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Message from the Editor-in-Chief

Message from the Editor-in-Chief,

Since February 6, 2023, we have been facing the consequences of a devastating earthquake covering a large region in Turkey. We are deeply sad about our losses and those who lost their people. From the first moment of the deadly destruction of all time in Turkey, our colleagues touched the lives of many injured people in the region. Many thanks to those who engaged so far. The professional activities of many scientists in the region will also be affected in the future, but we will be supporting them in every way.

In the current issue of the GMJ, we have interesting original articles, review articles and case reports. As the journal's publishing team, we tried to cover a broad field from different disciplines.

With this opportunity, I would like to express my gratitude to all submitting authors, reviewers, and editors for their contributions.

M. Ali Gülçelik, M.D., Prof.
Editor-in-Chief

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Uptake of high-dose folic acid decreases cell viability and proliferation via JAK/STAT pathway in human prostate cancer cells

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Keywords: Prostate cancer, folic acid, cell viability, cell proliferation

ABSTRACT

Aims: Several studies demonstrated that folic acid (FA) supplementation had some effects on prostate cancer initiation. In this study, the effect of FA concentration was evaluated on proliferation and viability in prostate cancer cells (PCCs). Additionally, we also determined the genes dysregulated by the uptake of a certain amount of FA in prostate cancer.

Methods: Changes in cell viability and proliferation were analyzed in PCC for low-dose (Group-1; 1 µM, 10 µM, 100 µM) and high-dose (Group-2; 1 mM, 10 mM) FA concentrations by Trypan blue staining and MTT assay, respectively. mRNA expression level of *FOLR1, FOLR2 FOLR3, JACK1, STAT3, STAT5/A, STAT5/B, PIAS1, PTPN1*, and *SOCS1* were determined by quantitative real-time polymerase chain reaction.

Results: Cell viability and proliferation were significantly lower than healthy prostate epithelial cells in high-dose FA-treated PCCs. mRNA expressions of *FOLR1, JAK1*, and *STAT3* were significantly upregulated in high-dose FA-treated PCCs compared with the controls. There were no significant alterations in the expression of *FOLR2-3, STAT5A/5B, PIAS1*, and *PTPN1* genes, however, *SOCS1* mRNA expression was significantly lower than the controls.

Conclusions: Low-dose FA showed no effect on cell viability and proliferation, whereas viability and proliferation were decreased by the uptake of high-dose FA that was supposed to stimulate the mRNA expression of *FOLR1* in PCCs. Decreased *SOCS1* and increased *JAK1* and *STAT3* gene expressions implicate the dosage-dependent FA effect on JAK/STAT signaling pathway in prostate cancer.

Introduction

There are three types of folate receptors (FR1-adult form, FR2-fetal form and FR3) that transport folate via endocytosis and are activated by folic acid (FA) in the cell (1). These receptors are encoded by *FOLR1*, *FOLR2* and *FOLR3* genes, respectively (2). FA acts as a cofactor in DNA synthesis, repair, and methylation (3,4). Several reports have indicated that the lack of FA results in epigenetic changes, inefficient DNA synthesis and defective cell proliferation (5-7). Additionally, folate deficiency is involved in various diseases such as neural tube defects, anemia, atherosclerosis, and several types of cancers (6,8-11). However, there are conflicting data regarding the effect of FA in the development of tumors. Kuo et al. (6) pointed out that FA inhibits colon cancer cell proliferation. In contrast, Hansen et al. (12) reported that FA activates JAK/STAT pathway and induces dose-dependent proliferation of FR1-positive HeLa cells. Hyperactivation of STAT transcription factors with FA stimulates hematologic malignancies and solid tumors including breast, lung, liver, head and neck, and stomach cancers (13). Moreover, increased activation of the JAK/STAT signaling is associated with a worse prognosis, increased recurrence, and poor overall survival (13,14).

Prostate cancer is one of the most common causes of cancer deaths in men (3). This type of cancer originates in the gland cells of the prostate. The epithelial cells in the prostate (basal, luminal, and neuroendocrine types) are the possible targets for cancer initiation and progression (15). Several authors have reported that dietary supplementation with vitamins or minerals does not affect tumor formation in the prostate (16,17). However, epidemiological studies demonstrated that while low-dose FA

could prevent prostate cancer, high-dose FA increases the risk of malignancy (18).

How FA induces tumor formation is not currently known. Various signaling pathways that halt cell growth and metastasis in prostate cancer have been elucidated by in vitro and in vivo studies (19,20). However, more studies are needed in a molecular aspect to understand whether FA has any effect on prostate cancer progression.

In addition to FA, several studies have been done to understand the molecular basis of prostate cancer and to improve therapeutic strategies by identifying molecular targets. Among them, PIAS1 (protein inhibitors of activated STAT) is a target that modulates various signaling pathways. It has been reported that PIAS1 expression is elevated in metastatic prostate cancer, and it has a significant role in tumor progression (21). Moreover, it has been demonstrated that overexpression of PTPN1 (protein-tyrosine phosphatase 1B) leads to neuroendocrine differentiation of prostate cancer cells (PCCs) (22) and it was indicated as a promoter of prostatic cell growth (23). Furthermore, *SOCS1* (suppressor of cytokine signaling 1) has also been demonstrated as a dysregulated tumor suppressor gene in prostate cancer and could be used as a prognostic biomarker (24). Additionally, all these proteins regulate JAK/STAT signaling pathway (25-27). However, no evidence has been reported regarding the role of FA on these factors associated with prostate cancer progression.

In this study, the effects of variable FA concentrations on the proliferation and viability of PCCs were evaluated and compared with prostate epithelial cells (PECs) to understand whether FA acts on prostate cancer progression. Moreover, we determined the expression level of potential genes related to FA transport and the JAK/STAT pathway.

