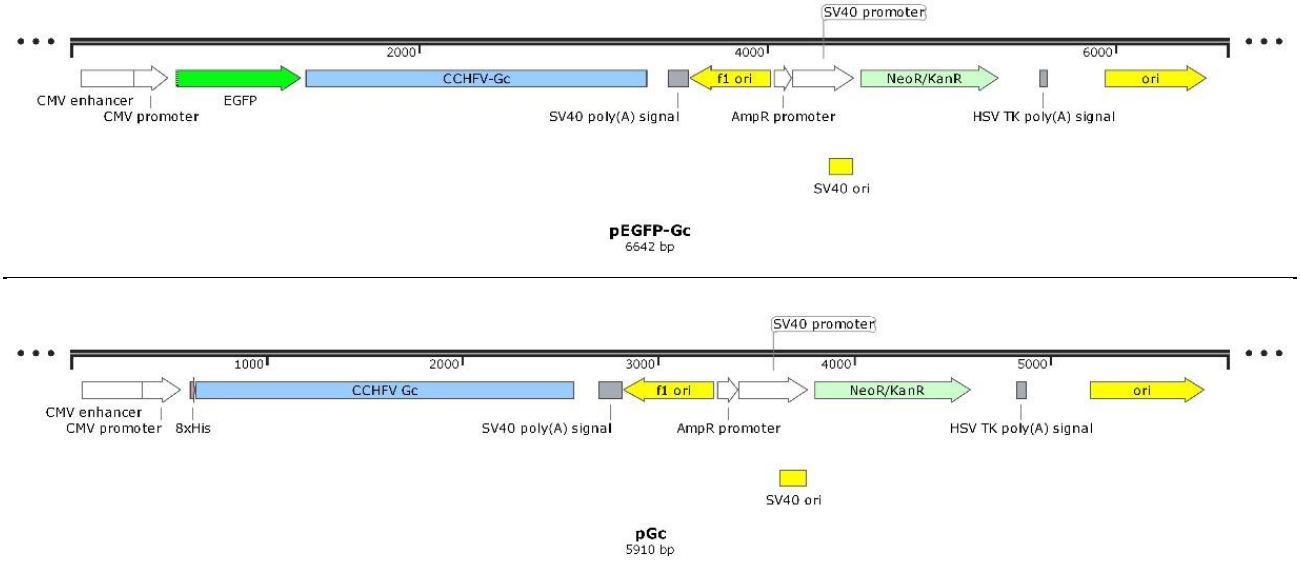

Figure 1. Maps of the **a)** pEGFP-Gc and **b)** pGc plasmids

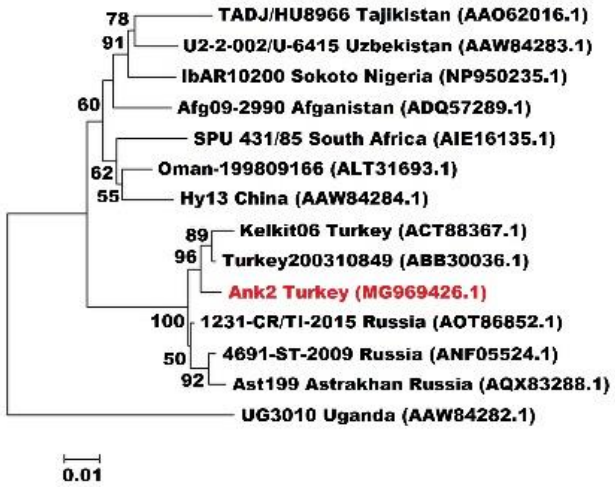

Figure 2. Phylogenetic tree of the CCHFV (Isolate Ank2) glycoprotein Gc constructed based on the Neighbor-Joining algorithm in MEGA7 software. The Genbank accession numbers of each sequence were given in brackets. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.28116628 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and were in the units of the number of amino acid substitutions per site. The analysis involved 14 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 644 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

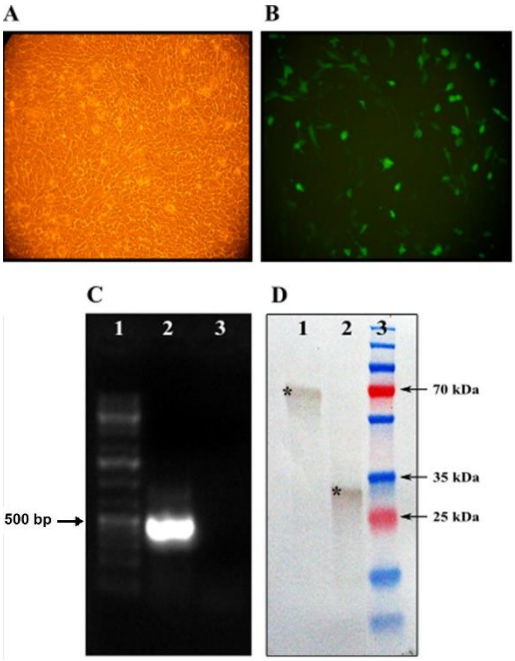

Figure 3. *In vitro* expression of pEGFP-Gc vector in BHK-21 cells **a)** simple light microscope and **b)** fluorescence microscope images of BHK-21 cells transiently transfected with pEGFP-Gc vector, **c)** Gel images of Gc transcript-specific RT PCR analysis conducted with RNA extracts of BHK-21 cells stably transfected with pEGFP-Gc vector, 1: DNA marker, 2: Amplicon of the Gc mRNA specific RT-PCR, and 3: RNA PCR control **d)** Western blot membrane images of EGFP-Gc fusion protein in protein extracts of BHK-21 cells stably transfected with pEGFP-Gc vector, 1: EGFP-Gc fusion product in cells stably transfected with pEGFP-Gc vector (indicated with asterisk) and 2: EGFP product in cells transiently transfected with pEGFP-C1 vector (indicated with asterisk), 3: Protein Marker

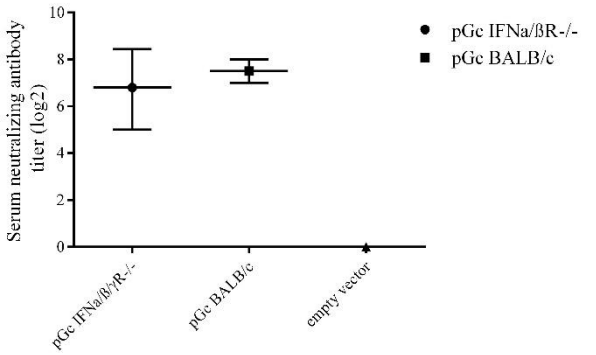

Figure 4. Determination of the neutralizing antibodies in the sera of BALB/c and IFN/α/βR-/- mice vaccinated with pGc vector using the micro-neutralization assay: Figure shows the lowest, the highest and the mean log2 neutralizing antibody titers observed in the sera of four BALB/c and four IFN/α/βR-/- mice received two consecutive pGc vaccine.

Cytokine Responses of Vaccinated Mice

The sera of mice of the immunization groups were examined for their Th1 and Th2 cytokine profiles. BALB/c mice vaccinated with pGc exhibited levels of the Th1 type cytokines IFN-γ, interleukin (IL-2), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) which were significantly higher than the mice received only the empty vector (Table 1, Figure 5a, 5b, 5f, 5h). Besides, in the vaccinated BALB/c mice, also significantly higher levels of Th2 type cytokines IL-4, IL-5 and IL-10 were observed compared to the mice received only the empty vector (Table 1, Figure 5c, 5d, 5e). On the other hand, IFN/α/βR-/- mice vaccinated with pGc responded with increased levels of the Th1 type cytokines IFN-γ and IL-6 which were significantly higher than the mice received only the empty vector (Table 2, Figure 6a, 6f). Also, in the vaccinated mice, significantly higher levels of Th2 type cytokines IL-4, IL-5 and IL-10 were observed compared to the mice received only the empty vector (Table 2, Figure 6c, 6d, 6e). In the light of these cytokine responses, it appears evident that our DNA vaccine vector elicited a balanced Th1/Th2 cellular response in both immunocompetent BALB/c and IFN/α/βR-/- mice.

Protective Efficacy of the pGc Mediated Immunization

IFN/α/βR-/- mice (n=4 per group) were vaccinated with the pGc vaccine two times at a dose of 100 µg DNA per each vaccination two weeks apart. Two weeks after the last vaccination, mice were challenged with a lethal dose (100LD50) of CCHFV-Ank2. Despite the generation of Gc specific humoral and cellular immunity after the vaccination schedule, all mice were defeated by lethal disease between days 3 and 6 post-challenge (Figure 6).

DISCUSSION

CCHF is the most geographically widespread tick-borne viral zoonosis. Expansion of the endemic areas and emergence of new foci, together with the increasing number of cases put this disease to the forefront with regards to an urgent need for developing effective preventive measures in order to reduce its impact on public health. Apart from the fact that the effective treatment of CCHF inevitably requires developing specific therapeutic agents, vaccination appears to be the most plausible strategy for controlling the disease. Recent reports on the development and efficacy of experimental vaccines against CCHF reveal promising results (11-18). However, currently there is no widely accessible, internationally approved, safe and efficient vaccine for CCHF and thus, in the context of revealing the immunizing and protective aspects of different vaccination approaches, vaccine development against CCHF is at present an actively studied research subject.

In the present study, we generated a DNA vaccine candidate (pGc) encoding for the envelope glycoprotein Gc of a cell adapted local CCHFV isolate (Ank2) and evaluated its immunizing and protective properties in both immunocompetent BALB/c and IFN/α/βR-/- mice by undertaking the homologous prime-boost vaccination regime.

Table 1. Cytokine data summary obtained from BALB/c mice received pGC vaccine or empty vector

Groups	Min	Q1	Median	Q3	Max	Mean	SD	p
IFN-γ (Vaccination)	54.4	54.4	54.4	68.82	83.24	64.01	16.65	**0.043**
IFN-γ (Empty Vector)	19.99	19.99	19.99	23.41	26.84	22.27	3.95	
IL-2 (Vaccination)	19.37	19.37	23.35	28.70	32.82	24.72	6.57	**0.031**
IL-2 (Empty Vector)	4.07	7.41	10.75	10.75	10.75	8.52	3.85	
IL-4 (Vaccination)	18.24	19.64	20.11	22.88	31.19	22.41	5.91	**0.019**
IL-4 (Empty Vector)	3.51	6.21	10.47	13.82	13.82	9.56	5.12	
IL-5 (Vaccination)	23.44	23.44	31.45	39.46	39.46	31.45	9.24	**0.026**
IL-5 (Empty Vector)	8.53	8.53	9.24	9.96	9.96	9.24	0.82	
IL-10 (Vaccination)	87.67	87.67	99.57	133.18	198.29	121.27	52.55	**0.031**
IL-10 (Empty Vector)	11.41	11.41	17.10	14.25	17.10	13.30	3.28	
IL-6 (Vaccination)	21.59	21.59	25.85	33.59	44.03	29.33	10.59	**0.028**
IL-6 (Empty Vector)	6.21	6.21	6.85	8.94	13.27	8.29	3.37	
IL-13 (Vaccination)	8.85	8.85	11.35	13.85	13.85	11.35	2.88	0.300
IL-13 (Empty Vector)	2.20	2.20	5.62	9.05	9.05	5.62	3.95	
TNF-α (Vaccination)	35.17	35.17	41.93	52.34	63.3	45.58	13.42	**0.019**
TNF-α (Empty Vector)	3.64	9.58	15.58	19.61	19.61	13.60	7.65	

Min: minimum; Q1: first quartile; Q3: third quartile; Max: maximum; SD: standard deviation

Table 2. Cytokine data summary obtained from IFN/α/βR-/- mice received pGC vaccine or empty vector

Groups	Min	Q1	Median	Q3	Max	Mean	SD	p
IFN-γ (Vaccination)	835.78	896.23	1288.03	1659.68	1659.68	1267.88	453.60	**0.027**
IFN-γ (Empty Vector)	722.34	722.34	722.32	822.30	822.30	772.32	57.71	
IL-2 (Vaccination)	11.98	11.98	13.68	15.38	15.38	13.68	1.96	0.659
IL-2 (Empty Vector)	5.50	11.38	13.34	13.49	13.94	11.53	4.029	
IL-4 (Vaccination)	64.22	64.80	65.00	76.46	110.86	76.27	23.06	**0.027**
IL-4 (Empty Vector)	49.20	49.20	52.99	56.78	56.78	52.99	4.37	
IL-5 (Vaccination)	23.36	25.82	30.49	34.34	34.34	29.67	5.55	**0.028**
IL-5 (Empty Vector)	11.56	11.95	12.09	14.56	22.00	14.43	5.049	
IL-10 (Vaccination)	157.90	157.90	181.75	205.60	205.60	181.75	27.53	**0.026**
IL-10 (Empty Vector)	91.66	91.66	96.34	101.02	101.02	96.34	5.40	
IL-6 (Vaccination)	14.64	14.64	15.46	16.28	16.28	15.46	0.94	**0.026**
IL-6 (Empty Vector)	12.40	12.40	12.76	13.13	13.13	12.76	0.42	
IL-13 (Vaccination)	77.02	91.48	145.03	193.76	193.76	140.21	62.33	0.663
IL-13 (Empty Vector)	73.77	106.78	118.42	128.33	156.16	116.69	33.69	
TNF-α (Vaccination)	31.56	32.49	36.61	40.42	40.42	36.30	4.78	0.105
TNF-α (Empty Vector)	30.00	30.00	31.30	32.61	32.61	31.30	1.50	

Min: minimum; Q1: first quartile; Q3: third quartile; Max: maximum; SD: standard deviation

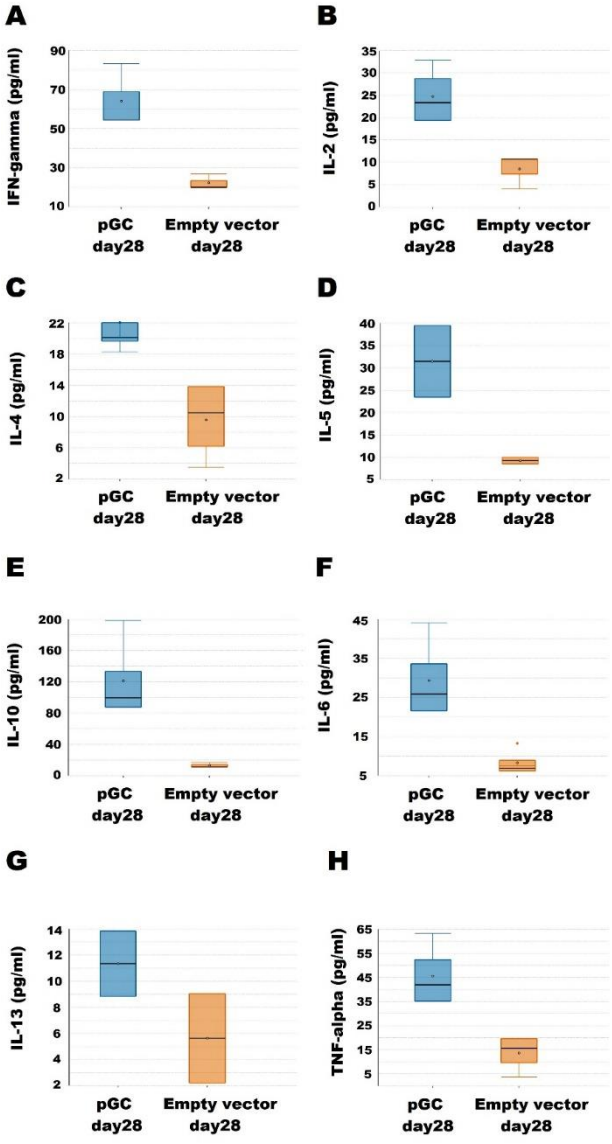

Figure 5. The Th1/Th2 cytokine levels in the sera of BALB/c mice vaccinated with the pGc vector: Levels of **a)** IFN-γ, **b)** IL-2, **c)** IL-4, **d)** IL-5, **e)** IL-10, **f)** IL-6, **g)** IL-13, and **h)** TNF-α

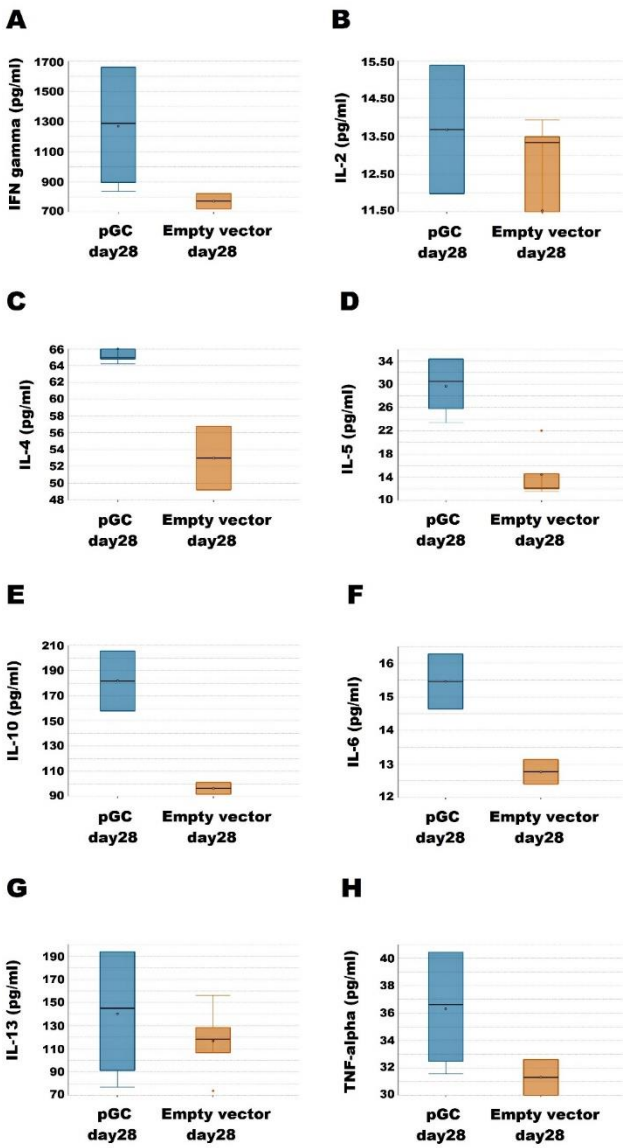

Figure 6. The Th1/Th2 cytokine levels in the sera of IFN/α/βR-/- mice vaccinated with the pGc vector: Levels of **a)** IFN-γ, **b)** IL-2, **c)** IL-4, **d)** IL-5, **e)** IL-10, **f)** IL-6, **g)** IL-13, and **h)** TNF-α

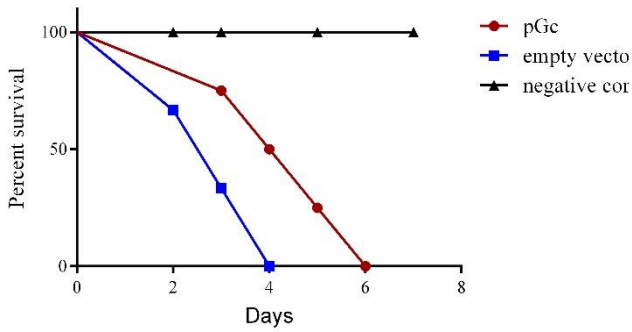

Figure 7. Efficacy of pGc vaccine in IFN/α/βR-/- mice challenged with CCHFV (Isolate Ank2): IFN/α/βR-/- mice were challenged with 100LD50 doses of CCHFV 14 days after the second vaccination with pGc (solid circles) and empty vector (solid squares). The control group received only saline solution was shown with solid triangles

For the immunogenicity of the pGc vaccine, we examined the antibody and cell-mediated immune responses in the immunocompetent BALB/c mouse model and got the similar results with those obtained in IFN/α/βR-/- mice (Figure 4, 5 and 6). Other researchers have also reported similar findings in studies with CCHFV viral vaccines expressing nucleoprotein (27).

In regards to the cytokine responses, mice received two consecutive doses of pGc yielded a cellular response with increased levels of both Th1 type and Th2 type cytokines which were evident from the cytokine profiles of the serum samples in both rodent models. Generation of a Th1 biased or balanced Th1/Th2 responses may be dependent on the antigen type used in the immunization process (28). For instance, studies on the development of DNA vaccines against respiratory syncytial virus (RSV) revealed that while DNA vaccines targeting the fusion (F) glycoprotein of RSV elicit a Th1 biased cellular responses, DNA vaccine constructs designed to express attachment (G) glycoprotein of RSV yield a balanced Th1/Th2 cellular responses in vaccinated mouse models (29,30). The protective aspects of the balanced Th1/Th2 responses primed by DNA based vaccines targeting different pathogen antigens were demonstrated in various *in vivo* infection settings (31-33). Thus it appears that the fail of pGc vaccine vector in conferring protection against lethal infection could not be solely attributed to the generation of balanced Th1/Th2 responses.

Two consecutive vaccinations of both BALB/c and IFN/α/βR-/- mice with pGc vector were found to elicit considerable amount of neutralizing antibody response, which was inferred from the micro virus neutralization assay. However, while the display of the glycoproteins on the surface of virions makes them a convenient target for the induction of neutralizing antibodies, antibody-mediated virus neutralization observed *in vitro* does not always correlate with the *in vivo* protection of antibodies specific to CCHFV (34). Nevertheless, the prolonged survival observed in some individuals of the vaccinated group in comparison to empty vector group might be a consequence of the presence of neutralizing antibodies.

Different than wild type mice, IFN/α/βR-/- mice mostly do not generate swift responses to infection and, furthermore, CCHFV infections create higher viral loads in IFN/α/βR-/- mice compare to the wild type mice (35). Increased obstacle in protecting IFN/α/βR-/- mice from infection appears associated with the deficiency in the immune responses, which is most likely caused by the insufficient cross-presentation of antigens by dendritic cells (36). Therefore, efficacy studies of vaccines in this disease model can be quite difficult as vaccine candidates must generate convenient immune responses by overcoming the IFN/α/βR-/- weakened antigen presentation in order to elicit protection. Furthermore, protective vaccines must trigger an effective adaptive immune response that can compensate the deprivation of the type I interferon mediated antiviral state activation in this disease model. Thus, IFN/α/βR-/- mice should be regarded as a higher bar to cross for efficacy studies of vaccines than the immunocompetent mouse models (18).

CCHFV vaccine development gets further difficult due to the scarcity of knowledge on involvement of both B and T cell epitopes for the establishment of an effective immune response; and the types of immune responses required for the disease protection. The virus like particles (VLP), modified vaccinia virus Ankara (MVA) and plasmid DNA vaccine platforms have exhibited success in protecting murine models from lethal CCHFV challenge, and the yielded protection was shown to be dependent on both arms of the adaptive immunity (13,15-17,27). Furthermore, all these platforms postulates that antibody and/or T cell mediated immune responses developed against the CCHFV glycoprotein precursor are crucial for protection in murine models (13-17,27,37).

Should the vaccine have exhibited any degree of protection, further work would have been done, for instance, by using a codon-optimized version of the pGc vaccine and/or electroporation assisted DNA vaccine delivery, which at least in part might have played a role in the better expression of the antigen *in vivo*. Besides, co-delivery of a relevant adjuvant and the pGc vaccine might have also improved the adaptive immune responses and consequently elicited a protective effect via the stimulation of the innate immunity. Although vaccination approaches that focus on a single antigen could be successful, an elegant vaccine candidate may effectively stimulate immune responses against multiple antigens and give protection with few doses. To this end, the pGc vaccine would be coupled with DNA vaccines or with other vaccine platforms that target CCHFV antigens different than Gc.

CONCLUSION

To the best of our knowledge, this is the first report of a DNA vaccine intended to target CCHFV based on the glycoprotein Gc. The pGc vaccine candidate exhibited antigen-specific immunity in IFN/α/βR-/- mice, but failed to confer a protection upon lethal challenge with the homologous CCHF virus. Once we gain more insight into the immune correlates of protection, the better we will have the chances to significantly improve the efficacy of vaccines.

Ethics Committee Approval: The study was approved by the Local Ethics Committee for Animal Experiments of Ankara University (17.12.2014, 23/155).

Conflict of Interest: None declared by the authors.

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The Evaluation of Core Needle Breast Biopsy Analyzes Performed with 14 and 18 Gauge Needles: A Single Center Experience for Eight Years

14 ve 18 Gauge İğnelerle Yapılan Kor İğne Meme Biyopsisi Analizlerinin Değerlendirilmesi: Sekiz Yıllık Tek Merkez Deneyimi

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ABSTRACT

Aim: Percutaneous imaging-guided core needle breast biopsy has become widely used as an alternative to incisional biopsy in the diagnosis of breast lesions. In this study, it was aimed to evaluate and report our core needle breast biopsy experiences performed with 14- and 18-gauge needles.

Material and Methods: Patients who underwent core needle breast biopsy between March 2012 and December 2019 in our radiology department and whose biopsy specimens were evaluated in the pathology department, of all age groups and both sexes, were included in this study. A total of 628 (615 female and 13 male) patients with breast masses were examined.

Results: The mean age of the patients was 52.20±13.94 (median= 51, range, 13-96) years, 90.4% (n=568) of the lesions were masses and the majority of lesions (53.2%, n=334) were 11-20 mm in size. The most of cases (47.2%, n=268) were BI-RADS 5. There was no significant difference between the two needles in terms of gender distribution, age, type of surgery, and core needle breast biopsy results. In 86.5% (n=141) of the patients, there was diagnostic accuracy between the surgical specimen and the core needle breast biopsy result.

Conclusion: We demonstrated that the 14-gauge and 18-gauge needles have similar performance of results. Smaller needles should be used for ultrasound-guided breast biopsies, which is less invasive, less painful, and creates less risk of hemorrhage. Moreover, no patient admitted to the emergency department because of the core needle breast biopsy acute complications such as hematoma, bleeding, etc. during this time.

Keywords: Core needle breast biopsy; breast masses; biopsy specimens.

ÖZ

Amaç: Perkutan görüntüleme eşliğinde kor iğne meme biyopsisi, meme lezyonlarının teşhisinde insizyonel biyopsiye alternatif olarak yaygın bir şekilde kullanılmaktadır. Bu çalışmada; 14 ve 18 gauge iğnelerle yapılan kor iğne meme biyopsisi deneyimlerimizin değerlendirilmesi ve sunulması amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmaya, Mart 2012 ve Aralık 2019 tarihleri arasında Radyoloji Anabilim Dalında kor iğne meme biyopsisi uygulanmış olan ve biyopsi örnekleri Patoloji Anabilim Dalında değerlendirilmiş olan, tüm yaş gruplarından ve her iki cinsiyetten hastalar dahil edilmiştir. Meme lezyonu olan toplam 628 (615 kadın ve 13 erkek) hasta incelenmiştir.

Bulgular: Hastaların ortalama yaşı 52.20±13.94 (medyan= 51, aralık, 13-96) yıl olup, lezyonların %90,4'ü (n=568) kitle lezyonu şeklindeydi ve lezyonların büyük çoğunluğu (53.1%; n=334) 11-20 mm boyutundaydı. Vakaların çoğu (%47,2; n=268) BI-RADS 5 idi. İki iğne arasında cinsiyet dağılımı, yaş, ameliyat türü ve kor iğne meme biyopsisi sonuçları açısından anlamlı bir farklılık yoktu. Hastaların %86,5'ünde (n=141) cerrahi olarak çıkartılan materyallerin histopatolojik sonucu ve kor iğne meme biyopsisi sonucu arasında tanısal doğruluk vardı.

Sonuç: 14-gauge ve 18-gauge iğnelerin benzer sonuç gösterme potansiyeline sahip olduğu tespit edilmiştir. Daha az invaziv, daha az ağrılı ve daha az kanama olma riski içeren ultrason eşliğindeki meme biyopsileri için daha küçük iğnelerin kullanılması daha uygundur. Ayrıca, çalışma süresi içinde hematom, kanama vb. gibi akut kor iğne meme biyopsisi komplikasyonları nedeniyle hiçbir hasta acil servise başvurmamıştır.

Anahtar kelimeler: Kor iğne meme biyopsisi, meme kitleleri, biyopsi örnekleri.

INTRODUCTION

Percutaneous imaging-guided core needle breast biopsy (CNB) has become widely used as an alternative to incisional biopsy in the diagnosis of breast lesions (1-3). For a decade, ultrasound-guided CNB (US-CNB) has been accepted as an alternative method with high sensitivity/specificity for the accurate diagnosis of breast cancers (4-7). It is faster, less invasive, and less expensive. Also, the increasing experience with this procedure makes the technique more preferable (8). Accurate targeting of the needle and adequate size of specimens are the most crucial factors affecting the underestimation rates and false-negative results (9,10).

The size of the needle used for CNB is one of the factors affecting the success of the biopsy. A variety of cutting needles (11-, 14-, 16-, and 18-gauge, respectively) being used by many centers, and the common reason to prefer larger needles is diagnostic quality. However, larger needle sizes have potential to increase complication rates, such as hematoma formation, bleeding, and vasovagal reactions (11,12). While large size needles are recommended for ≤10 mm or non-mass lesions, most physicians in many different centers have been recommended to use smaller needles such as 16-gauge or 18-gauge (13-15). Limited information about the effects of needle size on the accuracy of diagnosis is currently available, and this issue has not been adequately considered. At that point, a question may arise whether smaller or thinner needles (e.g., 18- and 16-gauge) can have diagnostic value for breast lesions or not (15).

US-CNB has been performed by our center for a long time. 14-gauge CNBs from March 2012 to December 2016 and 18-gauge CNBs from December 2016 to December 2019 were performed in our center. In this study, we aimed to evaluate and report our US-CNB experience with 14- and 18-gauge needles.

MATERIAL AND METHODS

Study Design and Setting

This study was performed with the approval of the local ethics committee of Düzce University (01.06.2020, 2020/73), and informed consent was waived because of the retrospective design of the study. A total of 665 US-CNBs (14-gauge CNBs from March 2012 to December 2016, and 18-gauge CNBs from December 2016 to December 2019) were performed in our institution. We retrospectively reviewed all the biopsy results and excluded cases that had missing data.

Participants and Measurements

Patients who underwent CNB between March 2012 to December 2019 in our radiology department and whose biopsy specimens were evaluated in the pathology department in this center, of all age groups and both sexes were included in this study.

Any CNB performed apart from this period, the cases whose biopsy materials were evaluated in another pathology center and patients whose files could not be accessed were excluded from the study.

Patients' data were recruited from the patient registry database system of radiology and pathology departments. The patients who underwent CNB procedure were screened retrospectively and the data of the patients were classified as follows;

- Patients' age, age group and gender
- Breast Imaging-Reporting and Data System (BI-RADS) class of the lesions;
  BI-RADS classification was routinely used for reporting the breast lesions; 0: assessment incomplete; 1: negative; 2: benign finding; 3: probably benign lesions; 4: suspicious of malignancy (possibility 3%-94%); and 5: highly suggestive of malignancy (>95%).
- The type of lesion (mass/non-mass)
- The size of the lesion
- Size of the needle (14-/18-gauge)
- Pathologic diagnosis (benign, malignant, others: borderline, unsatisfactory)
- If the patient has undergone a surgical operation, the type of procedure (total/partial mastectomy, lumpectomy)
- Pathologic diagnosis of the surgical specimen (SS)

Descriptive statistics of the obtained data and differences between two needle types (14- and 18-gauge) were compared.

An ultrasound-guided breast biopsy was performed at our department, and 14-gauge and 18-gauge needles were routinely used in the above-given time intervals. The choice of needle size is generally determined by the radiologist's preference. Two radiologists who have over five years' experience performed the CNBs. Ultrasound examinations were performed using a MicroMaxx HFL 38/13-6 scanner (SonoSite, USA) and a LOGIQ E-R7 (GE, USA). An automated core biopsy device with a 20-mm-long chamber was then used. Generally, at least three core samples were obtained in most of the breast lesions, and the puncture sites were compressed for 5-10 minutes to control bleeding after the biopsy procedure was completed (Figure 1). Pathological examinations were performed by a team of pathologists, and the results were reported as malignant or benign (fibroadenoma, ductal hyperplasia, fibrocystic change, granulomatosis mastitis, and abscess). Figure 2 shows the macroscopic and microscopic appearance of the biopsy material.

Statistical Analysis

The compliance of the data to normal distribution was tested with the Kolmogorov-Simirnov test. Student t test was used to compare normally distributed variables between two independent groups, and Mann-Whitney U test was used to compare non-normally distributed variables between two independent groups. Pearson chi-square or Fisher's exact tests were used to analyze categorical data. As descriptive statistics, mean±standard deviation, median, and minimum-maximum values for numerical variables, and number and percentage values for categorical variables were given. SPSS v.21.0 package program was used for statistical analysis and $p<0.05$ was considered statistically significant.

RESULTS

A total of 665 US-CNBs were performed in our center. Total 628 patients with breast masses were examined, 37 patients who had insufficient samples have excluded from the study. CNBs were performed with 14-gauge needles in 428 patients, while 200 were made with 18-gauge needles. The mean age was 52.20±13.94 (median= 51, range, 13-96) years, and most of patients (28.3%, n=178) were in 40-49 age group. 90.4 % (n=568) of the lesions were masses and

the majority (53.2%, n=334) of lesions were 11-20 mm in size. The BI-RADS score of 47.2% (n=268) of the patients was 5, and 14-gauge needles are mostly used in lesions size of >20 cm. Meanwhile the data of 14-gauge and 18-gauge needles were compared, no significant difference was found in terms of gender distribution, age, age distribution, lesion type, and BI-RADS class (Table 1).

Invasive breast carcinoma was the most frequent pathology results in total and in both needle groups (Table 2).

Total of 163 (25.9%) of the patients were underwent surgery in our center. Most of the operated patients were female (n=161, 98.8%) and the mean age was 57.40±12.66 (median= 56, range, 21-94) years. Total mastectomy was performed in 56.4% (n=92) of the patients, 80.4% (n=131) of these patients had malignant CNB results. When SSs

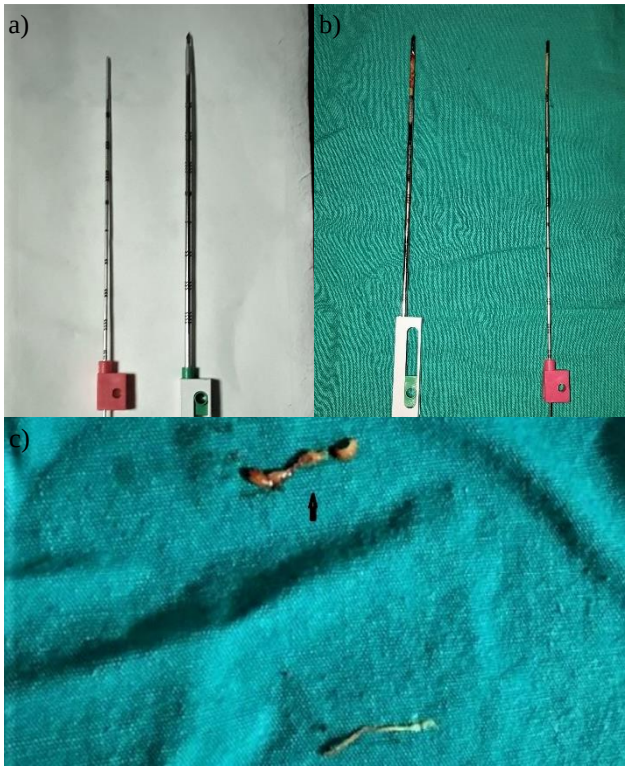

Figure 1. a) 14- and 18-gauge core biopsy needles, **b)** 14- and 18-gauge core biopsy needles with tissue, **c)** the arrow shows tissue sample taken with a 14-gauge needle and the other one is the tissue sample taken with a 18-gauge needle

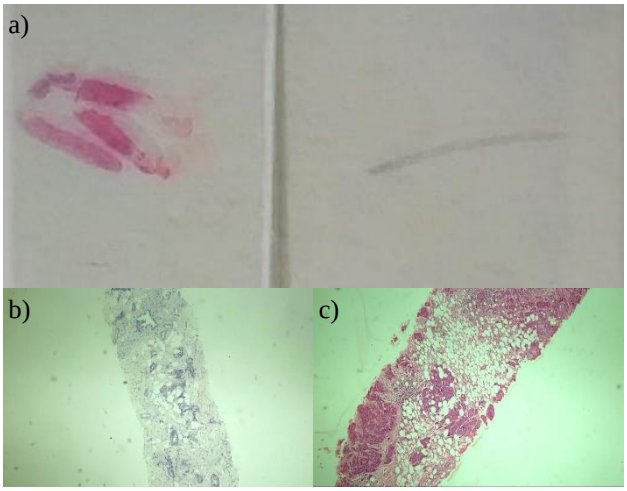

Figure 2. a) core biopsy breast specimens obtained using 14-gauge (left) and 18-gauge (right) needles, **b)** and **c)** photomicrographs show tumor cells infiltrating breast tissue with desmoplastic stroma (4x H&E)

Table 1. Descriptive and clinical characteristics of the patients

	Total (n=628)	**14-gauge (n=428)**	**18-gauge (n=200)**	**p**
Age (years), mean±SD	52.20±13.94	52.29±13.47	51.99±14.93	0.899
median (Q1-Q3) [min-max]	51 (42-61) [13-96]	51 (42-61) [21-96]	52 (42-61.5) [13-88]	
Age groups, n (%)				
<40	123 (19.6%)	81 (18.9%)	42 (21.0%)	
40-49	178 (28.3%)	123 (28.7%)	55 (27.5%)	
50-59	155 (24.7%)	109 (25.5%)	46 (23.0%)	0.876
60-69	108 (17.2%)	74 (17.3%)	34 (17.0%)	
≥70	64 (10.2%)	41 (9.6%)	23 (11.5)	
Gender, n (%)				
Female	615 (97.9%)	419 (97.9%)	196 (98.0%)	0.933
Male	13 (2.1%)	9 (2.1%)	4 (2.0%)	
Lesions type, n (%)				
Mass	568 (90.4%)	390 (91.1%)	178 (89.0%)	0.399
Non-mass	60 (9.6%)	38(8.9%)	22 (11.0%)	
Lesions size (mm), n (%)				
≤10	196 (31.2%)	125 (29.2%)	71 (35.5%)	
11-20	334 (53.2%)	222 (51.8%)	112 (56.0%)	**0.005**
21-50	73 (11.6%)	58 (13.6%)	15 (7.5%)	
>50	25 (4.0%)	23 (5.4%)	2 (1.0%)	
BI-RADS*, n (%)				
	n=568	**n=390**	**n=178**	
1	0 (0.0%)	0 (0.0%)	0 (0.0%)	
2	4 (0.7%)	4 (1.0%)	0 (0.0%)	0.610
3	40 (7.0%)	28 (7.2%)	12 (6.7%)	
4	256 (45.1%)	176 (45.1%)	80 (44.9%)	
5	268 (47.2%)	182 (46.7%)	86 (48.4%)	

SD: standard deviation; Q1-Q3: 1st - 3rd quartile, min-max: minimum-maximum, BI-RADS: breast imaging-reporting and data system, *: BI-RADS classification was not made for the pre-diagnosis of some lesions such as abscess and granulomatous mastitis

Table 2. Histopathological results of CNB materials

	Total (n=628)	14-gauge (n=428)	18-gauge (n=200)
Invasive breast carcinoma*	217 (34.6%)	153 (35.7%)	64 (32%)
Invasive lobular carcinoma	18 (2.9%)	12 (2.8%)	6 (3%)
Fibroadenoma	96 (15.3%)	66 (15.4%)	30 (15%)
Adenosis and fibrosis	70 (11.1%)	47 (11.0%)	23 (11.5%)
Abscess	30 (4.8%)	18 (4.2%)	12 (6%)
Granulomatous mastitis	30 (4.8%)	20 (4.7%)	10 (5%)
Ductal hyperplasia	19 (3%)	12 (2.8%)	7 (3.5%)
Fibrocystic disease	56 (8.9%)	37 (8.6%)	19 (9.5%)
Reactive lymph node	15 (2.4%)	9 (2.1%)	6 (3%)
Fat necrosis	11 (1.8%)	7 (1.6%)	4 (2%)
Gynecomastia	9 (1.4%)	5 (1.2%)	4 (2%)
Normal breast tissue	13 (2.1%)	9 (2.1%)	4 (2%)
Others	44 (7%)	33 (7.7%)	11 (5.5%)

CNB: core needle breast biopsy, *NOS: not otherwise specified

were examined, 86.5% (n=141) of the patients were consistent with the CNB result (Table 3). When the data of 14-gauge and 18-gauge needles are examined; there was no significant difference between the two groups in terms of gender distribution, age, type of surgery, and CNB biopsy results. Considering the results of SS and CNB biopsy, no significant difference was found between the two needles (Table 3).

During this time, no patient applied the emergency department owing to the CNB complication such as hematoma, bleeding, etc.

DISCUSSION

In this study, our eight years of CNB experiences were presented. CNB has become a standard alternative to excisional biopsy (2). CNB enables the evaluation of suspicious lesions with a reliable, fast, inexpensive, and less traumatic method (2). The BI-RADS is a scale that has been used for many years to standardize the reporting

Table 3. Descriptive and clinical characteristics of the patients operated

	Total (n=163)	14-gauge (n=94)	18-gauge (n=69)	p
Age (years), mean±SD	57.40±12.66	56.68±11.61	57.91±13.41	0.541
median (Q1-Q3) [min-max]	56 (47-68) [21-94]	56 (48-70) [21-94]	55.5 (46-64) [37-81]	
Gender, n (%)				
Female	161 (98.8%)	92 (97.9%)	69 (100.0%)	0.509
Male	2 (1.2%)	2 (2.1%)	0 (0.0%)	
Type of procedure, n (%)				
Total mastectomy	92 (56.4%)	58 (61.7%)	34 (49.3%)	0.114
Partial mastectomy	71 (43.6%)	36 (38.3%)	35 (50.7%)	
Histopathological results of CNB materials, n (%)				
Benign	28 (17.2%)	16 (17.0%)	12 (17.4%)	0.999
Malignant	131 (80.4%)	76 (80.9%)	55 (79.7%)	
Others	4 (2.4%)	2 (2.1%)	2 (2.9%)	
Conformity between the CNB and SS, n (%)				
Yes	141 (86.5%)	81 (86.2%)	60 (87.0%)	0.885
No	22 (13.5%)	13 (13.8%)	9 (13.0%)	

SD: standard deviation; Q1-Q3: 1st - 3rd quartile, min-max: minimum-maximum, CNB: core needle breast biopsy, SS: surgical specimen

of breast lesions, strengthen communication between physicians and reduce confusion about findings (16). BI-RADS 1-2 indicates that the lesion has no malignant potential, BI-RADS 3 score indicates a low (2%) risk of malignancy, but false-negative cases with CNB applied and detected malignancy are also shown (16,17). In a study including 9,068 cases, Jung et al. (2) reported 68.6% of the cases were BI-RADS 4 and the lesion size was less than 10 mm. In Wu et al.'s (7) study, most of the lesions were <20 mm and most of the cases were BI-RADS 4 (54.6%). In another study, 59.5% of the cases were BI-RADS 4 and most of the lesions were 21-50 mm in size (15). In our study, the majority (47.2%) of the patients were BI-RADS 5 and most (53.2%) of the lesions were 11-20 mm in size. Some patients had lesions BI-RADS 2 and underwent CNB for various reasons (such as transportation and follow-up difficulties, family history, suspicion, and insistence of the patient or surgeon). In field studies conducted in our country, it has been found that awareness of breast cancer and the number of women who undergo periodic breast control is low (18,19). We think that majority of our cases were identified as BI-RADS 5 due to this reason.

It is necessary to reduce the risk of complications and increase the accuracy of breast biopsies. It is well-known by most practitioners that large-gauge needles are related to higher complications (such as pain, hemorrhage, bruising, hematoma, infections), as a post-procedural complication (20,21). Pain is one of the most common complications and is related mostly to the depth of the lesion and the duration of the procedure (22). Patients do not need follow-up unless complications such as vasovagal syncope or persistent bleeding are seen (20). Large hematomas can be seen every 1/1000, but rarely require surgical drainage (21). Although it has been reported that the risk of complications is higher in thick needles, it is also argued that thick needles and thin needles have similar complication rates (23). Nguyen et al. (24) reported that 0.5% of the patients presented to the emergency service within 30 days after the non-vascular biopsy, the most common reason for application is bleeding. In this study, no serious complications and emergency applications have been identified among cases where CNB is applied.

Several studies at the literature examine the efficiency of needle size on biopsy success. Appropriate imaging of the

breast lesion, appropriate sample taking and histopathological diagnostic accuracy of the CNB material with SS is expected from a successful CNB procedure (15,25). The caliber of the needle, the size of the lesion, whether the lesion is mass or non-mass, and micro calcifications in the breast tissue are factors affecting the success of CNB (13,14,26-29). Many studies reported that 14-gauge long-throw biopsy needles provide the highest quality/quantity of biopsy samples than smaller needles (28,29). Giuliani et al. (25) has shown that thin needles have a similar performance with the thick (14-gauge) needles except for lesions under 10 mm and non-mass lesions in the breast. However, it has been shown that thin needles do not give the same results either in non-mass or mass lesions, but are more unsuccessful in non-mass lesions (15). Although CNB has high sensitivity and low false negativity rates, sometimes it may cause misleading results in borderline cases and biopsies with ductal carcinoma in situ (30). In such lesions, the false estimation rate can be up to 10-40% (30,31). Linda et al. (17) reported histopathological compliance in 87% of the CNB applied cases. It has been shown that CNB results obtained with 14-gauge, 16-gauge, and 18-gauge needles have similar diagnostic accuracy to the SS (25). It has also been found that the diagnostic accuracy is low, especially in non-mass lesions (15). The general characteristics of the cases performed biopsy with 14-gauge and 18-gauge needles are similar, and besides, the histopathological compatibility of the SS with the CNB result in cases undergoing surgery is also similar in this study. But it has been determined that the number of unsuitable samples is higher at biopsies taken with 14-gauge needles. This may be due to the use of a 14-gauge needle in 38 of 60 non-mass lesions. However, this result supports that it would be more appropriate to use thin needles.

CONCLUSION

Previous studies showed that US-CNBs had a high sensitivity, specificity, and accuracy using 14-gauge needles. However, we demonstrated that the 14-gauge and 18-gauge needles have similar performance. Smaller needles can be used for ultrasound-guided breast biopsies which is less invasive, less painful, and creates less risk of hemorrhage. However, no patient admitted to the emergency department because of the CNB acute complications such as hematoma, bleeding, etc., during this time.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (01.06.2020, 2020/73).

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Utilizing Formative Assessment to Encourage Study Strategy Modifications by Osteopathic Medical Students: An Observational Study

Osteopatik Tıp Öğrencilerinin Çalışma Stratejisi Değişikliklerini Teşvik Etmek için Biçimlendirici Değerlendirmeden Yararlanmak: Gözlemsel Bir Çalışma

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ABSTRACT

Aim: The standardized mode of delivering curriculum across the globe is didactic lectures. Formative and summative assessment methods are routine practices used to assess the students understanding and mastery in content and concepts which is delivered. The formative assessment methods are extremely popular in medical educational training but the data supporting such claims is largely lacking. The aim of this observational study was to determine if frequently provided formative assessment methods like practice quizzes, take home assignments can have any positive impact on student learning strategies and assessment outcomes in examination.
Material and Methods: A total of 145 students were included in this study. This study explored the relationship between student performance in various low stakes formative quizzes and high stakes summative examination. Based on the student performances across various formative assessment methods, the students were encouraged to modify or retain the study strategies and the effect of such recommendations were observed over the course of the semester.
Results: The data analysis showed positive relationship between the student performance on formative assessments and summative assessment before and after the intervention to study methods and strategies. Students in top, second, third and bottom quartile gained a cumulative average of 72.4%, 61.0%, 56.6% and 48.3% in the formative assessment and an average of 89.3%, 79.8%, 75.0%, and 65.7% on their summative examination which were used as tools for early intervention.
Conclusion: Following the early intervention and modifications in study strategies, there was a steady increase in student performance on high stakes examination.
Keywords: Formative assessment; audio visual resources; early intervention; osteopathic medical students; feedback loop; knowledge gap.

ÖZ

Amaç: Tüm dünyada müfredatın standart olarak veriliş şekli didaktik derslerdir. Biçimlendirici ve belirleyici değerlendirme yöntemleri, öğrencilerin verilen içerik ve kavramları anlama ve kullanma becerilerini değerlendirmek için kullanılan rutin uygulamalardır. Biçimlendirici değerlendirme yöntemleri tıp eğitim öğretiminde son derece popülerdir, ancak bu iddiaları destekleyen veriler açısından büyük ölçüde eksiklik söz konusudur. Bu gözlemsel çalışmanın amacı, uygulama sınavları, ev ödevleri gibi sıklıkla kullanılan biçimlendirici değerlendirme yöntemlerinin, öğrencilerin öğrenme stratejileri ve sınavdaki değerlendirme sonuçları üzerinde olumlu bir etkisi olup olmadığını belirlemektir.
Gereç ve Yöntemler: Bu çalışmaya toplam 145 öğrenci dahil edildi. Bu çalışmada, çeşitli düşük riskli biçimlendirici sınavlar ve yüksek riskli belirleyici sınavlardaki öğrenci performansı arasındaki ilişki araştırıldı. Çeşitli biçimlendirici değerlendirme yöntemlerinde öğrenci performanslarına dayalı olarak, öğrenciler çalışma stratejilerini değiştirmeye veya korumaya teşvik edildi ve bu önerilerin etkisi dönem boyunca gözlemlendi.
Bulgular: Veri analizi sonucunda, çalışma yöntem ve stratejilerine müdahaleden önce ve sonra öğrencilerin biçimlendirici değerlendirme ve belirleyici değerlendirme performansı arasında pozitif bir ilişki olduğu gösterilmiştir. Üst, ikinci, üçüncü ve alt çeyrekte yer alan öğrenciler, erken müdahale için bir araç olarak kullanılan biçimlendirici değerlendirmede kümülatif ortalama %72,4; %61,0; %56,6 ve %48,3 alırken belirleyici sınavlarında ise ortalama %89,3; %79,8; %75,0 ve %65,7 puan almışlardır.
Sonuç: Çalışma stratejilerindeki erken müdahale ve değişiklikler sonrasında, yüksek riskli sınavlarda öğrenci performansında sürekli bir artış olmuştur.
Anahtar kelimeler: Biçimlendirici değerlendirme; görsel-işitsel kaynaklar; erken müdahale; osteopatik tıp öğrencileri; geribildirim döngüsü; bilgi boşluğu.

INTRODUCTION

Medical education has become an assessment-driven system all over the globe. The pressure on the assessment methods and its success has been the focus as sound assessment of medical education serves the public interest. It is not a sudden change to assessment-based intrinsic motivation to study student learning but it has been a gradual transition, and it has come to the forefront as educational institutions strive to improve the effectiveness of student learning specially when the learning group is large with limited faculty (1-3). Stimulating student intrinsic motivation to study, could be an effective way to do so (3,4).

Medical students typically have different educational backgrounds and are the product of diverse systems, and have different aspirations, standards, learning methods, adaptive skills and mechanisms to cope with stress and rigors of the program (5). These influences, along with student motivation during their time at medical school, have an important role in their learning and drive to perform on high stake's assessment. In the academic world it is generally accepted that early intervention is important to improve the student success and helps the school and program retain students who are at risk of dropping from courses or programs altogether (6). The importance of early intervention by different forms of assessment has been obvious for a longtime, but has been under appreciated as a beneficial strategy. It has been stated by scholars "the quickest way to change student learning is to change the assessment system" (7). It is also known that the assessment is the single most powerful influence on learning. This holds true for any professional courses, including the medical field.

In academics the assessment can be broadly classified into four types: formative, summative, diagnostic, and benchmark/interim assessment (8). The basic sciences courses use formative and summative assessments to monitor the student's growth towards goals. They evaluate the quality of their work by using formative assessment and compare the student performance against a set of uniform standards by using summative assessment. Formative learning assessment is used to give feedback on their performance and to plan and identify strategies to improve. Typically, the formative learning assessment is carried out concurrently with instructions. Its main purpose is to modify teaching and learning to improve student's learning outcome. Formative assessment is conducted throughout the course or learning module. It is not used for decision making on students' academic progress. On the other hand, summative assessment is used to evaluate the effectiveness of educational environment and to sum up learning. Following a formative learning assessment method, a formative feedback is provided to a learner in a non-threatening and friendly environment which can be used by the student to make changes or modify the learning methods or resources throughout the learning process (9). The educators who have high standards of expectation of their learner group, intentionally invest a large amount of time and efforts in providing a formative feedback to their learner group which is effectively used by students in first or second quartile to optimize their learning (10). In general, if taken together, formative learning tools can be utilized by the faculty and learner to facilitate informed student action to improve student outcome.

Summative learning assessment is used to make decisions about the academic performance and progress of student in the professional program including pass/fail decisions or eligibility for licensure examinations. It determines whether the goals of education are being fulfilled. It is typically formal in nature and conducted at the end of the course or learning module or annually at the same time each school year.

Various researchers like Spolsky et al. (11) have suggested how the formative assessment can be used to provide essential feedback for teachers to assess the subsequent learning activities and experiences in their classroom (5). These activities can also aid to identify and remediate the deficiencies and difficulties in student learning and the knowledge gap. Cauley et al. (12) believed frequent formative assessment involving important concepts and information allows the students to have a better understanding and retention of learning material.

The primary focus of this study was about the effectiveness of formative assessment by the professors in classrooms on the summative high stake's assessment of students in basic science course at an osteopathic medical school (13). The various tools used in formative assessment are low stakes quizzes, team based learning or integrated case-based learning, clinical vignettes during the lecture with quick time or real time audience response system or student self-assessments by take home quizzes (14,15).

MATERIAL AND METHODS

This study was performed at a University which campuses osteopathic medical school, optometry college, nursing, and undergraduate college in Commonwealth of Kentucky. The university has adopted a discipline based, contextualized and competency driven curriculum which is delivered to osteopathic medical students over a period of 4 years. The medical knowledge to students in basic sciences subjects and clinical sciences subjects are mostly delivered by didactic lecture, but they are interspersed with active learning sessions like case discussions, modified TBL or flipped classroom. The biochemistry and genetics course is delivered in the first semester of the medical school. Every year between July and December, around 145 first year students complete their first semester at the university while gaining medical knowledge in various courses like biochemistry and genetics, gross anatomy and embryology, cell biology, osteopathic patient care, current issues in medicine and osteopathic manipulative medicine. The curriculum is structured as a traditional discipline based by using didactic lectures which are delivered over four blocks for dissemination of medical knowledge in all the courses. Some subjects also utilize active learning methods such as flipped classroom, team-based learning, and case discussions. Various forms of formative and summative assessments are practiced throughout the duration of each block, the types of formative assessment used for assessing medical knowledge are, quizzes using multiple choice questions, clinical vignettes using audience response system, case discussions, take-home quizzes (MCQ), and written assignments. Every student in the cohort had to go through same mechanism of

assessment. For this study, there was a very important formative quiz a week before the high stake's summative examination. The rationale behind having a formative quiz a week before the summative block exam was to determine students understanding of concepts and assess student's medical knowledge for the content delivered over the block and their readiness for the high stakes block examination (5,16,17) and for faculty or course director to intervene and work with students who may be at risk of gaining unsatisfactory grades. This was intended to help identify and triage students at risk and council them about modifications and methods to improve student understanding and learning of the content delivered to improve performance on high stakes summative assessment.

Each block is delivered over a period of 4-5 weeks and there is a summative examination at the end of each block which tests all the concepts learned in various subjects. The grades achieved by students in each block is utilized to assess student's level of medical knowledge for the block and the entire course overall. Each summative examination is a high stakes assessment delivered using multiple choice questions and is an important milestone in students' progress through each semester and medical school.

This study tried to explore a relationship between student performance in various low stakes quizzes and high stakes examination. The students were grouped into four groups based on their performance in various formative and summative assessment and the final grade average in the fall semester. The students were classified into top quartile who had an average between 91-100%, while 81-90% as 2nd quartile, 76-80% as 3rd quartile, and 70-75% as the bottom quartile.

The study was approved by the Institutional Review Board of University of Pikeville (06.03.2019, 19/0005).

Statistical Analysis

The statistical software SPSS v.27 was used to analyze the data from the study. The data is represented as mean percentage for the learner group, which was divided into four quartiles including the mean percentage for the assessment method and individual quartile group. The grade point average between the formative assessment and summative assessment were analyzed by using Pearson correlation.

RESULTS

This study was performed to test whether using a formative mode of assessment methods during the course of content and knowledge delivery would help faculty to identify the learners who may be at risk based on their study methods, understanding of concepts and medical knowledge or any such factors which may have an impact on their performance in high stakes examination. This study included all the 145 students enrolled in 1st year, which comprised of 51.7% (n=75) female and 48.3% (n=70) male students, who had finished the fall semester and were part of the cohort of the students who were included in this study and their data and grades analyzed. They all also had an average learning experience of minimum of 5 months and some had around 10-14 months. The general characteristics of the study group were matched by age and learning experience.

All the students have had similar academic experience during the semester with various formative and summative assessment methods. Every student has had four blocks over the course of the semester and there was at least one formative assessment which was mandatory for them to have attempted. The learner group was divided into four group and their grades in formative assessments and summative assessments were correlated to gain statistical information regarding the effect of intervention by the course faculty. As it is evident in the table below, students were first identified as at risk based on their cumulative performance on various formative assessment which was provided to learner group through the course of entire block using quizzes, ARS, clinical vignettes, before the summative examination. Based on analysis, the students grouped in the bottom quartile had strong correlation between their formative assessment results and summative assessment grades. The students in the bottom quartile gained an average of 48.3% on the formative assessment and 65.7% on the summative assessment. Similarly, students in top, second and third quartile gained an average of 72.4%, 61.0% and 56.6% in the formative assessment and gained an average of 89.3%, 79.8% and 75.0% on their summative high stake's examination. Following this, the students were identified who may be at risk and were advised about modification to study methods and resources being used or new strategy for conceptual understanding and knowledge retention. Following intervention and student advisement there was sustained and gradual improvement in students' performance over the following next formative assessments and high stakes summative assessments in biochemistry and genetics course.

The average student grades in formative assessments for students in bottom quartile were 67.1%, 71.7% and 78.0% and the respective grades in high stakes summative assessments were 74.9%, 77.9% and 79.9%. This proves that identifying students who may be at risk to gain unsatisfactory results may be assisted by early intervention. Comparatively the students in third quartile scored 74.7%, 80.0% and 82.9% on formative assessments and 80.7%, 82.5% and 85.0% on their summative assessment (Table 1).

The relative correlation coefficient for each subsection were also determined using Pearson correlation coefficient, between each formative block quizzes and summative high stakes examination were between moderate to strong correlation. The various r and p values were; r=0.354; p=0.073 for block I, r=0.549; p=0.048 for block II, r=0.742; p=0.038 for block III, and r=0.510; p=0.023 for block IV. The students in top and second quartile had similar improvements in their performance after early intervention by faculty or self, which was followed by modifications and better understanding of medical concepts and improved retention of knowledge.

DISCUSSION

Any university which has an excellent and successful medical curriculum should depend on a satisfactory and encouraging learning environment which includes teaching methodology and feedback system and a valid and acceptable assessment method. The various methods of formative assessment in a curriculum can be used as an

Table 1. Mean percentages for student groups

	Top Quartile	Second Quartile	Third Quartile	Bottom Quartile	Course Mean	r and p values
Block 1 Formative	72.4	61.0	56.6	48.3	60.2	r=0.354
Block 1 Summative	89.3	79.8	75.0	65.7	77.4	p=0.073
Block 2 Formative	82.9	78.0	74.7	67.1	75.7	r=0.549
Block 2 Summative	90.9	87.7	80.7	74.9	83.5	p=0.048
Block 3 Formative	90.0	84.3	80.0	71.7	81.4	r=0.742
Block 3 Summative	91.1	86.6	82.5	77.9	84.5	p=0.038
Block 4 Formative	88.2	85.4	82.9	78.0	83.6	r=0.510
Block 4 Summative	94.0	88.3	85.0	79.9	86.8	p=0.023

This table represents the mean percentages for the different block examinations, average percentages in each quartile group and the correlation between formative assessments and summative assessment percentages for each block for all the students enrolled in the course

effective tool to improve the student's learning outcome and satisfaction. It can also be used as an effective tool to identify at risk students and promote interventions and to aid students to improve their performance on various high stakes examinations.

According to many educators, providing specific feedback and early intervention is the single most important step an educator can do to help students or learner group. When we explain the importance of formative assessments in learning, feedback loop is often explained with it. It can also help most of the students to introspect about their study habits and methods and encourage them to modify them if they feel the result to be unsatisfactory. Based on the above discussion it was evident that students, following their formative assessment, can go back to the drawing board and start modifying the methods to learn the content, ways to improve retention of medical knowledge or identify innovative modes of learning. Over time the students learn to self-assess and both seek and use feedback to focus on improving all areas of their work.

Based on the data analysis for this study, as well it can be construed, this study too like many other studies, prove the importance of early intervention by using formative assessment tools (18-20). As explained before, the results for students who were at risk were offered feedback and enhanced assistance by various departments in terms of both tutoring, immediate feedback and resources which translated with gradual improvement in the student's academic performance. The results in this study were similar to those observed by Mitra et al. (18), which showed positive and significant improvement in summative scores on high stake examination following early and frequent formative testing. This was proved statistically by significant and positive correlation between the grades in formative and summative assessment.

It should be remembered that apart from measurable improvement in student performance, the formative assessment can also provide significant information about the existing learning gap which may not be measured by low or high stakes examination but may be essential for sustained improvement of student's knowledge. It is very important to close the gap between what students currently know and what they are expected to know by the end of the course or curriculum. This observation is similar to that published by Rushton (19), where they used formative assessment for providing constructive feedback and help student in deep learning of the concept and knowledge required. Thus, as noted by various authors, the use of formative assessment in assessing the student's learning or knowledge gap could be extremely impactful in making early intervention and providing feedback and modifying the learning environment to improve student performance(12,21,22).

CONCLUSION

Our data suggests that using formative assessment tools frequently, and providing directed feedback using results of the formative assessment as an early intervention strategy resulted in significant student performance improvements on summative examinations. In general, based on informal feedback, the students prefer increased use of formative assessment in terms of take home quizzes, pop quizzes, case discussions during lectures or team based/problem based sessions, assuming that the discussion with the learner group was relatively immediate. However, although almost all students were in favor of using this method for bridging the learning gap, a subset of students expressed reservations about the spontaneity of timing for such activities as it may distract from focus on other courses, or set the stage for self-doubt as the formative assessment is not a reflection of their knowledge gained for subsequent study. Though it should be stressed that formative assessments remain an important tool for the students to minimize their knowledge gap and encourages the development of critical thinking skills.

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Comparison of the Effects of Dialysis Type on Right Heart Functions in Chronic Renal Failure Patients

Kronik Böbrek Yetmezliği Hastalarında Diyaliz Tipinin Sağ Kalp Fonksiyonları Üzerindeki Etkilerinin Karşılaştırılması

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ABSTRACT

Aim: The aim of this study was to compare the effects of dialysis type on right heart functions with two-dimensional speckle tracking echocardiography (2D-STE).
Material and Methods: A total of 53 patients who have peritoneal dialysis and hemodialysis, applied to cardiology and nephrology outpatient clinics, were included in the study. Those with left heart failure, coronary artery disease, primary and secondary pulmonary hypertension, pulmonary embolism, deep vein thrombosis, moderate and severe heart valve failure and stenosis were excluded from the study. Sociodemographic characteristics and biochemical parameters of the patients were recorded. Right heart functions of the patients were evaluated using 2D-STE.
Results: There was no difference between groups with respect to age (p=0.496), the rate of male was higher in hemodialysis group (p=0.006). In comparison of 2D-STE measurements; right atrium (RA) maximum volume and RA minimum volume were significantly higher in hemodialysis patients (p=0.006, p=0.007, respectively). There were no difference between the groups in RA maximum volume index, RA minimum volume index and tricuspid annular plane systolic excursion (TAPSE). RAS strain and RAA strain were significantly lower in hemodialysis patients (p=0.001, p=0.012, respectively). A positive correlation was found between TAPSE and RA maximum volume in hemodialysis patients (r=0.484, p=0.036), and between TAPSE and RA minimum volume in peritoneal dialysis patients (r=0.486, p=0.025).
Conclusion: Right ventricular functions were found to be similar in peritoneal dialysis and hemodialysis patients. It was observed that right atrial functions were better protected in peritoneal dialysis patients than hemodialysis patients.
Keywords: Dialysis; right heart functions; speckle tracking echocardiography.

ÖZ

Amaç: Bu çalışmanın amacı diyaliz tipinin sağ kalp fonksiyonlarına etkilerinin iki boyutlu speckle tracking ekokardiyografi (2D-STE) ile karşılaştırılmasıdır.
Gereç ve Yöntemler: Kardiyoloji ve nefroloji polikliniklerine başvuran, periton diyalizi yapan ve hemodiyalize giren toplam 53 hasta çalışmaya dahil edildi. Sol kalp yetmezliği, koroner arter hastalığı, primer ve sekonder pulmoner hipertansiyon, pulmoner emboli, derin ven trombozu, orta ve ileri derecede kalp kapak yetmezliği ve darlığı olanlar çalışma dışı bırakıldı. Hastaların sosyodemografik özellikleri ve biyokimyasal parametreleri kaydedildi. 2D-STE ile hastaların sağ kalp fonksiyonları değerlendirildi.
Bulgular: Yaş açısından gruplar arasında fark yoktu (p=0,496), hemodiyaliz grubunda erkek oranı daha yüksekti (p=0,006). 2D-STE ölçümleri karşılaştırıldığında; sağ atriyum (right atrium, RA) maksimum hacmi ve RA minimum hacmi hemodiyaliz hastalarında anlamlı olarak yüksekti (sırasıyla p=0,006; p=0,007). RA maksimum hacim indeksi, RA minimum hacim indeksi ve triküspit anulusunun sistolde apikale yer değiştirmesi (tricuspid annular plane systolic excursion, TAPSE) açısından gruplar arasında fark yoktu. Hemodiyaliz hastalarında RAS strain ve RAA strain anlamlı olarak daha düşüktü (sırasıyla p=0,001; p=0,012). Hemodiyaliz hastalarında TAPSE ile RA maksimum hacmi arasında (r=0,484; p=0,036) ve periton diyalizi hastalarında TAPSE ile RA minimum hacmi arasında (r=0,486; p=0,025) pozitif korelasyon bulundu.
Sonuç: Sağ ventrikül fonksiyonları periton diyalizi ve hemodiyaliz hastalarında benzer bulundu. Sağ atriyal fonksiyonların periton diyalizi hastalarında hemodiyaliz hastalarına göre daha iyi korunduğu görüldü.
Anahtar kelimeler: Diyaliz; sağ kalp fonksiyonları; speckle tracking ekokardiyografi.

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INTRODUCTION

Renal replacement therapies such as hemodialysis (HD), peritoneal dialysis (PD) and kidney transplantation are required at the last stage of chronic kidney disease (end stage renal disease, ESRD) characterized by progressive and irreversible nephron loss. Cardiovascular diseases are important causes of mortality and morbidity in ESRD (1). In a study, it was found that cardiovascular mortality increased approximately ten times in HD patients compared to those who have not dialysis. Besides, cardiovascular diseases are responsible for approximately half of the deaths of ESRD patients (2,3). 15-28% of the patients who received dialysis indication have a diagnosis of heart failure (4,5). Therefore, early diagnosis of heart failure and taking the necessary precautions to prevent the progression of the disease are important with respect to mortality and morbidity in patients undergoing dialysis.

It is known that conventional echocardiographic methods may be insufficient to show subclinical cardiac dysfunction. Two-dimensional speckle tracking echocardiography (2D-STE), which is different from conventional methods, is a semi-automatic method that can measure cardiac functions (6). It has been shown in previous studies that this method can detect subclinical myocardial dysfunction earlier compared to conventional methods (7).

We aimed to evaluate the effect of dialysis type on right ventricular (RV) functions by 2D-STE in this study.

MATERIAL AND METHODS

Peritoneal dialysis and HD patients who applied to cardiology and nephrology outpatient clinics between January 2017 and August 2017 were screened retrospectively. Twenty-seven HD and twenty-six PD patients were included. These patients were those who had been on dialysis for at least one year. Patients with pulmonary hypertension, pulmonary embolism, deep vein thrombosis, coronary artery disease, moderate and severe heart valve failure and stenosis were excluded. In this study, patients with left ventricular systolic dysfunction (ejection fraction, EF<50%) were excluded then. The age, gender, height, weight (weights measured after dialysis treatment), smoking status and duration of dialysis of the patients were recorded. Whether they had diabetes mellitus, hypertension, hyperlipidemia diagnoses was recorded (all patients had diabetes mellitus). Body mass index (BMI) was calculated using the weight (kg) / height $(m)^2$ formula. Body surface areas were calculated using the Mosteller formula (height (cm) x weight (kg) / 3600)^{½}. Average of the arterial blood pressure measurements taken on different days were calculated. Patients with blood pressure higher than 140/90 mmHg were considered to be hypertensive. Diabetes mellitus was defined as a fasting blood glucose above 126 mg/dl and HbA1C=6.5.

While patients in the HD group received dialysis treatment at standard doses with a Kt/V ratio of 1.2 and above in 4-hour sessions 3 times a week, 33% hypertonic glucose dialysate and 95% icodextrin exchanges of all patients in the PD group were performed by continuous ambulatory peritoneal dialysis (CAPD).

The study was approved by the Ethics Committee of Ordu University Faculty of Medicine (18.05.2017, 2017/61).

Blood samples were taken into gel tubes (without anticoagulant) to measure biochemical parameters. After the serum were obtained from the blood samples, they were studied in the Abbott Architect 8000 automatic device. Blood samples were taken into EDTA tubes for hemogram measurement and studied in Abbott Cell-Dyn 3700 device. The glomerular filtration rate (GFR) was calculated with the Modification of Diet in Renal Disease (MDRD) formula as previously defined (8); GFR (mL/min/1.73 m^2) = 186 x serum creatinine$^{-1.154}$ x age$^{-0.203}$ x 0.742 (if female) x 1.212 (if black race).

Speckle Tracking Echocardiography

With a stable ECG recording of the patient holding his breath, RV free wall and right atrial (RA) functions were evaluated by conventional two-dimensional harmonic gray scale echocardiography in apical 4- and 2-chamber images (Philips IE 33 × Matrix, USA). Three cardiac cycles were recorded and averaged, with the frame rate adjusted between 60 and 80 frames per second. The recorded images were saved for later evaluation. Manual monitoring of RV and RA endocardial borders was performed for each view in the final systolic frame. Echocardiographic measurements of the PD patients were made in the morning when the peritoneum was empty before changing the peritoneal fluid. Echocardiographic measurements of HD patients were made in the middle of the week before HD. All echocardiographic measurements were performed according to the guidelines previously published by the American Echocardiographic Association (ASE).

Echocardiographic Measurements

All echocardiographic measurements were made with an echocardiography device (PhilipsIE 33Matrix, USA) equipped with 2.25 to 7.5 MHz imaging transducers. Measurements were made by a cardiologist who have blinded to the study design. Echocardiographic measurements were made the left decubitus position. Simpson's biplane method was used to calculate left ventricular ejection fraction (LVEF) from the apical 4-and 2-chamber views. Pulsed-wave Doppler tricuspid inflow velocities, including early (E) and atrial (A) waves were measured. Tissue Doppler imaging (TDI) measures of myocardial systolic, early diastolic and atrial velocities were assessed at the lateral tricuspid annulus wall. Tricuspid annular plane systolic excursion (TAPSE) was measured in a 4-chamber view by placing the 2D cursor at the tricuspid lateral annulus as previously defined the studies (9). Right atrium maximum volume index was calculated by dividing the RA maximum volume by surface area. RA minimum volume index was calculated by dividing the RA minimum volume by surface area.

Statistical Analysis

The data were examined in terms of normality with Kolmogorov-Smirnov test and variance homogeneity with Levene test. Variables fulfilling the assumptions were compared with Student's t test, while those not fulfilling the assumptions were compared with Welch's t test or Mann-Whitney U test. Categorical data were analyzed using the Pearson chi-square or Fisher's exact test, as appropriate. The numerical variables were summarized as mean±standard deviation and median, interquartile range, minimum-maximum, while the categorical variables were expressed as number and percentage. Pearson correlation coefficient was used for normally distributed variables, while Spearman's rank correlation coefficient was used for

non-normally distributed variables. SPSS v.25 (IBM Inc., Chicago, IL, USA) statistical software was used for statistical analyses. Results were evaluated at 95% confidence interval and the significance level was p<0.05.

RESULTS

The mean age of PD patients was 45.40±7.21 years and 8 (30.8%) of the patients were male. The mean age of HD patients was 43.22±11.89 years and 18 (69.2%) of the patients were male. There was no difference between groups with respect to age (p=0.496), and the rate of male was higher in the HD group (p=0.006). No difference was found between the groups in terms of hypertension, hyperlipidemia and smoking (Table 1). The duration of dialysis for PD patients was 3 years, the duration of dialysis of HD patients was 5 years, and there was a significant difference between the groups in terms of dialysis durations (p<0.001). BMI and heart rate were higher in PD patients (p=0.075, p=0.052, respectively). There was no difference between the groups in terms of surface areas (Table 1).

Ejection fraction of both groups was normal, but significantly higher in PD patients (61.15±5.16 vs. 54.26±10.44, p=0.004). In comparison of 2D-STE measurements between PD and HD patients; RA maximum volume and RA minimum volume were significantly higher in HD patients (p=0.006, p=0.007, respectively). There were no difference between the groups in RA maximum volume index, RA minimum volume index and TAPSE. Also, there was no difference between the groups in terms of RV strain. RAS strain and RAA strain were significantly smaller in HD patients (p=0.001, p=0.012, respectively). There was no statistically significant difference in terms of RV isovolumic contraction time (IVCT-RV), RV isovolumic relaxation time (IVRT-RV), and RV e-time (ET-RV) between the groups (Table 2). A positive correlation was found between TAPSE and RA maximum volume (r=0.484; p=0.036) in HD patients and between TAPSE and RA minimum volume (r=0.486; p=0.025) in PD patients (Table 3).

Table 1. Comparison of demographic, clinic and body characteristics, and biochemical parameters of groups

	n	Peritoneal Dialysis	n	Hemodialysis	p
Gender (male)	26	8 (30.8)	26	18 (69.2)	**0.006**[a]
Age (years)	25	45.40±7.21	18	43.22±11.89	0.496[b]
Body Mass Index (kg/m^2)	25	27.50±6.84	16	23.40±7.61	0.075[c]
Surface area (m^2)	25	1.79±0.23	16	1.75±0.36	0.672[c]
Hypertension	26	19 (76.0)	16	14 (87.5)	0.448[d]
Hyperlipidemia	25	8 (32.0)	16	2 (12.5)	0.265[d]
Smoking	25	6 (24.0)	14	4 (28.6)	0.999[d]
Dialysis year (years)	25	3 (1) [1-5]	18	5 (4) [2-12]	**<0.001**[e]
Hemoglobin (g/dl)	25	12.18±2.01	14	11.59±1.80	0.361[c]
GFR (mL/minute/1.73 m^2)	19	9.89±5.51	7	6.00±1.73	0.082[c]
Creatinine (mg/dl)	25	6.14±2.34	14	9.97±2.68	**<0.001**[c]
Heart rate (beats/min)	26	90.62±9.43	27	85.59±8.97	0.052[c]

GFR: glomerular filtration rate, [a]: Pearson's chi-square test, [b]: Welch's t test, [c]: Student's t test, [d]: Fisher's exact test, [e]: Mann-Whitney U test, descriptive statistics were given as n (%) for categorical variables, and mean±standard deviation or median (interquartile range) [minimum-maximum] values were used for numerical variables, as appropriate

Table 2. Comparison of two-dimensional speckle tracking echocardiography parameters of groups

	n	Peritoneal Dialysis	n	Hemodialysis	p
Ejection fraction (%)	26	61.15±5.16	27	54.26±10.44	**0.004**[a]
RA maximum volume (mL)	26	26.96±9.00	27	42.70±26.04	**0.006**[a]
RA minimum volume (mL)	26	15.85±4.06	27	27.19±19.63	**0.007**[a]
RA maximum volume index (mL/m^2)	19	14.67±4.90	8	25.81±20.10	0.163[a]
RA minimum volume index (mL/m^2)	19	8.82±2.71	8	16.38±15.95	0.223[a]
TAPSE (mm)	26	1.94±0.37	27	2.06±0.51	0.328[a]
RV strain (%)	26	-15.81±4.23	27	-14.86±5.64	0.494[b]
RAS strain (%)	26	30.34±8.24	27	23.65±5.98	**0.001**[b]
RAA strain (%)	26	21.93±7.75	27	17.17±5.16	**0.012**[a]
IVCT-RV (ms)	25	79.60±11.94	27	70.11±22.80	0.246[b]
IVRT-RV (ms)	24	84.75±20.22	27	90.78±24.48	0.171[b]
ET-RV (ms)	25	148.72±47.25	27	162.96±38.09	0.236[b]
Tricuspid E wave (m/s)	12	62 (19.5) [51-100]	9	68 (32) [56-100]	0.422[c]
Tricuspid A wave (m/s)	12	67 (34.5) [48-94]	9	64 (24) [42-90]	0.808[c]
Tricuspid E/A ratio	12	1.1 (0.5) [0.7-1.29]	9	1.2 (0.6) [0.8-1.47]	0.219[c]
Tricuspid deceleration time (m/s)	12	195.0 (39.3) [162-250]	9	190.0 (37.5) [166-216]	0.169[c]

RA: right atrium, TAPSE: tricuspid annular plane systolic excursion, RV: right ventricle, IVCT-RV: isovolumic contraction time-right ventricle, IVRT-RV: isovolumic relaxation time-right ventricle, ET-RV: ejection time-right ventricle, E: early, A: atrial, [a]: Welch's t test, [b]: Student's t test, [c]: Mann-Whitney U test, descriptive statistics were given as mean±standard deviation or median (interquartile range) [minimum-maximum], as appropriate

Table 3. Correlation coefficients between the variables

	TAPSE			
	PD		HD	
	r	p	r	p
RA maximum volume	0.292	0.199	0.484	**0.036**
RA minimum volume	0.486	**0.025**	0.345	0.148
RA maximum volume index	0.307	0.201	0.565	0.145
RA minimum volume index	0.386	0.103	0.626	0.097
RV strain	0.307	0.176	-0.052	0.832
RAS strain	-0.081	0.727	-0.072	0.770
RAA strain	-0.118	0.611	0.516	**0.024**

TAPSE: tricuspid annular plane systolic excursion, PD: peritoneal dialysis, HD: hemodialysis, RA: right atrium, RV: right ventricle

DISCUSSION

In this study, in which the effects of PD and HD on right heart functions were compared with 2D-STE, RV functions were similar. But, it was observed that RA functions were significantly better preserved in patients who had PD compared to HD.

Peritoneal dialysis and HD are well-known treatment methods for patients with ESRD. Despite advances in dialysis therapies, cardiovascular mortality in patients with ESRD is still very high. Especially, deterioration in cardiac functions is observed more frequently in dialysis patients than in the healthy population, and is the leading cause of death in this patient group (4). Besides traditional risk factors such as coronary artery disease, diabetes mellitus, and hypertension are more common in ESRD patients compared to the healthy population, these causes such as volume load, uremic cardiomyopathy, hypotension attacks, anemia, oxidative stress, arteriovenous (AV) fistula, left ventricular hypertrophy can cause deterioration in cardiac functions (9,10).

Over hydration is one of the major problems in the development of cardiovascular problems in patients who had HD and PD. (11-13). Even though the RV works against low pressure under normal conditions, it can adapt to changes in volume load. Right heart dysfunction may develop in patients whose volume load might increase too much, such as ESRD. Such situations may impair the quality of life by causing the already increased peripheral ponding to become more prominent in right heart failure. However, it has not been clearly elucidated which HD or PD provides better volume control even if recent studies PD is thought to provide better fluid control than HD (15). In studies in the literature, it was thought that while RV functions were relatively protected in PD, the adverse effects of RV functions in HD patients may be due to the presence of AV fistula that causes high output in this group (16,11).

Transthoracic echocardiography is the most common diagnostic method used in clinical practice for evaluation of RV functions. Some studies have evaluated the changes in left ventricular parameters caused by a decrease in preload however the results regarding the RV are not clear (17,18). Therefore, it is important which parameters and echocardiographic examinations to use when evaluating RV functions. Although tissue Doppler parameters can be used in the evaluation of RV, systolic and diastolic functions, it is controversial whether these parameters are independent of preload (19,20). However, some studies have found that sudden decreases in preload cause a decrease in tricuspid lateral annulus systolic velocity in patients with normal RV function (21,22). Functional evaluation of the RV is very difficult due to the complex geometric structure of the RV, which is in the form of a half-moon, and because the RV is load dependent, it can be exposed to pericardial effects and right-sided volume and pressure load. (23-25). Techniques such as magnetic resonance imaging and radionuclide ventriculography used for quantitative calculation of right ventricular ejection fraction (RVEF) are invasive, relatively expensive, time-consuming and affected by the complex geometry of the RV (11,26). These methods cannot be applied very much in clinical practice. There are different echocardiographic parameters to evaluate RV systolic functions. One of the most frequently used methods is TAPSE (15). Many studies have found a very strong correlation between TAPSE and RV systolic functions (27). TAPSE values greater than 20 mm indicate normal functions of the RV, while a value smaller than 20 mm indicates impaired functions in varying degrees. In our study, TAPSE value, which is an indicator of RV systolic functions, was similar in patients who had PD and HD. In a previous study, it has been shown that over hydration caused by AV fistula impairs significantly RV systolic functions in HD patients than PD patients (28). Similarly, in a study comparing PD patients with healthy people, it was observed that RV systolic functions were preserved (29). It was thought that one of the reasons why PD preserves RV functions better than HD may be that sudden decreases in preload are less common in this patient group. However, RV functions were similar in PD and HD patients in our study. Although severe and advanced ventricular dysfunction can be detected in ESRD patients using different echocardiographic parameters, it may be insufficient to detect early ventricular dysfunction (26). STE conventional echocardiography is widely used to detect normal appearing systolic dysfunction (30). Early diagnosis of subclinical myocardial dysfunction has been possible in ESRD patients with the help of new echocardiographic methods such as STE (31). In this study, when PD and HD patients were evaluated with 2D-STE, which showed more sensitive cardiac functions, ventricular functions were found to be similar.

In our study, RA strain values were found to be significantly better in patients who had PD compared to HD while RV strain values were determined similar in PD patients and HD patients. When the studies in the literature and our study results are evaluated together, PD seems to be superior in protecting RA functions. In previous studies, the RA not only acts as a reservoir and channel, but reduces the pressure in the central venous circulation, acts as a buffer while supporting RV filling, and helps prevent acute increases in RV diastolic pressure (32-34). Atrial function impairments are known to be an early indicator of ventricular systolic dysfunction, and atrial strain values have been shown to be associated with RV functions (35). Therefore, monitoring of atrial functions is important for early detection of RV systolic dysfunction in ESRD patients receiving dialysis treatment. In our study, it was

found that RA functions were better preserved in PD patients. RA global strain values were better in PD patients compared to HD patients. RA volume values were higher in HD patients than PD patients. Since long-term volume burden will affect the right heart functions in these patients, the follow-up of RA functions will be useful especially in the early prediction of subclinical systolic dysfunctions in this patient group.

Limitations

Our study includes small number of patients, and there was no long-term clinical outcome data such as cardiovascular event rates and survival evaluation. These seems to be the most important study limitation. Another limitation of this study is represented by its retrospective design. Additional limitations include the lack of using cardiac magnetic resonance imaging for RV functional assessment and the 2D-strain method for the determination of right heart function.

CONCLUSION

Most of the previous studies have evaluated the effect of dialysis type on left ventricular functions. A few studies have compared right heart functions in patients who had PD and HD. In this study, it was shown that RV functions were similar protected in patients who underwent PD and HD. But RA functions were better protected in patients who underwent PD than HD. More studies are needed to confirm the clinical implications of our findings.

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Effectiveness of Bilateral Greater and Lesser Occipital Nerve Blocks in the Prophylaxis of Episodic Migraine

Epizodik Migren Profilaksisinde Bilateral Büyük ve Küçük Oksipital Sinir Bloğunun Etkinliği

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ABSTRACT

Aim: In recent years, many studies have been conducted on the effectiveness of occipital nerve block (ONB) especially in the prophylaxis of chronic migraine. In this study, it was aimed to investigate the effectiveness of bilateral greater and lesser ONBs in the prophylaxis of episodic migraine without aura.

Material and Methods: This retrospective study included patients with episodic migraine without aura who underwent bilateral greater and lesser ONBs between January 2018 and December 2019. Information about headache frequency, attack duration, and pain severity (VAS score) of the patients in the last month before nerve block and 1 month after nerve block was retrieved from the database and recorded for statistical analysis.

Results: A total of 17 patients aged 26-57 years were included in this study. None of the patients developed any drug side effects or complications associated with ONB. With ONB, the median value of headache attack frequency dropped from 5 (range, 4-14) to 2 (range, 0-6) per month ($p=0.001$), the median value of pain duration dropped from 12 (range, 6-14) to 4 (range, 0-9) days ($p<0.001$), and the median value of VAS pain severity score dropped from 9 (range, 7-10) to 5 (range, 0-10) for the patients ($p=0.001$), compared with the pretreatment values.

Conclusion: Bilateral greater and lesser ONBs are effective in the prophylaxis of episodic migraine without aura. ONB can be considered as a treatment option in patients with episodic migraine without aura who do not respond to conventional treatments (oral drugs) or do not accept conventional therapy.

Keywords: Occipital nerve block; episodic migraine without aura; headache; prophylactic treatment.

ÖZ

Amaç: Son yıllarda oksipital sinir bloğunun (OSB) özellikle kronik migren profilaksisindeki etkinliği konusunda çok sayıda çalışma yapılmıştır. Bu çalışmada, epizodik aurasız migren profilaksisinde bilateral büyük ve küçük OSB'nin etkinliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu geriye dönük çalışmaya, Ocak 2018 ile Aralık 2019 tarihleri arasında bilateral büyük ve küçük OSB uygulanmış olan epizodik aurasız migren hastaları dahil edilmiştir. Hastaların sinir bloğu uygulanmadan önceki son ay ile sinir bloğu uygulandıktan sonraki bir aylık baş ağrısı sıklığı, atak süresi ve ağrı şiddeti (VAS skoru) hakkındaki bilgileri veri tabanından alındı ve istatistiksel analiz için kaydedildi.

Bulgular: Bu çalışmaya yaşı 26 ile 57 yıl arasında olan toplam 17 hasta dahil edilmiştir. Hastaların hiçbirinde OSB ile ilişkili herhangi bir ilaç yan etkisi ya da komplikasyon gelişmemişti. Tedavi öncesi değerler ile karşılaştırıldığında, hastaların OSB ile baş ağrısı atak sıklığının medyan değeri ayda 5 (aralık, 4-14)'ten 2 (aralık, 0-6)'ye ($p=0,001$), ağrı süresinin medyan değeri 12 (aralık, 6-14)'den 4 (aralık, 0-9) güne ($p<0,001$) ve son olarak VAS ağrı şiddeti skoru medyan değeri ise 9 (aralık, 7-10)'dan 5 (aralık, 0-10)'e düşmüştü ($p=0,001$).

Sonuç: Bilateral büyük ve küçük OSB epizodik aurasız migren profilaksisinde etkilidir. Konvansiyonel (oral ilaçlar) tedavilere yanıt vermeyen veya konvansiyonel tedaviyi kabul etmeyen epizodik aurasız migreni olan hastalarda OSB bir tedavi seçeneği olarak düşünülebilir.

Anahtar kelimeler: Oksipital sinir bloğu; epizodik aurasız migren; baş ağrısı; profilaktik tedavi.

Preliminary results of this study was presented as an oral presentation at the 5th International Hippocrates Congress on Medical and Health Sciences (December 18-19, 2020)

INTRODUCTION

Migraine is a common headache disorder with an annual prevalence of 16.4% in Turkey. It is more common in female (8.5% in male, 24.6% in female), especially between the ages of 35-40 (1). Chronic migraine is defined as a headache that has occurred for >3 months with a frequency of ≥15 days per month, with a migraine headache at least 8 days per month. Otherwise, if a headache occurs <15 days per month in a patient with migraine, it is defined as episodic migraine (2). Occipital nerve block (ONB) has been used as a peripheral nerve block method for a long time. Numerous studies have been conducted on its use in the treatment of migraine (3,4). Most of these studies have focused on chronic migraine and the greater occipital nerve (4-6). However, as far as we have investigated in the literature, no data have been found regarding the effectiveness of bilateral greater and lesser ONBs in the prophylaxis of episodic migraine without aura. This situation has led us to design this study.

In this study, it was aimed to investigate the effectiveness of bilateral greater and lesser ONBs performed with a mixture of methylprednisolone, prilocaine, and bupivacaine in the prophylactic treatment of episodic migraine without aura.

MATERIAL AND METHODS

This retrospective study included patients with migraine who underwent bilateral greater and lesser ONBs at the Neurology and Neurosurgery Clinic, Aksaray University Training and Research Hospital, between January 2018 and December 2019. Among these patients, those aged >18 years, who were diagnosed with episodic migraine without aura according to the International Classification of Headache Disorders (ICHD-3) beta version (7), had >4 headache attacks per month with an attack duration of at least 1 day, and whose pain score according to the Visual Analog Scale (VAS) was severe (VAS >6) were included in the study. Patients who had previously undergone ONB and those with bleeding diathesis, local active infection in the occipital region or systemic infection, uncontrolled diabetes mellitus, uncontrolled hypertension, liver disease, congestive heart failure, history of psychiatric disease, and renal failure, as well as alcohol or substance addiction, antiaggregant or anticoagulant use, history of cranial and occipital surgery, coronary artery disease, history of allergic reaction to the drugs to be administered, and those who were pregnant and breastfeeding were excluded from the study.

Before ONB was administered, all patients were using at least one of the migraine prophylactic drugs (flunarizine, topiramate, amitriptyline, and propranolol) regularly for at least 6 months. Bilateral greater and lesser ONBs were performed after all patients were given detailed information about the procedure to be performed along with its risks, and their consents were obtained. There is no established consensus about the method of ONB. When the literature is examined, it can be seen that it was done with many different methods (3). Our application method is as follows: A 10-ml mixture consisting of 1 ml methylprednisolone acetate (40 mg/ml), 4 ml 2% prilocaine hydrochloride (20 mg/ml), and 5 ml 0.5% bupivacaine hydrochloride (5 mg/ml) was prepared; bilateral greater and lesser occipital nerve areas were determined; and each area was injected with 2.5 ml of the mixture using a 25 gauge, 90-mm spinal needle in each session in patients who accepted ONB treatment and from whom consent was obtained. Precautions were taken in terms of possible allergic reactions and complications, and patients were kept under observation for 1 h after the procedure. ONB was applied in three sessions, once every 15 days (on days 0, 15, and 30) in the first month. When the expected effect was not observed in patients who were called to the neurology outpatient clinic for follow-up 1 month after the last session (at least 50% reduction in the frequency, duration, and severity of attacks was taken as a criterion for treatment success), an additional 4th session of ONB was performed using the same procedure. Patients were given detailed information and asked to keep a monthly headache diary, including headache frequency, pain severity, and duration. Patients who underwent ONB were followed up for 3 months.

We used the information from our database regarding the headache frequencies, attack duration, and pain severity of the patients in the last month before nerve block was performed and in the third month after nerve block was performed for statistical analysis.

The study was approved by the Aksaray University Ethics Committee (December 18, 2020, 13-23) and the study was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

The Shapiro-Wilk test was used to examine the distribution pattern of the data. The Wilcoxon test was used to evaluate the effectiveness of ONB since data not distributed normally. Descriptive statistics were presented with median, quartiles and minimum-maximum. Statistical analyses were performed using SPSS v.23.0 software for Windows. $p<0.05$ was considered statistically significant.

RESULTS

Of the 252 migraine patients followed in our outpatient clinic, a total of 17 patients, including 15 female and two male patients, who received ONB treatment, were included in the study. The median age of the patients was 37 (range, 26-57). The median disease duration of the patients was 7.5 years (range, 1-30).

In Table 1, the comparison of frequency, duration, and severity of the patients' headaches before and after bilateral greater and lesser ONBs is shown. The median headache frequency of the patients was 5 (range, 4-14) per month before treatment, whereas it was 2 (range, 0-6) after ONB, and this was statistically significant ($p=0.001$). While the median duration of pain before ONB was 12 (range, 6-14) days/month, it was found to be 4 (range, 0-9) days/month after ONB, and this was statistically significant ($p<0.001$). Finally, with ONB treatment, the median VAS pain severity score of the patients was decreased statistically significantly ($p=0.001$) from 9 (range, 7-10) to 5 (range, 0-10).

DISCUSSION

In the present study, we found that the VAS score and the severity and frequency of pain significantly decreased after ONB in patients with episodic migraine without aura. In previous studies, ONB was performed for the bilateral greater occipital nerve (4-6,8). In this study, bilateral nerve

Table 1. Comparison of the headache frequency, attack duration, and pain severity before and after ONB

	Before ONB	After ONB	p
Headache frequency (day/month)	5 (4-6.5) [4-14]	2 (1-3) [0-6]	**0.001**
Duration of headache (day)	12 (10-14) [6-14]	4 (1.25-5.5) [0-9]	**<0.001**
Headache severity (VAS score)	9 (8-10) [7-10]	5 (4-7) [0-10]	**0.001**

ONB: occipital nerve block, VAS: visual analog scale, descriptive statistics were presented as median (1^{st} - 3^{rd} quartiles) [minimum-maximum]

block was performed for both greater and lesser occipital nerves. In addition, although there is no consensus in the literature for ONB (9), we used a mixture of methylprednisolone, prilocaine, and bupivacaine.

There are many studies on the effectiveness of ONB in chronic migraine prophylaxis (4-6). On the other hand, there are a few studies investigating the effectiveness of ONB in the prophylaxis of episodic migraine without aura (10,11). In a randomized, double-blind, and placebo-controlled study by Dilli et al. (10), which included patients with both episodic and chronic migraine together, a single greater ONB did not provide a short-term protective effect on moderate and severe migraine days compared with placebo in patients with episodic or chronic migraine. However, Dilli et al. (10) did not differentiate between the patients as episodic and chronic, and they performed a greater ONB only once. In a single-blind, randomized, and placebo-controlled study of greater occipital and supraorbital nerve block by Özer et al. (11), they showed that greater ONB and supraorbital nerve block with lidocaine was more effective than placebo in the prophylactic treatment of both episodic and chronic migraine. In the present study, we found that bilateral greater and lesser ONBs that we applied in three sessions (with a mixture of methylprednisolone, prilocaine, and bupivacaine) at 15-day intervals were effective and safe in the prophylaxis of episodic migraine without aura.

In the literature, development of complications or drug side effects during ONB has been reported in a small number of patients (3). Most of these were mild or transient effects (3). No drug side effects or complications developed during or after nerve block procedure in the patients in the present study. Drug side effects or complications are more likely to occur in studies with much larger numbers of patients. The patient should nevertheless be informed in detail about the effects that may occur with ONB, and the clinician applying the treatment should be prepared in this respect.

This study is an important study for providing new information to the literature due to the fact that nerve block was performed for both bilateral greater and lesser occipital nerves and that it was conducted only in episodic migraine without aura. Nevertheless, this study has few limitations. First, the study was conducted on a small number of patients and was retrospective. Second, we did not follow up the patients for >3 months after ONB.

CONCLUSION

Based on the results of this study, it has been considered that bilateral greater and lesser ONBs are effective and safe in the prophylaxis of episodic migraine without aura. ONB can be considered as a treatment option in patients with episodic migraine without aura who do not respond to conventional treatments (oral drugs) or do not accept conventional therapy.

Ethics Committee Approval: The study was approved by the Ethics Committee of Aksaray University Faculty of Medicine (18.12.2020, 13-23).

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The Relationship Between Clinical Phenotypes and Chromosomal Microdeletions/Duplications in Pediatric Neurology

Pediatrik Nörolojide Klinik Fenotipler ve Kromozomal Mikrodelesyon/Duplikasyonlar Arasındaki İlişki

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ABSTRACT

Aim: The aim of this study was to determine the diagnostic utility of chromosomal microarray analysis (CMA) in daily pediatric neurology practice and to identify the guiding clinical parameters for patients requiring this test.
Material and Methods: The CMA results for 91 patients with global developmental delay/intellectual disability (GDD/ID) admitted to our pediatric neurology clinic for various reasons between 2018 and 2020 were examined. Demographical and clinical data for 34 patients (37.4%) in whom del/dup was determined at CMA and 57 patients (62.6%) with normal CMA were compared.
Results: There was no statistically significant difference between two groups in terms of demographic characteristics such as age, gender, type of delivery, gestational age, etc. Dysmorphisms, hypotonia, myelination abnormalities were significantly more frequent in patients with del/dup than in patients with normal result. The frequency of macrocephaly and obesity was higher in the normal group, and that of generalized seizures was higher among epileptic patients in this group. Nineteen (55.9%) of the 34 cases who have del/dup detected at analysis were regarded as pathogenic, 15 (44.1%) as uncertain clinical significance (likely pathogenic, likely benign and no subclassification).
Conclusion: Since CMA is an expensive, laborious, and time-consuming test, considering clinical parameters when requesting CMA will yield high diagnostic efficiency. A high possibility of copy number variants may be predicted in GDD/ID patients with dysmorphisms, hypotonia, and myelination delay. CMA should represent the genetic analysis of choice in pediatric neurology practice in case of no finding suggesting a different etiology in these patients.
Keywords: Chromosomal microarray analysis; pediatric neurology; myelination delay; hypotonia; global developmental delay; intellectual disability.

ÖZ

Amaç: Bu çalışmanın amacı, günlük pediatrik nöroloji pratiğinde kromozomal mikrodizi analizinin (chromosomal microarray analysis, CMA) tanısal kullanışlılığını saptamak ve bu testi gerektiren hastalar için kılavuz olan klinik parametreler belirlemektir.
Gereç ve Yöntemler: Pediatrik nöroloji kliniğimize 2018 ve 2020 yılları arasında çeşitli nedenlerle başvuran global gelişme geriliği/zihinsel yetersizlik (global developmental delay/intellectual disability, GDD/ID) olan 91 hastanın CMA sonuçları incelendi. CMA'da del/dup tespit edilen 34 (%37,4) hastanın ve normal CMA'ya sahip olan 57 (%62,6) hastanın demografik ve klinik verileri karşılaştırıldı.
Bulgular: İki grup arasında yaş, cinsiyet, doğum şekli, doğum zamanı gibi demografik özellikler bakımından istatistiksel olarak anlamlı bir farklılık yoktu. Dismorfizm, hipotoni ve miyelinizasyon anormallikleri CMA'da del/dup olan hastalarda normal CMA'lı hastalara göre önemli ölçüde daha sıktı. Normal CMA grubunda makrosefali ve obezite sıklığı daha yüksekti ve bu grupta epileptik hastalardaki generalize konvulsiyon sıklığı daha yüksekti. Analizde del/dup saptanan 34 vakadan 19'u (%55,9) patojenik, 15'i (%44,1) klinik önemi bilinmeyen (muhtemelen patojenik, muhtemelen iyi huylu ve sınıflandırılamayan) olarak kabul edildi.
Sonuç: CMA pahalı, zahmetli ve zaman alan bir test olduğundan, CMA talep edilirken klinik parametrelerin dikkate alınması yüksek tanısal verimlilik sağlayacaktır. Dismorfizm, hipotoni ve miyelinizasyon gecikmesi olan GDD/ID hastalarında kopya sayısı değişiklikleri yüksek olasılıkla saptanabilir. Bu hastalarda farklı bir etyoloji düşündüren herhangi bir bulgunun olmadığı durumlarda, CMA pediatrik nöroloji pratiğinde tercih edilebilir bir genetik analizdir.
Anahtar kelimeler: Kromozomal mikrodizi analizi; pediatrik nöroloji; miyelinizasyon geriliği; hipotoni; global gelişme geriliği; zihinsel yetersizlik.

INTRODUCTION

Global developmental delay (GDD) refers to significant retardation in two or more areas of development (gross or fine motor skills, speech and language, cognition, personal and social interactions, and activities of daily living). It affects 1-3% of children, many of whom exhibit intellectual disability (ID) subsequently (1,2). The term GDD is generally used for the under-five age group, while the term ID is used at older ages due to the applicability of intelligence quotient testing (3).

Chromosomal microarray analysis (CMA) has become a routine and recommended first step test in both GDD and ID (4,5). The frequency of detection of deletions or duplications in CMA increases in pediatric neurology patients with GDD/ID, hypotonia, epilepsy, and dysmorphic findings at childhood age group. In case of no additional finding leading to diagnosis, CMA can therefore be used as a screening procedure in the presence of these clinical findings. However, limited accessibility restricts the use of this test in some centers.

The purpose of present study was to identify clues capable of predicting the probability of detection of deletion and duplication in patients with this manifestation at the CMA test. We also reported data for duplications and deletions together with the cases' clinical summaries.

MATERIAL AND METHODS

Patients

Ninety-one patients (43 female and 48 male) aged 1-15 years who admitted to our pediatric neurology outpatient clinic for various reasons, such as dysmorphisms, microcephaly macrocephaly, epilepsy, hypotonia, motor retardation, gait disturbance, and speech retardation, and identified as requiring CMA following observation of GDD/ID between 2018 and 2020 were included in the study. Children with inborn errors of metabolism and previous causative genetic diagnoses explaining their clinical findings were excluded.

This study was approved by the local ethics committee of Düzce University (01.06.2020, 109). The research made in accordance with the Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects.

Chromosomal Microarray Analysis

Chromosomal microarray analyses have requested for all patients. Individual informed consent for medical examinations, genomic analyses, and case presentations were obtained from the parents. Genomic DNA was extracted from peripheral leukocytes of fresh blood samples collected from the patients, and chromosomal analysis was performed. DNA was isolated from peripheral blood samples, and CMA was performed using Agilent ISCA v2 Human Genome 8x60k oligonucleotide array.

Study Design and Clinical Data

CMA results of the 91 patients were evaluated retrospectively. Patients without submicroscopic deletions and/or duplications based on the CMA results were determined as Group 1, and those with submicroscopic deletions and/or duplications as Group 2.

The Denver II developmental test (6), Stanford-Binet Intelligence Scales, 5th edition (SB-5) test (7), and Wechsler Intelligence Scale for Children, 3rd edition (8), were used for the diagnosis of GDD and ID, respectively.

Age, gender, anamnesis, family history, parents' ages, and consanguinity between the parents were investigated. Anthropometric measurements, physical and neurological examinations were performed. Patients in whom micro-macrocephaly, hypotonia, dysmorphisms, low weight, tall or short stature, obesity, and spasticity were identified based on the examination findings were investigated. Patients with GDD/ID, autism, attention deficit and hyperactivity disorder (ADHD), speech disability were identified. These disorders assessed also in accordance with the criteria of Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (9).

All patients underwent metabolic workup (complete blood count, blood biochemistry, lactate, pyruvate, ammonia, serum amino acids, urine organic acids, free and total carnitine analysis, plasma acylcarnitine analysis, uric acid, and biotinidase activity), cranial magnetic resonance imaging (CMRI), abdominal ultrasonography, echocardiography, and ocular and hearing examinations. The CMRI results were divided into groups, and patients with corpus callosum abnormality, vascular abnormality, cortical abnormality, myelination delay, brainstem abnormality, cerebral atrophy, dilated ventricle, cavum septum pellucidum et vergae, arachnoid cyst, and normal imaging were identified.

Age at onset of seizures and seizure types (motor, non-motor, focal or generalized seizure) were determined in epileptic patients in both groups. The epilepsy subtype classifications were based on the terminology proposed by the Commission on Classification and Terminology of the International League against Epilepsy (10). Among the patients with epilepsy, individuals with drug-resistant epilepsy and epilepsy under-control were identified based on failure of adequate trials of two tolerated, appropriately chosen, and used anticonvulsant drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom (11). Electroencephalography (EEG) examinations were performed on all epileptic patients. EEG signals were recorded for a minimum of 30 min from 19 scalp electrodes, based on the International 10-20 System (Galileo NT Mizar-Sirius 33 Channels; EBNeuro) (12). Patients with focal spike, generalized spike, background abnormality, or normal findings were identified. All parameters were compared between the groups. The CMA results of the patients in Group 2 were also assessed according to the American College of Medical Genetics guidelines (13). The results were grouped in line with pathogenic, uncertain clinical significance (likely pathogenic, likely benign, no sub-classification) and benign definitions. The case characteristics in this group are presented in Table A1 in the appendix.

Statistical Analysis

The data were evaluated using Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) for Windows v.22.0 software. The descriptive statistical methods (mean, standard deviation, median, interquartile range, minimum-maximum values, number, and percentages) was used. The Kolmogorov-Simirnov and Shapiro-Wilk tests were used to examine normality assumption. Since we found that the data were not normally distributed Mann-Whitney U test was used for a

pairwise comparison of groups. Pearson chi-square and Fisher's exact tests were applied to analyze categorical data, and odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated. p values <0.05 were considered statistically significant.

RESULTS

The mean age of the patients was 5.2±3.7 (median=4, min-max=1-17) years. The CMA results of 57 (62.6%) patients revealed no submicroscopic deletions or duplications (Group 1), while 34 (37.4%) patients exhibited at least one of the submicroscopic deletions or duplications (Group 2).

No statistically significant difference was found between two groups in terms of age, gender, type of delivery, gestational age, birth weight, length of hospital stay during neonatal period, family history of neurological or psychiatric disease, parents' ages, or parental consanguinity (Table 1).

There was no statistically significant difference between the groups in terms of macrocephaly, microcephaly, low weight, tall stature, short stature, or spasticity parameters (Table 2).

The dysmorphology rate was significantly higher in Group 2 than in Group 1 (p=0.003). When the dysmorphology condition was considered, the risk of dysmorphology was 4.865 times higher in Group 2 than Group 1 (OR=4.865, 95% CI=1.647-14.364). A significantly higher number of obese patients was observed in Group 1 compared to Group 2 (p=0.023).

The hypotonia rate was significantly higher in Group 2 than in Group 1 (p=0.047). The risk of hypotonia was 2.682 times higher in Group 2 than in Group 1 (OR=2.682, 95% CI=1.067-6.744). GDD/ID ratios were similar in the two groups. No difference was also determined in proportions of patients with autism, ADHD, and speech disability (Table 2).

Table 1. Demographical characteristics of patients

	Group 1 (n=57)	Group 2 (n=34)	p
Age (years)	4 (5.25) [1-17]	4 (5.13) [1-14]	0.231
Gender			
Female	26 (45.6)	17 (50.0)	0.685
Male	31 (54.4)	17 (50.0)	
Type of delivery			
CS	36 (63.2)	17 (50.0)	0.218
NSD	21 (36.8)	17 (50.0)	
Time of delivery			
Preterm	11 (19.3)	8 (23.5)	0.631
Term	46 (80.7)	26 (76.5)	
Hospitalization	20 (35.1)	9 (26.5)	0.393
SGA	9 (15.8)	7 (20.6)	0.561
Family history	17 (29.8)	9 (26.5)	0.732
Mom age	30 (8) [19-41]	26.5 (6) [17-43]	0.170
Dad age	33 (9) [24-46]	32 (7) [24-53]	0.529
Consanguinity			
No	42 (73.7)	30 (88.2)	
1st degree	9 (15.8)	2 (5.9)	0.242
2nd degree	6 (10.5)	2 (5.9)	

CS: caesarean section, NSD: normal spontaneous delivery, SGA: small for gestational age, descriptive statistics were presented as n (%) for categorical variables, and as median (interquartile range) [minimum-maximum] for numerical variables

The distributions of patients with corpus callosum abnormality, vascular abnormality, cortical abnormality, myelination delay, brainstem abnormality, cerebral atrophy, dilated ventricle, cavum septum pellucidum et vergae, arachnoid cyst, and normal imaging were similar in two groups, as per the CMRI results (Table 2). However, the rate of myelin abnormalities was significantly higher in Group 2 than in Group 1 (p=0.014). The risk of myelin abnormalities was 4.77 times higher in Group 2 than in Group 1 (OR=4.770, 95% CI=1.339-16.988).

The frequency of epilepsy was similar between the groups (p=0.304). Nineteen of the 34 patients with epilepsy were from Group 1 and 15 were from Group 2. When epileptic patients were compared, no statistically significant difference was found between the groups in terms of age at onset of seizures, response to antiepileptic drugs, and EEG abnormalities (Table 3). In terms of seizure types, the frequency of generalized seizures in Group 1 was higher than in Group 2 (p=0.011).

Table 2. Comparison of patients with and without submicroscopic deletions and/or duplications, n (%)

	Group 1 (n=57)	Group 2 (n=34)	p
Short Stature	15 (26.3)	15 (44.1)	0.081
Tall Stature	1 (1.8)	2 (5.9)	0.553
Macrocephaly	9 (15.8)	1 (2.9)	0.084
Microcephaly	22 (38.6)	8 (23.5)	0.139
Dysmorphology	31 (54.4)	29 (85.3)	**0.003**
Nonambulatuar	19 (33.3)	9 (26.5)	0.493
Spasticity	2 (3.5)	1 (2.9)	0.999
Obesity	8 (14.0)	0 (0.0)	**0.023**
Hypotonia	29 (50.9)	25 (73.5)	**0.047**
GDD/ID	50 (87.7)	32 (94.1)	0.475
GR	15 (26.3)	15 (44.1)	0.081
Speech disability	41 (71.9)	29 (85.3)	0.143
CC abnormality	10 (17.5)	8 (23.5)	0.488
Vascular abnormality	1 (1.8)	1 (2.9)	0.999
Cortical abnormality	2 (3.5)	0 (0.0)	0.527
Cardiac abnormality	5 (8.8)	5 (14.7)	0.492
Renal abnormality	2 (3.5)	1 (2.9)	0.999
Vision abnormality	10 (17.5)	2 (5.9)	0.199
Hearing abnormality	4 (7.0)	2 (5.9)	0.999
Myelin abnormality	4 (7.0)	9 (26.5)	**0.014**
Cavum septum	3 (5.3)	3 (8.8)	0.668
Arachnoid cyst	9 (15.8)	4 (11.8)	0.760
Brainstem abnormality	6 (10.5)	3 (8.8)	0.999
Cerebral atrophy	6 (10.5)	5 (14.7)	0.741
Dilated ventricle	7 (12.3)	3 (8.8)	0.738
HSM	3 (5.3)	3 (8.8)	0.668
PVL	0 (0.0)	2 (5.9)	0.137
Normal CMRI	32 (56.1)	18 (52.9)	0.767
Epilepsy	19 (33.3)	15 (44.1)	0.304

GDD/ID: global developmental delay/intellectual disability, GR: growth retardation, CC: corpus callosum, HSM: hepatosplenomegaly, PVL: periventricular leukomalacia, CMRI: cranial magnetic resonance imaging

Table 3. Comparison of epileptic patients with and without submicroscopic deletions and/or duplications (n=34)

	Group 1 (n=19)	Group 2 (n=15)	p
Age at seizure onset	1.5 (2.5) [0-9.5]	1.75 (2) [0-11]	0.557
Focal spike	9 (47.4)	8 (53.3)	0.730
Generalized Spike	8 (42.1)	3 (20.0)	0.271
Normal EEG	3 (15.8)	4 (26.7)	0.672
Background abnormality	8 (42.1)	6 (40.0)	0.901
Controlled epilepsy	11 (57.9)	9 (60.0)	0.901
Resistant epilepsy	8 (42.1)	6 (40.0)	0.901
Motor seizure	16 (84.2)	13 (86.7)	0.999
Non-motor seizure	7 (36.8)	3 (20.0)	0.451
Focal seizure	3 (15.8)	4 (26.7)	0.672
Generalize seizure	19 (100)	10 (66.7)	**0.011**

EEG: Electroencephalography, descriptive statistics were presented as n (%) for categorical variables, and as median (interquartile range) [minimum-maximum] for numerical variables

Clinical Characteristics and CMA Results of Patients with Submicroscopic Deletions and/or Duplications

Nineteen patients' mutations detected were considered pathogenic (55.9% of the Group 2, 20.9% of the total), fifteen patients' mutations were designated as uncertain clinical significance (44.1% of the Group 2, 16.5% of the total). These were separated as likely pathogenic, likely benign and no subclassification among themselves. The clinical features of these patients and their CMA results are shown in Table A1 in the appendix.

DISCUSSION

No etiology can be determined in some pediatric neurology patients. Although the possibility of diagnosing some patients has been increased by novel genetic technologies introduced in recent years, the use of these tests s restricted by cost and transportation problems. CMA was performed on patients with GDD/ID and admitted to the pediatric neurology outpatient clinic of our hospital for various reasons. Statistically significant findings emerged in some clinical data when patients with duplication/deletion were compared with those with normal CMA results.

Numerous studies in recent years have shown the sensitivity of CMA analysis in patients with GDD/ID, autistic spectrum disorder (ASD), and dysmorphic findings, and have recommended its use as a first-line screening test (4,5). CMA is 100 times more sensitive than karyotype analysis and makes a 15-20% contribution to diagnosis (4). In a recent study, the diagnostic efficiency of CMA was found 31.7% on the patients who have GDD/ID (14). Shoukier et al. (15), observed more microcephaly, short stature, failure to thrive, and especially congenital heart defects, in patients with pathology detected as a result of CMA analysis compared to patients with normal results in a series of 342 cases with GDD/ID. Even though no statistically significant difference was observed between two groups in terms of microcephaly and macrocephaly in the present study, the rate of macrocephaly was higher markedly in Group 1. In terms of the genetic causes of macrocephaly, the etiology includes neurocutaneous and neurometabolic diseases, which are generally caused by single-gene disorders, and syndromes with overgrowth caused by a single gene disorder (16). In other words, macrocephaly is frequently seen with chromosomal del/dup.

Studies have reported a high frequency of congenital anomalies in patients with pathogenic copy number variants (CNVs) (17,18). In one recent piece of research, patients who underwent CMA were divided into four groups as isolated ID/DD, DD/ID with multiple congenital anomalies (MCA), isolated ASD, and DD/ID with epilepsy. CNV rates were significantly higher in the DD/ID with MCA group than in the other three groups (19). Consistent with the previous literature, the rate of dysmorphisms was significantly higher in Group 2 compared to Group 1 in the present study.

Failure to thrive may frequently accompany chromosomal diseases. Shoukier et al. (15), observed higher birth weight and subsequent failure to thrive in patients with pathology at CMA compared to patients with normal CMA. The role of CNVs was also emphasized in a study conducted with newborns to determine the etiology of small for gestational age (20). Although failure to thrive is commonly observed with pathological CNVs, new submicroscopic deletions and duplications are also implicated at the etiology of obesity (21,22). In the present study, although there was no difference between the two groups in terms of low weight, tall stature, short stature, or birth weight, the obesity rate was higher in Group 1. While no statistically significant difference was determined, low weight and short stature rates in Group 2 were higher than in Group 1. This result may suggest that del/dups detected for pediatric neurology patients with GDD/ID mostly create a failure to thrive.

Despite the fact that structural chromosomal abnormality is among the known etiological factors in hypotonia, this can also be caused by several neurological diseases (23). The hypotonia rate in Group 2 was statistically significantly higher than in Group 1. In other words, it may be concluded that hypotonia increases the risk of deletion/duplication detection 2.68-fold at CMA.

The importance of rare CNVs in generalized or focal childhood epilepsy is well known (24). CNVs have also been associated with epileptic encephalopathy (25), atypical rolandic epilepsy (26), epilepsy with intellectual disability (27), absence epilepsy (28) and fever-related syndromes (29) in previous studies. We observed no significant difference in epilepsy rates between our patient groups with and without CNV. Seizure patterns were generalized in all patients in Group 1, while Group 2 contained patients with focal seizures. This difference was statistically significant. We attribute this result to the study being record-based. Although no previous research has shown a relationship between seizure types and CNV, studies have investigated the association between focal and generalized epilepsy types and CNV. Perez et al. (30), reported 1.139-fold greater risk of microdeletion development in patients with epilepsy than in a control group (micro del carrier rates were 4.85% in the epilepsy group and 3.47% in the control group). They also determined that microdeletions had an essential role in the genetic structure in the genetic generalized epilepsy group, and made a minor contribution in rolandic epilepsy and adult focal epilepsy.

Coppola et al. (31), recent study emphasized the importance of CNVs in patients defined as epilepsy plus (epilepsy and comorbid features), and reported new pathogenic CNV and candidate genes. They also reported approximate CNV rates of 12% in patients with co-morbidities with epilepsy of unknown cause and recommended that these be investigated.

The importance of CNVs in this group was emphasized in another study with a reported diagnostic yield of approximately 15% in a group of 92 patients with epilepsy and ID (32). In the present study, 15 (44.1%) of the 34 patients in Group 2 had epilepsy. This shows that CNVs are commonly encountered in epilepsy patients with ID/GDD and whose etiology has not been determined, and are necessary for diagnosis.

Cranial magnetic resonance imaging is performed as part of the evaluation of patients with GDD/ID in the presence of additional neurological findings (abnormal head circumference, focal neurological signs, or epilepsy). Detection of CMRI abnormalities in patients with specific genetic syndromes, inborn errors of metabolism, or perinatal acquired injury helps to establish the diagnosis (33). Heide et al. (34), found CNV in 13% of patients with both corpus callosum abnormality and intellectual disability and thought that this might explain the possible cause of the disease. In another study, CNV was detected in 14% of 108 patients with corpus callosum anomalies who underwent CMA, and was found to be responsible for the pathogenesis in half of these cases (35). In a study investigating the relationship between cerebellar anomalies and CNV, the authors determined that cerebellar lesions will not be supported by the presence of CNV other than patients with Dandy-Walker malformation or complex poly-malformative phenotypes (36). In the present study, comparison of the CMRI findings of patients with corpus callosum abnormality, vascular abnormality, cortical abnormality, brainstem abnormality, cortical atrophy, dilated ventricle, periventricular leukomalacia, cavum septum pellucidum et vergae, arachnoid cyst, normal imaging parameters, revealed no differences between the two groups. However, rates of white matter disorders such as periventricular myelin changes, myelination delay, and non-specific myelin defects were higher in patients with del/dup in CNV than in those with normal CNV. Determination of deletion or duplication in patients with CNV increased the risk of myelination disorders 4.77-fold compared to the normal group.

Vigdorovich et al. (37), also determined that different chromosomal micro-rearrangement syndromes create nonspecific multifocal and especially periventricular white matter changes as a common feature at CMRI. The authors also reported that these lesions may also be accompanied by corpus callosum dysgenesis or gray matter loss. These findings support our own study.

We detected pathological deletion/duplication in 20 (22.0%) of the 91 patients in this study. This rate was higher than average in terms of diagnostic yield, according to the previous literature. This may be due to CMA, the third step, being necessarily performed in patients with GDD/ID who present to our hospital with various neurological problems after other etiological investigations have been performed. A diagnostic yield of 10.2% (0-50%) was reported in a report of 18 studies of patients with GDD/ID with facial dysmorphism, congenital anomalies, or neurological symptoms (38). We detected CNV in 34 patients (37.4%) (pathogenic and unknown clinical significance). D'Arrigo et al. (39), found pathological significance in 16% and unknown clinical significance in 31% of 329 cases.

The most important limitation of our study is its retrospective nature. Also, the number of our patients was small. We also realize that our classification is imperfect, and we were only able to perform parental CMA in a small number of cases.

CONCLUSIONS

This study demonstrates the importance of the use of CMA in the first step in patients with dysmorphisms, hypotonia, or myelination disorders with GDD/ID. We also think that CNVs will be more common in pediatric neurology patients with microcephaly and failure to thrive. The role of CMA in the diagnosis of epileptic patients with undetermined etiology and GDD/ID association should not be ignored. The increasing identification of new CNVs and the growing identification of pathogenic CNVs will finally determine the place of CMA analysis in pediatric neurology patients.

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The Appendix:

Table A1. Clinical and genetic features of patients whose chromosomal microarray analysis results were evaluated

No	Sex/Age	Clinical Manifestations	Neuroimaging	CMA results/ Significance	Size	Start Stop	Responsible genes
1	M/7.5	Drug-resistant epilepsy (myoclonic-astatic seizures), Macrodactyly and syndactyly on the 2nd and 3rd toes of the left food ID, ATX gene normal	Normal	2q13 UCS;NS 6p21.32 UCS;LP	0.3MB dup 0.2MB del	(110783236-111103309)x3 İnherited, (32688930-32956557)x1 De novo	**2q13:** *MALL,NPHP* **6p21.32:** *HLA-DQA2,HLA-DQB2,HLA-DOB,TAP2,PSMB8,TAP1,PSMB9,HLA-DMB,HLA-DMA,BRD*
2	M/2.5	Hypotonia, GDD, Atypical autism, SD, Left ear SNHL	Normal	15q13.2q13.3 Pathogenic	1.5MB del	(30654726-32509926)x1 unknown	*ARHGAP11B,CHRFAM7A,CHRNA7,FAN1,KLF13,OTUD7A,TRPM1*
3	M/7.5	GDD, Diplegia, Spasticity Autism, SD, Epilepsy (generalized motor seizures), FD, Microcephaly, GR, SGA, Epilepsy	Cerebral atrophy Thin CC	12q13.12q13.13 UCS;LP 13q13.1 UCS;NS	2.4MB del 0.4MB del	(50157351-52618686)x1, (32533833-32937352)X1 De novo	**12q13.12q13.13:***TBMIM6,NCKAPL5,FAIM2, AQP2,AQP5, SMARCD1, GPD1, COX14* **13q13.1:***CERS5,LIMA1,DIP2B,ATF1,TFCP2 ,BIN2,CELA1,GALNT6,SLC4A8,ACVRL1, ACVR1B,GRASP, NR4A1, ATG101, KRT80*
4	F/2	Rubinstein-Taybi, Drug-resistant epilepsy (focal motor seizures), GDD, FD, Microcephaly, GR, SGA, SD, Right eye cataract, PS/ASD	Thin CC, myelination delay	16p13.3 Pathogenic	52KB del	(378,3001-383,5116)x1 unknown	*CREBBP*
5	F/3.5	GDD, SD, FD, Microcephaly, Epilepsy (non-motor seizures), Simian sign, Broad thumbs and first toes	Thin CC, myelination delay	15q11.2 Pathogenic	0.5MB del	(227,656,28-233,00,287)x1 unknown	*TUBGCP5,CYFIP1,NIPA2,NIPA1*
6	M/3	GDD, FD Epicanthus, Upslanted palpebral fissure, Micrognathia, SS, Epilepsy (hot water epilepsy), SGA, Unsteady gait, Foot and toe deformity, Brainstem hypoplasia	Cerebral and cerebellar atrophy, increased subarachnoid space, cystic dilated fourth ventricle, hypoplasia of brainstem	Xp22.33 UCS;LP Yp11.32 UCS:LB	0.1MB dup	602488-733497)x3 Maternal inherited 552488-683497)x3 Denovo	*SHOX* *No gene*
7	M/1.5	GDD, GR and Short stature, Microcephaly, Recurrence infection, Di George syndrome Telangiectasia in the ear, ATX gene normal, CFTR gene normal	Normal	22q11.21 Pathogenic	2.8MB del	(18628019-21440514)x1 unknown	*USP18, DGCR6, PRODH, DGCR2, TSSK2, GSC2, SLC25A1, CLTCL1, HIRA, MRPL40, UFD1L, CLDN5, SEPT5, GP1BB,TBX1, GNB1L, TXNRD2, COMT, ARVCF, TANGO2, MIR185, DGCR8, TRMT2A, RANBP1, ZDHHC8, RTN42, DGCRGL, RIMBP3, ZNF74, SCARF2, MED15, PI4KA, SERPIND1, SNAP29, CRKL, LZTR1, SLC7A4*
8	F/5.5	SD, FD, High palate, low set ears, triangular facial shape, Hypertelorism, ID, SGA	Periventricular myelination abnormality	19p12q11 UCS;LP	3.7MB del	(24378197-28095812)x1 unknown	no known gene in this region
9	F/4.5	GDD, GR, Microcephaly FD, Coarse facial features	Normal	4p16.1 UCS;NS	0.7MB dup	(9766686-10520199)x4 Unknown	DRD5, SLC2A9, WDR1, CLNK

10	F/7	GDD, FD, Teeth abnormality, Low set ears, low posterior hairline, Coarse face, hypertelorism, prominent beak nose, SD, Cleft lip and palate, Epilepsy(non-motor and generalized motor seizures), ID, Foot deformity, Scoliosis, scapula alata, Clinodactyly, retrognathia	Partial agenesis of CC, cavum septum pellucidum et vergae, colpocephaly, increased subarachnoid space	6q26 UCS;NS	0.4MB dup	162619277-163055489)x3 Unknown	*PARK2*
11	F/2.5	Phelan McDermid syndrome, Anithelix Prominent intertriginous folds, Clinodactyly Unsteady gait, SD, GDD	Normal	22q13.31q13.33 Pathogenic	3.1MB del	(47987698-51169045)x1 unknown	*ADM2, ALG12, ARS, BRD1, CHKB, CPT1B, CRELD2, FAM19A5, HDAC10, IL17REL, MAPK11, MAPK12, MAPK8IP2, MIOX, MLC1, MOV10L1, NCAPH2, PANX2, PIM3, PLXNB2, PPP6R2, SBF1, SCO2, SHANK3, SYCE3, TUBGCP6, TYMP, ZBED4*
12	F/13	SS, Overweight, Hypochondroplasia, Coarse face, Steady gait, ID	Normal	19p12 UCS:LB	0.7MB del	(20216230-21001208)x1 Paternal inherited	*ZNF90,ZNF737*
13	F/1	Strabismus, Optic nerve pathology, FD, Ptosis of the right eye, Long eyelashes, Low-set ears, Mongoloid eyes, Sacral dimple, GR, GDD	Normal	4q28.2q28.3 4q28.3q31.21 4q32.1 4q34.3 Pathogenic	3MB, 9MB, 1.8MB, 2.6MB del	129223349-132575313)x1 (134924076-144240473)x1 (158444071-160299086)x1 (177605696-180305067)x1 Unbalanced segregation of a balanced translocation	*JADE1,SCLT1/CCRN4L,CLGN,ELMOD2,IL15,INPP4B,MAML3,MGST2,NAA15,PCDH18, RAB33B,SETD7,SLC7A11,TBC1D9,UCP1,USP38,ZNF330/C4orf46,ETFDH,PPID,RAPGEF2,RXFP1/AGA,NEIL3,VEGFC*
14	M/11.5	Epilepsy (generalized motor seizures), ADHD, FD, Downslanted palpebral fissure, Tall stature, Arachnodactyly, ID, Polythelia	Normal	15q13.3 Pathogenic	0.49MB del	(32018731-32515681)x1 unknown	*CHRNA7, OTUD7A*
15	F/2	GDD, Hiperlaxity, Pes planovalgus, GR, FD	Thin CC, dilated lateral ventricles, Arachnoid cyst on posterior fossa, cavum septum pellucidum et verge	9p24.3p13.1 Pathogenic	40MB dup	(46587-40294324)x3 Unbalanced segregation of a balanced translocation(maternal)	*ACER2, ACO1, ADAMTSL1, AK3, ALDH1B1, APTX, AQP3, AQP7, B4GALT1, BAG1, BNC2, C9orf72, CA9, CBWD1, CCIN, CCL19, CCL21, CCL27, CD274, CD72, CDC37L1, CDKN2A, CDKN2B, CER1, CHMP5, CLTA, CNTFR, CNTLN, CNTNAP3, CREB3, DCTN3, DDX58, DMRT1, DMRT2, DMRT3, DMRTA1, DNAI1, DNAJA1, DNAJB5, DOCK8, ELAVL2, ENHO, EQTN, ERMP1, EXOSC3,FAM154A, FANCG, FBXO10, FOCAD, FOXD4, FREM1, FRMPD1, GALT, GBA2, GLDC, GLIPR2, GLIS3, GNE, GRHPR, HAUS6, HINT2, IFNA1, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA2, IFNA21, IFNA4, IFNA5,*

							IFNA6, IFNA7, IFNA8, IFNB1, IFNE, IFNK, IFNW1, IFT74, IGFBPL1, IL11RA, IL33, INSL4, INSL6, JAK2, KANK1, KCNV2, KDM4C, KIAA0020, KIAA1161, KIAA1432, KIF24, KLHL9, LINC00961, LINGO2, LURAP1L, MELK, MLANA, MLLT3, MOB3B, MPDZ, MSMP, MTAP, NDUFB6, NFIB, NFX1, NOL6, NPR2, NUDT2, PAX5, PDCD1LG2, PIGO, PLAA, PLGRKT, PLIN2, PPAPDC2, PRSS3, PSIP1, PTPLAD2, PTPRD, RCL1, RECK, RFX3, RGP1, RLN1, RLN2, RNF38, RPS6, RRAGA, RUSC2, SH3GL2, SHB, SIGMAR1, SIT1, SLC1A1, SLC24A2, SMARCA2, SMU1, SNAPC3, SPAG8, SPINK4, STOML2, TAF1L, TEK, TESK1, TLN1, TMEM261, TMEM8B, TOMM5, TOPORS, TPD52L3, TPM2, TTC39B, TUSC1, TYRP1, UBAP1, UBE2R2, UHRF2, UNC13B, VCP, VLDLR, ZBTB5, ZDHHC21
16	M/7	DiGeorge syndrome SD GDD, ID, FD, Prominent ears, Sparse eyebrows, Widely space nipples, Left renal agenesis, Recurrence infection, Cardiac abnormality	Normal	22q11.21 Pathogenic	2.56MB del	(18901004-21462353)x1 Un known	*AIFM3, ARVCF, CDC45, CLDN5, CLTCL1, COMT, CRKL, DGCR14, DGCR2, DGCR6, DGCR6L, DGCR8, GGTLC3, GNB1L, GP1BB, GSC2, HIRA, KLHL22, LZTR1, MED15, MRPL40, P2RX6, PI4KA, PRODH, RANBP1, RIMBP3, RTN4R, SCARF2, SEPT5, SERPIND1, SLC25A1, SLC7A4, SNAP29, TANGO2, TBX1, THAP7, TRMT2A, TSSK2, TXNRD2, UFD1L, ZDHHC8, ZNF74*
17	F/2.5	FD, Low-set ears, High palate, Sleep problem, GDD, SD, Hypotonia, Atrial septal defect, GR	Dilated frontal Horne of the lateral ventricle	6q15q16.3 Pathogenic	11.73KBdel	(89,181,413-100,914,602)x1	*PNRC1, PROL2, PM20D2, ACY1L2, GABRR1, GABRR2, UBE2J1, UBC6E, PNRC1, PROL2, SIM1, GRIK2, MCHR2, ASCC3, EPHA7*
18	F/2	FD (caput quadratum, trigonocephaly, downslanted palpebral fissure, low posterior hairline, high nasal root), GR, SS, GDD, Febrile status, epilepticus (generalized motor seizure)	Delayed myelination (level of centrum semiovale, especially frontal and parietal deep white matter)	17p13.3 UCS;LP 17q12 UCS;LP	0.8MB del, 0.9MB dup	(1251996-2084712)x1 (31993787-32911168)x3 unknown	**17p13.3:** *CRKDPH1, HIC1, INPP5K, MYO1C, OVCA2, PITPNA, PRPF8, RILP, RPA1, RTN4RL1, SCARF1, SERPINF1, SERPINF2, SLC43A2, SMG6, WDR81, TWHAE /* **17q12:** *ASIC2, CCL1, CCL11, CCL13, CCL2, CCL7, CCL8, TMEM132E*
19	M/3	SD, GDD, FD (hypotelorism, plump lip, bitemporal narrowing, high and narrow palate), waddle walk, asthenia	Normal	10q11.22 UCS;NS 13q34 UCS;NS	0.7MB 0.3MB dup	(46972140-47701570)x3 (113922447-114288971)x3 unknown	**10q11.22:** *GPRIN2,NPY4R* **13q34:***ADPRHL1,LAMP1,TFDP1,TMCO3*

20	M/4	FD, High palate, Low-set ears, Frontal bossing, Arachnodactyly, Undescended testicle, GDD, SD	Dilated lateral and third ventricle, Increased subarachnoid space, Mega cisterna magna, Increased prepontine and suprasellar cistern space	9p24.3p13.1 Pathogenic	38.5MB dup	(204,198-387,414,37)x4 Unbalanced segregation of a balanced translocation	*ACER2, ACO1, ADAMTSL1, AK3, ALDH1B1, APTX, AQP3, AQP7, B4GALT1, BAG1, BNC2, C9orf72, CA9, CBWD1, CCIN, CCL19, CCL21, CCL27, CD274, CD72, CDC37L1, CDKN2A, CDKN2B, CER1, CHMP5, CLTA, CNTFR, CNTLN, CNTNAP3, CREB3, DCTN3, DDX58, DMRT1, DMRT2, DMRT3, DMRTA1, DNAI1, DNAJA1, DNAJB5, DOCK8, ELAVL2, ENHO, EQTN, ERMP1, EXOSC3,FAM154A, FANCG, FBXO10, FOCAD, FOXD4, FREM1, FRMPD1, GALT, GBA2, GLDC, GLIPR2, GLIS3, GNE, GRHPR, HAUS6, HINT2, IFNA1, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA2, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNB1, IFNE, IFNK, IFNW1, IFT74, IGFBPL1, IL11RA, IL33, INSL4, INSL6, JAK2, KANK1, KCNV2, KDM4C, KIAA0020, KIAA1161, KIAA1432, KIF24, KLHL9, LINC00961, LINGO2, LURAP1L, MELK, MLANA, MLLT3, MOB3B, MPDZ, MSMP, MTAP, NDUFB6, NFIB, NFX1, NOL6, NPR2, NUDT2, PAX5, PDCD1LG2, PIGO, PLAA, PLGRKT, PLIN2, PPAPDC2, PRSS3, PSIP1, PTPLAD2, PTPRD, RCL1, RECK, RFX3, RGP1, RLN1, RLN2, RNF38, RPS6, RRAGA, RUSC2, SH3GL2, SHB, SIGMAR1, SIT1, SLC1A1, SLC24A2, SMARCA2, SMU1, SNAPC3, SPAG8, SPINK4, STOML2, TAF1L, TEK, TESK1, TLN1, TMEM261, TMEM8B, TOMM5, TOPORS, TPD52L3, TPM2, TTC39B, TUSC1, TYRP1, UBAP1, UBE2R2, UHRF2, UNC13B, VCP, VLDLR, ZBTB5, ZDHHC21*
21	M/4	Epilepsy (generalized motor seizures), GDD, FD, Camptodactyly, strabismus, SD	Normal	7q31.1q31.31 Pathogenic	10MB dup	(107593989-118062404)x1 unknown	*PPP1R3A,MET,FOXP2,CAV1,ANKRD7,ASZ 1,C7orf60,CAPZA2,CAV1,CAV2,CFTR,CTTN BP2,DNAJB9,DOCK4*
22	M/6	SD, Autism, GDD, ID, Prominent beak nose	Normal	7q35 UCS;LP Xq27.1q27.2 Pathogenic	0.13MB del, 1.2MB dup	(145878672-146017091)x1 (139584651-140801014) 2x3 unknown	**7q35:** *CNTNAP2* **Xq27.1q27.2:** *SOX3, CDR1, SPANXB1, LDOC1, SAPNXA1, SAPNXA2, SPANXD, SPANXC*

23	F/4	GDD, SD, FD (happy face, low-set ears)	Corpus callosum hypoplasia (body) and agenesis (splenium)	Xq25 UCS;NS	0.4MB del	(122869800-123283576)x4 unknown	*XIAP, STAG2*
24	M/3.5	SD, FD (hypotelorism, macrocephaly, high palate, low set ear, flat occiput, left epicanthus, synophrys, local alopecia on temporal area), GDD, Autism	Normal	12q24.13q24.21 UCS;LP	0.28 MB dup	(114267718-114552522)x3 unknown	*RBM19*
25	M/7	SD, ID, FD, Macrocephaly, Ataxia ATX gene: normal	Frontotemporal atrophy, Cavum septum pellucidum et vergae	1q21.1 Pathogenic	0.36MB del	(14538817-145755813)x1 unknown	*CD160,HFE2,ITGA10,PDZK1,PEX11B,PIAS3,POLR3C,POLR3GL,RBM8A,TXNIP*
26	F/2	GDD, SD, GR, Microcephaly, FD, Low-set ears Abnormal earlobe shape	Cerebral atrophy Myelination delay	1q21.1q21.2 Pathogenic	3.5MB del	(145415190-148936712)x4 unknown	*NBPF20,NBPF10,TXNIP,RBM8A,GNRHR2, PEX11B,ITGA10,PIAS3,CD160,PDZK1,GPR89,NBPF11,NBPF12,PRKAB2,FMO5,CHD1L,BCL9,ACP6,GJA5,GJA8,GPR89B,NBF8,PPAL4A,NBPF14,NBPF9,NBPF15*
27	F/2.5	Wolf Hirchorn syndrome, Epilepsy (focal motor seizures), GR Microcephaly SD, Cleft palate FD, GDD	Thin CC, Myelination delay	4p16.3p16.1 Pathogenic	10MB del	(72447-103,372,96)x3 unknown	*ZNF141, PDE6B, ATP5I, MYL5, CPLX1, GAK, TMEM175, DGKQ, IDUA, FGFRL1, RNF212, SPON2, CTBP1, MAEA, UVSSA, CRIPAK, SLBP, TMEM129, TACC3, FGFR3, LETM1, WHSC1, C4orf48, NAT8L, POLN, HAUS3, ZFYVE28, RNF4, TNIP2, SH3BP2, ADD1, NOP14, GRK4, HTT, RGS12, HGFAC, DOK7, LRPAP1, ADRA2C, OTOP1, ZBTB49, MSX1, CYTL1, EVC2, EVC, CRMP1, JAKMIP1, WFS1, PPP2R2C, S100P, BLOC1S4, TBC1D14, TADA2B, GRPEL1, SORCS2, AFAP1, ABLIM2, MIR95, HTRA3, ACOX3, GPR78, CPZ, HMX1, USP17L9P, DRD5, SLC2A9, WDR1*
28	F/9.5	1p36 syndrome, Triple X syndrome, Moya moya syndrome, ID, Drug resistant epilepsy (focal motor, seconder generalized motor and non-motor seizures), GDD, SD, FD	Thin CC (Corpus and splenium, Ivy sign of moya moya.	1p36.32p36.32 Pathogenic Xp22.33q28 Pathogenic	1.8MB del	(2951244-4763189)x1 (93118-155235833)x3 De novo	*AJAP1,CEP104,DFFB,MEGF6,PRDM16,SMIM1,TP73,TPRG1L,WRAP73*
29	F/9.5	Epilepsy (generalized motor seizure, drug-resistant epilepsy), FD (coarse face, plump lip, short filtrum, prognathia), GDD, SS	Cerebellar tonsils 5 mm below the level of the foramen magnum.	2q37.3 UCS;NS	0.5MB del	242517966-243029573)x1 unknown	*THAP4,ATG4B,DTYMK,ING5,D2HGDH,GAL3ST2,NEU4,PDCD1*

30	M/12	FD, High and narrow palate, Flat occiput, Deep-set eyes, Hypotelorism, Microcephaly, Fusiform finger shape, Micropenis GDD, ID	Normal	Xp21.31q22.2 Pathogenic	12.7MB dup	(90301668-103038108)x4 unknown	*PABPC5, PCDH11X, NAP1L3, DIAPH2, RPA4, PCDH19, TNMD, TSPAN6, SRPX2, SYTL4, NOX1, XKRX, DRP2, TAF7L, TIMM8A, BTK, RPL36A, GLA, ARMCX1, ARMCX2, ARMCX3, NXF5, BEX5, NXF2, TMSB15A, NXF4, GPRASP1, BHLHB9, RAB40AL, BEX1, NXF3, BEX4, BEX2, TCEAL7, BEX3, TCEAL1, PLP1*
31	M/8	SD, FD (brachydactyly, teeth hypoplasia, short fingers), ADHD, SS, ID	Normal	5p15.33 UCS;NS 8q22.1 UCS;NS	0.29MB dup 1.1MB dup	(956671-1255361)x3 (9716932-98301557)x3 unknown	**5p15.33:** *NKD2, SLC12A7, SLC6A19, TERT /* **8q22.1:** *GDF6, MTERFD1, PTDSS1, SDC2, TSPYL5, UQCRB*
32	M/14	ID, Drug-resistant epilepsy (focal motor seizures) SD	Normal	17q12q21.2 UCS;LP	2.7MB dup	(385,802,44-386,06,106)x3 unknown	*DDX52, HNF1B, TBC1D3C, TBC1D3H, TBC1D3, TBC1D3G, TBC1D3E, MRPL45, GPR179, ARHGAP23, MLLT6, CISD3, PSMB3, RPL23, LASP1, FBXO4, PLXDC1, CACNB1, RPL19, FBXL20, MED1, CDK12, NEUROD2, STARD3, TCAP, PNMT, PGAP3, ERBB2, GRB7, IKZF3, ZPBP2, GSDMB, ORMDL3, GSDMA, CSF3, MED24, THRA, NR1D1, MSL1, SDO6, RARA, GJD3, TOP2A, IGFBP4,*
33	F/12	ID, SD, FD (big prominent ears, flat nasal root), GR, Epilepsy (motor seizure)	Normal	10q21.1q21.2 Pathogenic 10q21.2q22.1 UCS;LP	3.7MB dup 7.6MB dup	(59897533-63600048)x3 (63685902-71376096)x3 unknown	**10q21.1q21.2:***ANK3,BICC1,CCDC6,CDK1, CISD1, IPMK, LINC00948, RHOBTB1, SLC16A9,TFAM, TEMEM2 6, UBE2D1* **10q21.2q22.1:** *ADO, ARID5B, ATOH7, CCAR1, CTNNA3, DDX21, DDX50 DNA2 DNAJC12, EGR2, HERC4, HK1, HKDC1, HNRNPH3, JMJD1C, KIAA1279, LRRTM3, MYPNA, NEUROG3, NRBF2, PBLD, REEP3, RTKN2, SIRT1, SLC25A16, SRGN, STOX1 SUPV3L1, TACR2, TET1, TSPAN15, VPS26A, ZNF365*
34	M/2.5	Epilepsy (generalized motor seizures), FD, Synophrys, Micrognathia, Prominent, beak shape nose, GDD NIPBL gene: normal	Normal	15q13.3 UCS;NS	0.49MB dup	(32020066-32515681)x3 unknown	*CHRNA7, OTUD7A*

ADHD: Attention deficit hyperactivity disorder, CC: Corpus callosum, FD: Facial dysmorphism, GDD: Global developmental delay, GR: Growth retardation, ID: Intellectual disability, PS / ASD: Pulmonary stenosis / atrial septal defect, SD: Speech delay, SGA: Small gestational age, SNHL: Sensorineural hearing loss, SS: Short stature, UCS;LP: Uncertain clinical significance; likely pathogenic, UCS;NS: Uncertain clinical significance; no sub classification, UCS;LB: Uncertain clinical significance; likely benign

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Comparison of Rehabilitation Programs After Arthroscopic Rotator Cuff Repair in Terms of Timing

Artroskopik Rotator Manşet Onarımı Sonrası Rehabilitasyon Programlarının Zamanlama Açısından Karşılaştırılması

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ABSTRACT

Aim: The aim of this study was to determine and compare the effects of early and delayed passive joint rehabilitation protocol on functional and quality of life outcomes in patients following arthroscopic rotator cuff repair (RCR).
Material and Methods: A total of 202 patients who underwent arthroscopic RCR were included into the study. Ninety eight patients who started the rehabilitation program just after the arthroscopic RCR were comprised as early rehabilitation (ER) group, while 104 patients whose shoulder joint motion was not allowed for 3 weeks after surgery as delayed rehabilitation (DR) group. Demographic characteristics, preoperative and postoperative American Shoulder and Elbow Surgeons (ASES) score, Constant Murley (CM) score, visual analogue scale (VAS), and the 36-item Short Form Health Survey (SF-36) scores were evaluated.
Results: There was no significant difference between the ER and the DR groups in terms of improvement of ASES, CM, VAS and SF-36 scores after arthroscopic RCR. There was no difference between two groups in terms of complications such as re-tear, frozen shoulder and infection that developed during the follow-up period. Both rehabilitation protocols were found to have a similar effect on patient-reported outcomes.
Conclusion: At a mean follow-up time of 13 months, early and delayed onset postoperative rehabilitation programs are associated with similar functional and quality of life outcomes, and complication rates. Therefore, DR can be preferred primarily in patients with large tears. ER can be an option for the patients with small tears who has anticipation of early return to work and daily life.
Keywords: Shoulder; arthroscopy; rotator cuff injuries; rehabilitation.

ÖZ

Amaç: Bu çalışmanın amacı, artroskopik rotator manşet onarımını (RMO) takiben hastalarda erken ve ertelemeli pasif eklem rehabilitasyon protokolünün fonksiyonel sonuçlar ve yaşam kalitesi üzerindeki etkilerini belirlemek ve karşılaştırmaktır.
Gereç ve Yöntemler: Çalışmaya artroskopik RMO uygulanan toplam 202 hasta dahil edildi. Artroskopik RMO'dan hemen sonra rehabilitasyon programına başlayan 98 hasta erken rehabilitasyon (early rehabilitation, ER), ameliyat sonrası 3 hafta omuz eklem hareketine izin verilmeyen 104 hasta ise gecikmiş rehabilitasyon (delayed rehabilitation, DR) grubunu oluşturmaktaydı. Demografik özellikler, ameliyat öncesi ve ameliyat sonrası Amerikan Omuz ve Dirsek Cerrahları (American Shoulder and Elbow Surgeons, ASES) skoru, Sabit Murley (Constant Murley, CM) skoru, görsel analog skala (visual analogue scale, VAS) ve 36 maddelik Kısa Form Sağlık Anketi (SF-36) skorları değerlendirildi.
Bulgular: Artroskopik RMO sonrası ASES, CM, VAS ve SF-36 skorlarının iyileşmesinde ER ve DR grupları arasında anlamlı bir fark saptanmadı. İki grup arasında takip süresince gelişen tekrar yırtık nüksü, donuk omuz ve enfeksiyon gibi komplikasyonlar açısından fark yoktu. Her iki rehabilitasyon protokolünün de hasta tarafından bildirilen sonuçlar üzerinde benzer bir etkiye sahip olduğu görüldü.
Sonuç: Ortalama 13 aylık bir takip süresinde, erken ve gecikmiş başlangıçlı postoperatif rehabilitasyon programları, benzer fonksiyonel ve yaşam kalitesi sonuçları ve komplikasyon oranları ile ilişkilidir. Bu nedenle DR, özellikle büyük yırtıklara sahip hastalarda öncelikli olarak tercih edilebilir. ER, işe ve günlük hayata erken dönüş beklentisi olan küçük yırtıklara sahip hastalar için bir seçenek olabilir.
Anahtar kelimeler: Omuz; artroskopi; rotator manşet yaralanmaları; rehabilitasyon.

INTRODUCTION

In the treatment of patients undergoing rotator cuff repair (RCR), physical therapy is generally an important component (1). The purpose of rehabilitation after RCR is to prevent re-rupture, reduce pain, increase range of motion (ROM) and return to normal functional activities as soon as possible (2). The effects of rehabilitation techniques on postoperative recovery have been increasingly considered (3,4). It has been stated that the timing of rehabilitation programs is important for early joint movement acquisition and strengthening (5). There is a consensus that early onset of passive ROM reduces the risk of joint stiffness and provides earlier functional activity gain (6). However, it has been emphasized that this rehabilitation can put excessive stress on the repair area and increase the risk of anatomical failure (7). Due to concerns about tendon healing, delayed rehabilitation protocol with early immobilization has started to gain popularity (8). It is thought that delayed rehabilitation protocols can prevent situations that may adversely affect tendon healing such as micro-motion and cavity formation in the repair area. However, delayed joint motion may increase the risk of joint stiffness after surgery and potentially delay the return of shoulder function (9). Because of these conflicting findings, there is no definitive consensus on the initiation of a rehabilitation after RCR (3). The aim of this study is to determine and compare the effects of early-onset passive joint rehabilitation and late-onset rehabilitation after immobilization on quality of life and clinical outcomes after arthroscopic RCR.

MATERIAL AND METHODS

Following the approval of Uludağ University Clinical Research Ethics Committee (08.07.2020, 12/12), archive records were retrospectively reviewed, and 237 patients who underwent arthroscopic RCR due to full-thickness isolated supraspinatus rupture between January 2017 and January 2020 were identified. Early rehabilitation (ER) was applied to 125 of the patients and delayed rehabilitation (DR) was applied to 112 of them. Twenty four patients with an L or inverted L-shaped tear pattern, associated labral pathology, glenohumeral arthritis, cervical spine pathologies, or degenerative joint diseases were excluded. Of the remaining 213 patients, 11 were excluded due to follow-up incompatibility. Subsequently, 202 patients aged between 18-70 years, with full thickness isolated supraspinatus tendon rupture on magnetic resonance imaging (MRI) and confirmed arthroscopically by isolated full-thickness crescent-shaped supraspinatus rupture of the rotator cuff were included (Figure 1).

Postoperative Rehabilitation Protocols

All operations were performed using the same surgical technique and patients were referred to two different rehabilitation protocols.

Early rehabilitation (ER): After the arthroscopic RCR, they immediately started the rehabilitation program. On the postoperative 1st day, passive shoulder exercises were initiated as much as the patients could tolerate. Pendulum exercises were initiated at the first week. Passive shoulder joint exercises have been continued under the supervision of a physiotherapist until the 6th week. The patients were advised to continue their exercises at home in sets of 10, 5 times a day. Active shoulder motions were allowed at the end of the 6th week and strengthening exercises were started at the 3rd month. When not exercising, a shoulder arm sling was used in the neutral position for the first 6 weeks.

Delayed Rehabilitation (DR): Shoulder joint motion was not allowed for 3 weeks after arthroscopic RCR, but elbow and wrist joint movements were allowed. After 3 weeks, passive shoulder exercises were initiated for the patients to the extent that they could tolerate, and pendulum exercises were started. The patients were advised to continue their exercises at home in sets of 10, 5 times a day. Active shoulder motions were allowed after the 6th week and strengthening exercises were started at the 3rd month. A shoulder arm sling was used for all patients in the neutral position for the first 6 weeks.

Evaluation of Demographic and Clinical Findings

In both groups, age, gender, operated side, status of dominance in surgical side, duration of shoulder symptoms, follow-up period, tear size, working status, tear chronicity (<3 months acute, >3 months chronic) and complications (frozen shoulder, re-tear, infection) were compared. Rotator cuff rupture was classified by using MRI. According to DeOrio and Cofield classification; tears of 1 cm and less were classified as small, 1-3 cm medium, 3-5 cm large tears, and massive tears greater than 5 cm (10). The results of the American Shoulder and Elbow Surgeons (ASES) score, constant Murley (CM) score, visual analogue scale (VAS), and the 36-item Short Form Health Survey (SF-36) questionnaire recorded by an independent observer were used in clinical evaluation.

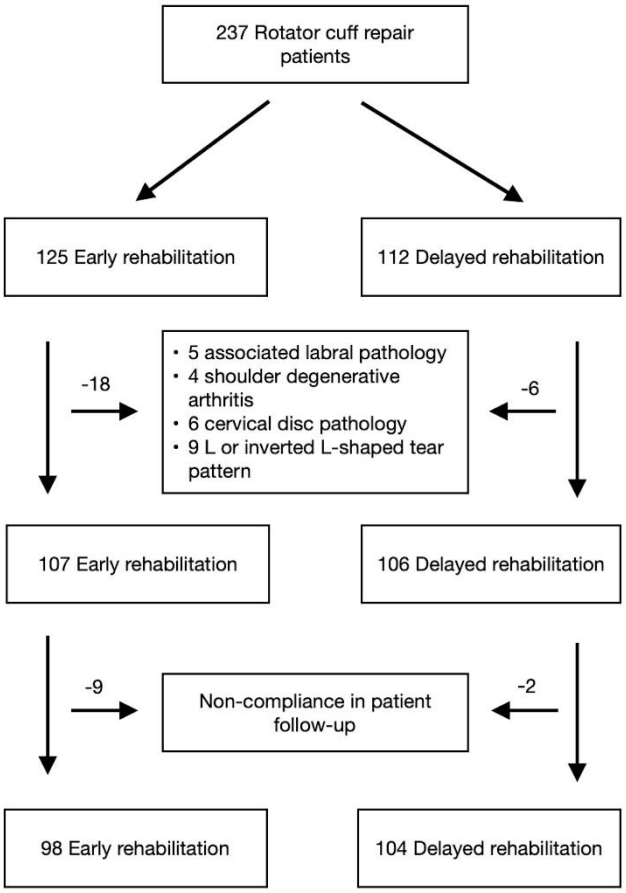

Figure 1. Flowchart of the study design, exclusion steps and the numbers of excluded patients

Surgical Technique

All patients were operated by two experienced surgeons under general anesthesia in beach chair position. Diagnostic arthroscopy was initially performed to assess the size of the rotator cuff tear, any lesions in the biceps tendon, and other associated lesions. Radiofrequency cauterization and adrenaline-supplemented irrigation fluid were used for bleeding control, and systolic blood pressure was controlled by anesthesiology. The fluid pressure was kept at an average of 40 mmHg with the arthroscopic pump. Rotator cuff repair was performed by using a Smith&Nephew (London, UK) TWINFIX® suture anchor with an ULTRABRAID® suture or a FOOTPRINT PK® suture anchor. Following RCR, the anterior aspect of the coracoacromial ligament was released in 76 patients, and subacromial decompression was performed in 113 patients.

Statistical Analysis

The mean, standard deviation, frequency and percentage values were used in the descriptive statistics. Kolmogorov-Smirnov test was used to evaluate distribution of the data. Independent samples t test was used to compare quantitative data, and Pearson chi-square or Fisher's exact test was used to compare qualitative data between groups. Pre till postoperative changes were evaluated by using two-way repeated measures analysis of variance. All statistical analyzes were performed by using SPSS v.24 (SPSS Inc., Armonk, NY) statistical package, and p values <0.05 were considered as statistically significant.

RESULTS

There were 98 (52 female/46 male) patients in the ER group and 104 (58 female/46 male) patients in the DR group. Follow-up time was 14.85±4.88 months in ER group, and 13.14±4.25 months in DR group (p=0.006). The duration of the symptoms for the patients in ER group was 6.44±2.51 months and 84 (85.7%) patients had chronic tears. In 60 (61.2%) patients, the tear was on the dominant side. The mean duration of symptoms in DR group was 5.86±2.31 months. 85 (81.7%) patients' tears in this group were chronic and 57 (54.8%) of them were on the dominant side. No significant difference was found in both groups in terms of age, gender distributions, working status, surgical side, status of dominance in surgical side, duration of symptoms, tear chronicity or tear size (Table 1).

Mean preoperative ASES, CM and VAS values were 41.76±7.26, 41.36±5.91 and 6.33±0.89 in ER group, respectively, while they were 43.09±7.02, 41.72±5.13 and 6.06±0.78 in DR group. Mean postoperative ASES, CM and VAS values were 77.62±11.77, 76.92±11.82 and 2.03±1.46 in ER group, respectively, while they were 77.63±12.22, 77.48±11.64 and 1.99±1.31 in DR group. There were statistically significant improvements in ASES, CM, VAS, SF-36 scores in both groups. However, there was no significant difference between two groups in terms of preoperative and postoperative clinical scores (Table 2).

Table 1. Demographics and disease-specific characteristics

	ER Group (n=98)	DR Group (n=104)	p
Age (years), mean±SD	62.72±6.36	62.88±6.38	0.859
Gender, n (%)			
Female	52 (53.1)	58 (55.8)	0.699
Male	46 (46.9)	46 (44.2)	
Working status, n (%)			
No/Housewife	20 (20.4)	19 (18.3)	0.844
Officer	25 (25.5)	22 (21.2)	
Worker	22 (22.4)	28 (26.9)	
Retired	31 (31.6)	35 (33.7)	
Injured side, n (%)			
Right	53 (54.1)	48 (46.2)	0.260
Left	45 (45.9)	56 (53.8)	
Injured side, n (%)			
Dominant	60 (61.2)	57 (54.8)	0.356
Non-dominant	38 (38.8)	47 (45.2)	
Tear chronicity, n (%)			
Acute	14 (14.3)	19 (18.3)	0.444
Chronic	84 (85.7)	85 (81.7)	
Tear size, n (%)			
Small	24 (24.5)	30 (28.8)	0.770
Medium	54 (55.1)	53 (51)	
Large	20 (20.4)	21 (20.2)	
Duration of symptoms (months), mean±SD	6.44±2.51	5.86±2.31	0.087
Follow-up time (months), mean±SD	14.85±4.88	13.14±4.25	**0.006**

ER: early rehabilitation, DR: delayed rehabilitation, SD: standard deviation

Table 2. Comparison of the clinical scores within and between the groups

	ER Group (n=98)		DR Group (n=104)		p_W	p_B
	Preoperative	Postoperative	Preoperative	Postoperative		
ASES score	41.76±7.26	77.62±11.77	43.09±7.02	77.63±12.22	**<0.001**	0.611
CM score	41.36±5.91	76.92±11.82	41.72±5.13	77.48±11.64	**<0.001**	0.644
VAS	6.33±0.89	2.03±1.46	6.06±0.78	1.99±1.31	**<0.001**	0.246
SF-36						
Physical functioning	58.92±8.13	83.87±11.54	60.19±9.00	85.62±9.96	**<0.001**	0.584
Role limitations due to physical health	16.58±15.20	73.72±15.80	21.44±18.32	76.44±15.11	**<0.001**	0.496
Role limitations due to emotional problems	40.15±27.09	86.86±19.18	39.08±25.62	86.95±17.85	**<0.001**	0.747
Energy/fatigue	29.94±10.7	75.00±13.86	30.67±11.27	75.52±14.26	**<0.001**	0.766
Emotional well-being	32.73±12.21	66.36±17.38	34.69±11.41	68.73±17.14	**<0.001**	0.547
Social functioning	27.99±9.63	72.96±13.37	28.81±10.82	73.55±12.95	**<0.001**	0.909
Pain	22.75±9.98	73.96±21.50	23.85±9.72	75.68±19.40	**<0.001**	0.502
General health	28.46±12.56	75.66±15.60	28.99±13.71	76.34±15.90	**<0.001**	0.623
Health change	19.13±15.14	80.36±18.73	20.67±17.57	81.25±17.33	**<0.001**	0.838

ER: early rehabilitation, DR: delayed rehabilitation, ASES: American Shoulder and Elbow Surgeons, CM: Constant Murley, VAS: Visual analogue scale, SF-36: 36-item Short Form Health Survey, p_W: within group p value, p_B: between group p value, descriptive statistics were given as mean±standart deviation

Complications

Superficial infection occurred in 5 (2.4%) of all patients. Of these, 2 (2.04%) were in ER group and 3 (2.94%) were in DR group (p=0.999). All patients recovered with systemic antibiotics and surgical debridement was not required for any of them. Frozen shoulder occurred in 16 patients (7.9%) postoperatively; 6 (6.1%) of them were in ER group and 10 (9.6%) of them were in DR group (p=0.358). Re-tears occurred in 13 (6.4%) patients; 8 (8.2%) of them were observed in ER group and 5 (4.8%) of them were in DR group (p=0.331). The rate of re-tear increased as the initial size of the tear increased. While no re-tear was observed in the small tear group, it was observed in 2 (1.9%) of medium tear and 11 (26.8%) of large tear groups (Table 3).

Table 3. Comparison of complications between the groups

	ER Group (n=98)	DR Group (n=104)	p
Superficial infection	2 (2.04)	3 (2.94)	0.999
Frozen shoulder, n (%)	6 (6.1)	10 (9.6)	0.358
Re-rupture, n (%)	8 (8.2)	5 (4.8)	0.331
Tear size, n (%)			
Small (n=54)	0 (0.0)	0 (0.0)	
Medium (n=107)	1 (0.9)	1 (0.9)	
Large (n=41)	7 (17.1)	4 (9.8)	

ER: early rehabilitation, DR: delayed rehabilitation

DISCUSSION

The most important finding obtained in this study is that no significant clinical and functional difference was found between ER and DR in mid-term follow-up after RCR.

Currently, arthroscopic repair is widely used in the treatment of the rotator cuff tears. Physical rehabilitation after repair has a very important role in the recovery of patients (1). However, the timing of postoperative rehabilitation in these patients is still a matter of debate (8,11). In many studies, any important advantage or disadvantage of early or late-onset rehabilitation after arthroscopic RCR compared to each other could not be revealed (12-15). However, there are studies that prefers the ER protocol because it provides better range of motion, causes less shoulder stiffness and muscle atrophy, increases patient satisfaction and facilitates return to daily life (16,17). It was stated that patients who received a supervised early exercise program after RCR would benefit more in terms of shoulder function, pain reduction and range of motion compared to those who received a standard exercise program (18). In a review, it was reported that good clinical and functional results and range of motion were obtained in the early postoperative period with ER protocols, by the way similar results were achieved between 3-6 months with DR protocols (19). On the other hand, Longo et al. (20) achieved better clinical and functional results in patients with limited rehabilitation compared to patients who received early aggressive rehabilitation. Also Koh et al. (12) demonstrated that DR provides similar clinical outcomes without an increase in postoperative structural failure. The DR protocol after RCR is preferred due to the concern that early movement may adversely affect tendon healing from micro-movement and cavity formation in the repair area. However, delayed motion can increase the risk of joint stiffness after surgery and potentially delay the return of shoulder function (9). Some studies have indicated that the ER protocol after arthroscopic RCR increases the risk of re-tear, especially in patients with large tears between 3 cm and 5 cm (21,22). On the contrary, some authors found that there was no difference in terms of re-tear between ER and DR applied in patients with small and moderate size tears (5,23,24). In the review of Bakti et al. (25), it was stated that DR after RCR will reduce the risk of re-tear and provide improvement in subjective outcome measures. It has been emphasized that the stiffness that may be encountered in DR is insignificant compared to the clinical difficulties in re-tear. Unrelated to physical therapy after surgery, shoulder activity in the first 2 years after surgery was also associated with a higher risk of re-tear in patients who underwent RCR (26). Beneficial effects of a period of immobilization on the structural quality and strength of the healing tissue have been provided by animal model studies and that it is not detrimental to ROM (27,28). Kovacevic et al. (29) found that by reducing the load, the quality of tendon-bone healing increased. It was reported that immobilization plays a role in allowing healing and the natural phases of inflammation and proliferation (30). However, the healing process differs between animal models and human subjects. While the rotator cuff muscles of humans do not contract during passive ROM, these muscles contract unless the animals are under anesthesia. The rehabilitation initiation time after rotator cuff surgery is an important point in determining which benefits and side effects may occur. These contradictory findings lead to a lack of definitive conclusions as to whether early-onset rehabilitation protocols are harmful or beneficial after RCR (3).

In present study, mean follow-up time was 13.97 months. The rate of re-tear during this period was 6.4%. This rate was significantly lower than the 20% to 90% re-rupture rate reported in previous studies (31). The data obtained in this study showed that re-tears may occur in both rehabilitation protocols in patients with large tears. Due to the absence of MRI or any other radiological evaluation after arthroscopic repair, the actual incidence of re-tear may be higher than detected. Asymptomatic partial re-tears might have been missed. With postoperative control MRI and longer follow-up time, higher rates of re-tear may be detected. Also, there was no difference between the groups in terms of other postoperative complications such as superficial infection and frozen shoulder. Most studies showed comparable outcome scores, quite a few studies used a validated quality-of-life score. Mazzocca et al. (32) reported that there was a clinically significant difference between the early and late movement groups in their study using the WORC score. We used SF-36 for this purpose. There was no significant difference between ER and DR groups in terms of the preoperative ASES, CM, VAS and in all sub-scales of the SF-36 questionnaire. Similarly, there was no significant difference between the two groups in postoperative ASES, BM, VAS and all subscales of the SF-36 questionnaire. The improvements in all clinical scores in both groups were similar, suggesting that both rehabilitation protocols had similar effects on patient-reported outcomes. Risk stratification can be applied to decide the best rehabilitation option for a particular patient.

Given that DR does not pose an additional risk for joint stiffness and has a lower potential for structural deterioration, it may be preferable for large, full-thickness tears for a more successful outcome. On the other hand, ER may be an option in a selected patient population with small tears with anticipation of early return to work and early return to daily life.

To eliminate the risk of bias in the study, the presence of tears were confirmed on MRI images of each patient participating in the study by an independent blinded researcher. The adequacy of the repairs was confirmed by surgical video recordings. Then, clinical and functional scores of all patients were made by a blinded investigator. We think that this study model and the number of patients included in the study increase the power of the study.

The limitations of this study are that the patients did not have pre- and postoperative range of motion measurements and had a relatively short follow-up period. Apart from this, postoperative MRI scanning was not performed to determine subclinical RCR failure. Longer follow-up, detailed post-rehabilitation ROM measurements, and detailed radiological examinations could reveal more information about the benefits and side effects of each protocol.

CONCLUSION

At a mean follow-up of 13 months, early and delayed onset postoperative rehabilitation programs are associated with similar functional and quality of life outcomes and complication rates. Therefore, delayed rehabilitation can be preferred primarily in patients with large tears. Early rehabilitation can be selected for a limited patient population with small tears with anticipation of early return to work and daily life.

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Suicidal Major Depression in a Healthcare Worker Associated with the COVID-19 Pandemic: A Case Report

Bir Sağlık Çalışanında COVID-19 Pandemisi ile İlişkili İntihar Düşüncesinin Eşlik Ettiği Majör Depresyon: Olgu Sunumu

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ABSTRACT

Novel coronavirus disease (COVID-19) pandemic has been affecting the whole world since December 2019. During the pandemic, healthcare professionals are at the forefront of the fight against COVID-19, and many healthcare workers died during this fight. Healthcare workers, who are at the forefront of the pandemic, are under intense mental and physical stress along this period. This disease makes increase psychiatric symptoms for general population and especially in health professionals at pandemic conditions. In particular, the symptoms of burnout, depression, and anxiety have rised significantly. With this case report, we present a physician who had symptoms that started during the COVID-19 pandemic, had depression accompanied by suicidal thoughts, and went into complete remission with electroconvulsive therapy. The mental health effects of healthcare workers who have had COVID-19 are discussed within the frame of this case report.
Keywords: Heathcare workers; COVID-19; pandemic; depression; suicidal thoughts.

ÖZ

Yeni koronavirüs hastalığı (COVID-19) pandemisi Aralık 2019'dan bu yana tüm dünyayı etkilemektedir. Pandemi sürecinde sağlık çalışanları COVID-19 ile mücadelede en önde savaşmaktadır ve birçok sağlık çalışanı bu mücadele sırasında hayatını kaybetmiştir. Pandeminin ön saflarında yer alan sağlık çalışanları bu dönemde yoğun ruhsal ve fiziksel stres altındadır. Pandemi dönemlerinde genel popülasyonda ve özellikle sağlık çalışanlarında psikiyatrik belirtiler ve hastalıklar artmaktadır. Özellikle tükenmişlik, depresyon ve anksiyete belirtileri önemli ölçüde artar. Bu olgu sunumunda, COVID-19 pandemisi sırasında semptomları başlayan, intihar düşüncelerinin eşlik ettiği depresyonu olan ve elektrokonvülsif terapi ile tam remisyona giren bir hekim sunulmaktadır. COVID-19 pandemisinin sağlık çalışanlarının ruh sağlığı üzerindeki etkileri bu olgu sunumu çerçevesinde tartışılmaktadır.
Anahtar kelimeler: Sağlık çalışanları; COVID-19; pandemi; depresyon; intihar düşüncesi.

INTRODUCTION

In December 2019, a severe acute respiratory syndrome (SARS) outbreak caused by a new coronavirus started in Wuhan, China. On March 11, 2020, the World Health Organization declared coronavirus disease 2019 (COVID-19) as a pandemic (1). Healthcare workers, who had been on the front line of the fight against the pandemic since the first day, are exposed to intense physical and mental stress, mainly due to heavy workload and the fear of contamination. Experiences and observations from previous pandemics show that healthcare workers are more susceptible to psychiatric disorders in such situations (2). Recent studies published shortly after the onset of the COVID-19 pandemic similarly reveal that healthcare workers' mental health is negatively affected (3). The risk of suicide probability increases with the long-term effects of the pandemic on the general population. Especially societies with significant social and economic losses are considered more risky in this regard. That

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situation, which is defined as "suicide storm", which may increase after the pandemic, causes serious concerns (4). Throughout the pandemic period, an increase in psychiatric diseases and suicidal behavior can be observed. Healthcare workers and the elderly, who are among the particularly vulnerable groups, are above all at risk for suicide (5). Researches are commonly screen studies of mental symptoms, and we do not yet have definitive data on the prevalence of psychiatric morbidity associated with the pandemic. Assuming that the epidemic continues at the same pace, these data will be completed is also uncertain. In this case report, we present a case of suicidal depression in a physician whose symptoms started soon after the pandemic outbreak and who recovered after electroconvulsive therapy (ECT) and aim to discuss the impact of the COVID-19 pandemic on the mental health of health workers.

CASE REPORT

A 62-year-old male patient works as an anesthesiologist at a private hospital. The patient admitted to the psychiatric outpatient clinic with suicidal thoughts, anhedonia, fear of contracting COVID-19, and fear of transmitting COVID-19 to other people. The patient had no previous psychiatric illness history until three months ago, just when the COVID-19 was officially announced in Turkey. After putting a patient with an upper respiratory tract infection to sleep, he started to experience similar symptoms, which led him to worry that he was infected with the COVID-19. Since an antiviral treatment for his nasopharyngitis did not improve his symptoms, the patient underwent a negative polymerase chain reaction (PCR) test for COVID-19. However, he was still preoccupied with worries about being infected, which then turned to a growing fear of death. Fearing that he might infect other people, he isolated himself from everyone, including his wife. Simultaneously, the patient started to feel depressed and was even intolerant of his beloved granddaughter's presence. Things he used to enjoy were a source of boredom partly because he was easily distracted from what he was doing. Reading news and articles about COVID-19 was almost his only daily activity. He did not eat much, but this was mostly due to abdominal pain and increased intestinal motility, especially after eating. However, endoscopic and colonoscopic evaluations revealed no pathology. He had difficulty falling asleep; thus, he started taking alprazolam 0.5 mg/day, which was ineffective for his abdominal pain kept him awake all night. The patient began to feel useless and thought that it would be better to die. Occasionally, these thoughts would intensify to such an extent that he made suicide plans. There was no suicide attempt. The patient had a plan to suicide by taking anesthetic agents.

The patient was born in a small Aegean town to a low-income family with three children. During times of childhood, he used to take on responsibilities incomparable to his young age. His academic life has always been a success story, and unlike his other two siblings, he left his hometown to continue his university education. His mother was a disciplined and overprotective person who kept the patient and his siblings under control, which was to be passed later to the patient as he grew old. It can be said that the over controlling attitude of the mother leads to a perfectionist personality in the patient. Perfectionism may contribute to the development of depressive symptoms and suicidal ideation by interacting with the stress of success. He described his siblings as always depressive personalities, and his sister also had COVID-19 anxiety similar to the patient.

He had a meticulous, controlling but also cheerful and extrovert premorbid personality. He was loved and trusted by his colleagues and thought himself as a good parent to his children. The patient, who had hypertension and subclinical hyperthyroidism, had no history of addiction.

At first glance, his overuse of protective equipment against COVID-19 was notable. He felt worthless and suicidal and was excessively preoccupied with COVID-19 transmission. In the mental state examination, his self-care was bad, and he had a depressed mood. His thought structure was normal and his thought content had intense suicidal thoughts. He did not have any psychotic symptoms. Affect was depressed. He had mild psychomotor retardation. The patient was diagnosed with major depression according to DSM-5 diagnostic criteria. Detailed blood tests and brain imaging also revealed no pathology. His Hamilton depression (HAM-D) rating score was 41 points, whereas he got 12 points from the Beck scale for suicide ideation (BSSI). Since he was severely depressed with intense suicidal thoughts, the patient was hospitalized. The patient had a serious suicide risk and therefore ECT was chosen primarily. The patient was discharged on the 4th day of hospitalization at his own request and the request of his family. He continued to receive ECT. He received ten sessions of ECT, at the end of which he was in complete remission. Every other day, 3 times a week and bilateral ECT protocol was applied. He was put on a daily dose of 100 mg oral sertraline. The patient was followed up in the outpatient clinic monthly after discharge. At the 6th month follow-up, the patient's HAM-D score was 2 and BSSI score was 0. The patient had no complaints.

DISCUSSION

In this case report, we presented the case of a healthcare worker who suffered from a severe suicidal depressive episode, which we thought to be precipitated by the COVID 19 pandemic. After a suspicious contact in the first days of the COVID-19 pandemic, fear of being infected turned to a severe form of health anxiety in the patient who had no previous psychiatric history, with ambiguous somatic complaints such as abdominal pain. Soon, a full-blown depressive episode manifested itself and led the patient to seek for professional help. Although it is impossible to establish a definite relationship, the triggering factor here seems to be the patient's fear of being infected with the coronavirus. Indeed, a sudden and life-threatening epidemic can place enormous strain on healthcare professionals because of factors such as increased workload, fear of contagion, and the need to make ethically difficult decisions (6,7). It is reported that during the COVID-19 pandemic, most healthcare professional experienced mild symptoms of both depression and anxiety and that the prevalence of moderate-to-severe symptoms was low (8). However, in our case, the clinical picture went far beyond mild symptoms, and ended up in a suicidal depression.

Physicians have a higher risk of suicide compared to the normal population. Factors such as psychological pressure and an intense fear of death during the epidemic might have increased the risk (9). The case of Dr. Lorna Breen

was both a painful and alarming example in this regard. She was the medical director of the emergency department in a community hospital in New York, where she contracted coronavirus from one of her patients and ended her life in April 2020 (10). Our patient and Dr. Breen show us the extent of psychiatric risk the healthcare workers might be facing during the COVID-19 pandemic, which is still full speed.

Our patient had several risk factors that might have predisposed him to depression. One such factor is his having comorbid physical illnesses, namely essential hypertension, and subclinical hyperthyroidism. Indeed comorbid physical illness was one of the reported risk factors for psychiatric morbidity among healthcare workers during the COVID-19 outbreak (11). High levels of social media exposure with pandemic content are also reported to be positively associated with mental health problems during the pandemic. In this regard, the patient's extensive internet use to get information about COVID-19 might have been a permanent source of anxiety and provoked his depression (12).

The case we presented here is a severe depression that psychiatric professionals may often encounter in their practice, and also full remission of symptoms is not exceptional, given the high efficiency of ECT. However, almost all experts agree that the COVID-19 pandemic will not end any time soon. This means that healthcare workers will continue to be in the field and will be even more mentally vulnerable (13).

CONCLUSION

This is a warning case which shows the extent of psychiatric morbidity in healthcare workers associated with the pandemic. Questioning suicidal thoughts in evaluating the mental symptoms of healthcare workers who have taken an active role in the pandemic period can be life-saving. Thus healthcare professionals should be closely monitored in terms of early diagnosis and treatment of psychiatric disorders, and protective measures should be taken. Studies with large samples evaluating the suicide risk of healthcare workers are needed during the pandemic period.

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Can Prevention of Erection with Sulpride be a Way to Increase Success in Hypospadias Surgery? A Case Report

Sülprid ile Ereksiyonun Önlenmesi Hipospadias Cerrahisinde Başarıyı Artırmanın Bir Yolu Olabilir mi? Bir Olgu Sunumu

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ABSTRACT

Hypospadias is treated surgically and different methods can useable for this way. Due to involuntary erections during adolescence and later, neourethra seems at risk until wound healing is complete. Erections following penile surgery are painful and can affect the healing process negatively, because the stitches may not withstand a strong erection. Therefore, prevention of erection and management of pain are extremely important after the hypospadias surgery; especially in adolescents. Preventing erection may increase the chance of success from surgery. Short-term use of antipsychotics may be beneficial to prevent erection. In this case report, the use of sulpride in an eighteen-year-old patient after hypospadias repair and the effect of this treatment on the results of the surgery was presented.
Keywords: Sulpride; hypospadias; erection prevention; adolescent.

ÖZ

Hipospadias cerrahi olarak tedavi edilmekte ve cerrahide farklı yöntemler kullanılabilmektedir. Ergenlik döneminde ve daha sonra erişkinlikte meydana gelen istem dışı ereksiyonlar nedeniyle yeni oluşturulan üretra yara iyileşmesi tamamlanana kadar risk altındadır. Penis ameliyatı sonrası oluşan ereksiyonlar ağrılıdır ve dikişlerin dayanıklılığını azaltması nedeniyle iyileşme sürecini olumsuz şekilde etkileyebilir. Bu nedenle, özellikle ergenlik döneminde, hipospadias cerrahisinden sonra sertleşmenin önlenmesi ve ağrı yönetimi son derece önemlidir. Ereksiyonun önlenmesi ameliyattan elde edilecek başarı şansını artırabilir. Antipsikotiklerin kısa süreli kullanımı ereksiyonu önlemek için faydalı olabilir. Bu olgu sunumunda on sekiz yaşındaki bir hastada hipospadias onarımı sonrası sülprid kullanımı ve bu tedavinin ameliyatın sonuçlarına olan etkisi sunulmaktadır.
Anahtar kelimeler: Sülprid; hipospadias; ereksiyon önlenmesi; ergenlik.

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INTRODUCTION

Hypospadias is a congenital disease of the penis and urethra occurring when the distal urethra cannot complete its development normally and the urethral meatus can be observed anywhere between the perineum and the glans (1). Its incidence is reported as 1:300 (2). Hypospadias is not an isolated anomaly, however is associated with urological, sexual, physiological and psychiatric problems.

Hypospadias is treated surgically and different methods can be used. The aim of the surgery is to create a functional and aesthetically acceptable new urethra. The most common complications of the surgery are urethral stenosis and urethrocutaneous fistula (2-4).

Due to involuntary erections during adolescence and later, neourethra is at risk until wound healing is completed. Erections following penile surgery can be painful

and affect the healing process negatively. Therefore, prevention of erection and management of pain are extremely important issues after the repair of hypospadias; especially in adolescents (2-4). Preventing erection may increase the chance of success from surgery.

Artificial erection may be required for fixation of the cordial during the operation, but erection which will develop during and after the operation will both increase risks of bleeding and the reopening of the suture line.

In this case report, prevention of erection with sulpride after surgery in an 18-year-old hypospadias case will be presented.

CASE REPORT

An eighteen year old male patient who has been operated for acute appendicitis and right inguinal hernia previously has also experienced two unsuccessful operations due to mid penile hypospadias. During early postoperative period, the sutures in the neourethra completely opened due to erections and the operation site was degenerated.

The patient had undergone psychiatric examination before surgery and there wasn't any findings of psychopathology. A motivational interview was held to resolve the patient's uncertainties over his reservations and fears about the surgery. The patient was informed about psychotropic drugs to be given in order to prevent postoperative erection. Hypospadias operation has been successfully completed with the Warren Snodgrass technique.

The patient started to take sulpride, a second generation antipsychotic, at a dose of 50 milligrams per day (mg/d) right after the operation. During the follow-up, he had two erections, once in the post-op first day and the second one in fourth day. Because the nocturnal penile tumescence test was not available in our center, the number of erections was evaluated taking into account the statements of the ward nurse, patient's relatives and the patient. Erection quality was evaluated based on the patient's statement. The patient was asked to give 10 points for the strongest erection and 1 point for the weakest. He gave scores to both erections' qualities between 4 and 5. It was evaluated that the duration of erections lasted a maximum of one minute. Sulpiride was continued for 15 days postoperatively. The urinary catheter was removed ten days after the surgery. It was observed that the patient showed a complete recovery on the surgical site. No complications were detected in the postoperative twentieth day and after second month controls. No urethral stricture or fistula was detected. The patient did not describe any voiding or sexual dysfunction. When EMG assisted uroflowmetry applied, it was revealed that voiding calibration and curve were both normal and there seemed to be no residual urine in the bladder.

DISCUSSION

Hypospadias is an extremely sensitive condition for children and families. It affects both the patient and their relatives not only physically but also psychologically. It is aimed to prevent physical and psychological problems that may occur by diagnosing and treating the disease in early childhood. Both physical and psychological problems can be experienced especially in patients who are operated after puberty. In this case, as well, hypospadias negatively affected adolescent identity acquisition. However, previous negative surgery attempts caused the patient to have prejudices that he could not improve. This influence has been evaluated as a psychological effect in accordance with the literature (2). The therapeutic interview was conducted with the patient regarding his concerns about the surgery and postoperative expectations.

One of the factors that worries the physician after the surgery is the pain experienced by the patient. To our knowledge, acute pain is a phenomenon that can be strongly triggered by emotional elements such as fear, anxiety, or depression and previous pain experience (5). In this context, medical treatments that will relieve anxiety and calm the patient should also be considered. In fact, it will be appropriate to provide evaluation by a psychiatrist in terms of ruling out any psychiatric disorders before surgery. If necessary, the management or treatment of anxiety should be planned.

There are many studies about the follow-up of post-operative erectile and sexual functions (6,7). Some factors such as the surgery technique, development of complications and wound healing were found to be effective on erectile function (8,9). Generally, erectile dysfunction is more common in these patients than normal population (10). Erectile function and one's sex life depend not only on a functional penis and hormones, but also on psychosocial factors (11). For this reason, the future sexual experiences of the individual may be negatively affected after an unsuccessful operation.

Due to involuntary erections, it becomes difficult to maintain the suture line and control bleeding in the early postoperative period. For this reason, it is important to secure the suture line and reduce bleeding by preventing erection during the first ten days when the incision is expected to heal. There are very few studies related to the prevention of involuntary erection after the operation. These few studies are mostly related to anesthetic techniques and drugs that have been shown to prevent postoperative erection (12,13).

In general, many different drugs are known to inhibit sexual functions. Antidepressants, antipsychotics, anxiolytics and mood stabilizers particularly act on sexual function through various mechanisms in the central nervous system. Postoperative use of psychotropic drugs can be considered in this context.

As defined by Kaplan (14), sexual activity consists of three phases: the desire, the arousal, and the orgasm. Drugs affect these phases in different sizes and ways. According to Stahl (15), neurotransmitters show different mechanisms of action on the three phases of the human sexual response cycle. In the first phase (desire), dopamine (DA), melanocortin, testosterone and estrogen show positive effects, while prolactin and serotonin (5HT) have a negative effect. The second stage, arousal, is associated with erection in men and lubrication of vagina in women. Various neurotransmitters facilitate sexual arousal, including nitric oxide (NO), norepinephrine (NE), melanocortin, testosterone, estrogen, acetylcholine (Ach), and dopamine. While dopamine and nitric oxide have a weak positive effect on the third stage (orgasm) -which is associated with ejaculation in men- this stage is inhibited by serotonin and facilitated by norepinephrine (15).

The pharmacodynamic effects of different antidepressants varies, suggesting that multiple receptor systems play a

role in the etiology of sexual dysfunction. In general, the inhibitory effect on erection occurs particularly through the activation of serotonine 5HT2 receptors (15). Other inhibitory mechanisms of erection and libido includes the effects via blockade of noradrenergic α-1, anticholinergic, antihistaminergic, anitidopaminergic receptors and increased prolactin levels (15-17). An inhibitory effect of nitric oxide synthase has also been hypothesized (18). Possible mechanisms of psychotropic drug induced sexual dysfunction are as follow, pharmacological effect (19,20):

- Cholinergic blockade
- α1-adrenergic blockade
- Hyperprolactinemia
- Inhibition of serotonin reuptake (Indirect stimulation of 5-HT2 receptors)
- Antihistaminergic effect
- Antidopaminergic effect in mesolimbic areas
- Antidopaminergic and antinoradrenergic effect in medulla spinalis
- Decrease in NO as a result of NO synthetase inhibition
- Increase in opioid levels
- Increase in cortisol levels

Sexual dysfunction rates in meta-analysis studies are as follows: for moclobemide and agomelatine 4%, bupropion 10%, mirtazapine 24%, fluvoxamine 26%, escitalopram 37%, duloxetine 42%, imipramine 44%, fluoxetine 70%, paroxetine 71%, citalopram 79%, venlafaxine and sertraline 80% (21). It should be kept in mind that priapism may occur as a rare side effect of sertraline (22).

Adverse reactions of sexual function caused by antipsychotics are generally in inhibitory nature and depend on their effects on all phases of the sexual response cycle. These effects include decreased sexual desire (libido), difficulty in erection, orgasm and sexual satisfaction (23). We can say that all antipsychotics are associated with decreased sexual desire due to their antidopaminergic nature. In agents that show partial agonism to dopamine such as aripiprazole and agents with atypical antipsychotic properties, sexual reluctance will not be as much as the negative symptoms we expect to see as a side effect in typical antipsychotics (24).

However, most antipsychotic agents with typical properties (i.e. chlorpromazine, pimozide, thioridazine, sulpiride) cause erectile dysfunction. Another point that should not be forgotten is that all antipsychotics can cause priapism. In some case reports, it was reported that priapism can also be observed with aripiprazole, clozapine, quetiapine, risperidone and ziprasidone (25). However, it appears as a more frequently expected side effect with agents such as pimozide (26).

In a study conducted by Dossenbach et al. (27) with 3838 patients, it was observed that sexual problems were common among all patients taking antipsychotics, but kind of the drug used did not make a significant difference.

Sulpride, like a typical antipsychotic, is known to act with postsynaptic D2 blockade at high doses. In this case, it was assumed that it prevented erection via antidopaminergic pathways. While high doses cause behavioral changes that predict antipsychotic effect in laboratory tests, it has been shown that they do not cause catalepsy, in other words, they have a low tendency to cause extrapyramidal side effects (28).

CONCLUSION

Although our patient had been operated twice with the same surgical technique, the suture line was completely opened in the early postoperative period due to involuntary erections resulting in an unsuccessful outcome. In the last operation, the involuntary erections had been reduced with medication, so the incision line could heal completely and the neourethra was formed without any complications.

The use of sulpride, especially after late operations such as post pubertal period, is a method that can increase the success rate of the surgery. Further studies related to this subject are needed.

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AUTHOR GUIDELINES

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ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including last table.
- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.


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