Methods

Cell culture

Healthy human PEC line (ATCC® PCS-440-010™) and human PCC line (ATCC® PC3-CRL-1435™) were cultured in RPMI-8226 1640 (Sigma-Aldrich-R8758) including 10% (v/v) FBS (BiochromAG, Germany) and 1% (v/v) gentamicin (Biological Industries, Israel) at 37 °C in 5% CO_2. Two groups [(Group-1; low-dose FA (1 µM, 10 µM, 100 µM) and Group-2; high-dose FA (1 mM, 10 mM)] were established for each cell line.

Preparation of FA solution

FA (Sigma) was diluted in RPMI-8226 1640 (Sigma-Aldrich-R8758) at different concentrations (1 µM, 10 µM, 100 µM, 1 mM, 10 Mm).

Cell viability assay

Trypan blue (Sigma) was used to assess cell viability. It was diluted at 0.8 mM in PBS and mixed with the cells in a 1:1 ratio. In this method, live (viable) and dead (non-viable) cells were counted on a hemocytometer (28).

MTT assay

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) is a colorimetric assay to measure the metabolic activity of cells, and it was used as an indicator of cell proliferation in the current study. Cell proliferation was estimated by MTT after $3x10^4$ cells in a culture flask were treated with variable concentrations of FA (1 µM, 10 µM, 100 µM, 1 mM, 10 mM) for 24 h.

RNA isolation and cDNA synthesis

Cells were harvested using trypsin/EDTA solution (Sigma Aldrich/T4049) after 24 h. Total RNA was extracted in each group via a High Pure RNA Isolation kit (Roche). cDNAs were synthesized using the RevertAid First Strand cDNA synthesis kit (ThermoFisher). The quality of cDNAs was checked with 2% agarose gel.

Quantitative real-time polymerase chain reaction

Selected genes [*FOLR1* (OMIM: 136430), *FOLR2* (OMIM: 136425), *FOLR3* (OMIM: 602469), *JAK1* (OMIM: 147795), *STAT3* (OMIM: 102582), *STAT5/A* (OMIM: 601511), *STAT5/B* (OMIM: 601512) *PIAS1* (OMIM: 603566), *PTPN1* (OMIM: 176885) and *SOCS1* (OMIM: 603597)] were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). *ACTB* (OMIM: 102630) was used as an internal control. All the forward and reverse primer sequences were retrieved from the PrimerBank database (https://pga.mgh.harvard.edu/primerbank/). Each qRT-PCR was performed in a 20 µL reaction by using LightCycler® 480 System. To get optimum results, qRT-PCR reactions were performed six times for each gene and condition. mRNA expression levels of *FOLR1-3*, *JAK1*, *STAT3*, *STAT5/A-B*, *PIAS1*, *PTPN1,* and *SOCS1* were determined in FA-treated cells compared to control (untreated PCCs). Mean values were obtained in all groups.

Statistical Analysis

The statistical significance was determined by two-tail Student's t-test in Microsoft Excel. $P<0.05$ was considered significant.

Results

Cell viability was over 85% (between 85-92%) in normal and PCCs in all groups (Figure 1). In Group 1, the cell viability ratio was 89-92%. Cell viability was significant only in 10 µM FA-treated PCCs in Group-1 (Figure 1A). However, cell viability was lower in high-dose FA-treated PCCs compared with PECs (Figure 1B). All other concentrations showed similar viability ratios compared to the no FA-treated group in each PCC line (Figure 1).

In the MTT assay, FA dosage for LD 50 (lethal dose 50) was determined as 10 mM. Additionally, all ratios on the MTT assay were statistically insignificant except 100 µM FA-treated PCC in

Group-1 (Figure 2A). However, cell proliferation was reduced in parallel with the elevation of FA concentration in PCCs compared to PECs (Figure 2B). Overall, cell viability and proliferation were not affected in Group 1, but they were significantly decreased in Group 2.

mRNA expression levels of selected genes were studied in PCCs and controls for 1 µM, 1 mM, and 10 mM FA concentrations by qRT-PCR (Figure 3). *FOLR1* expression was significantly upregulated in PCCs compared to PECs at all FA concentrations (Figure 3A). No significant difference was determined for *FOLR2* and *FOLR3* gene expression (Figure 3A). Additionally, *JAK1* mRNA expression was slightly but significantly increased compared to controls (Figure 3B). However, *STAT3* gene expression was significantly upregulated with increasing concentrations of FA (Figure 3B). No significant difference could be obtained for *STAT5/A* and *STAT5/B* gene expression (Figure 3B). Furthermore, *SOCS1*, an inhibitor of the JAK/STAT signaling pathway, *PTPN1*, an inducer of PCC growth and *PIAS1*, an inhibitor of the activated STAT pathway, were also studied at the mRNA level. Although no significant difference was obtained for *PTPN1* and *PIAS1* gene expression, mRNA expression of *SOCS1* was downregulated with increasing concentrations of FA (Figure 3C).

Discussion

In this study, low-dose FA showed no effect on cell viability and proliferation in PCCs. However, the uptake of high-dose FA decreased cell viability and proliferation in PCCs. Moreover, decreased *SOCS1* and increased *JAK1* and *STAT3* gene expressions indicated a dose-dependent effect of FA on the JAK/STAT signaling pathway in PCCs.

Folate is a substance naturally found in fruits and vegetables. The synthetic form of folate is FA. As a source of folate, FA is used in dietary supplements (29). It is also essential for cell growth and division (6). The low level of FA leads to defects in DNA replication, methylation, and repair in the cell (30,31). Furthermore, excessive FA levels can enhance tumor growth in the colon, polyomavirus middle-T-induced breast, and prostate

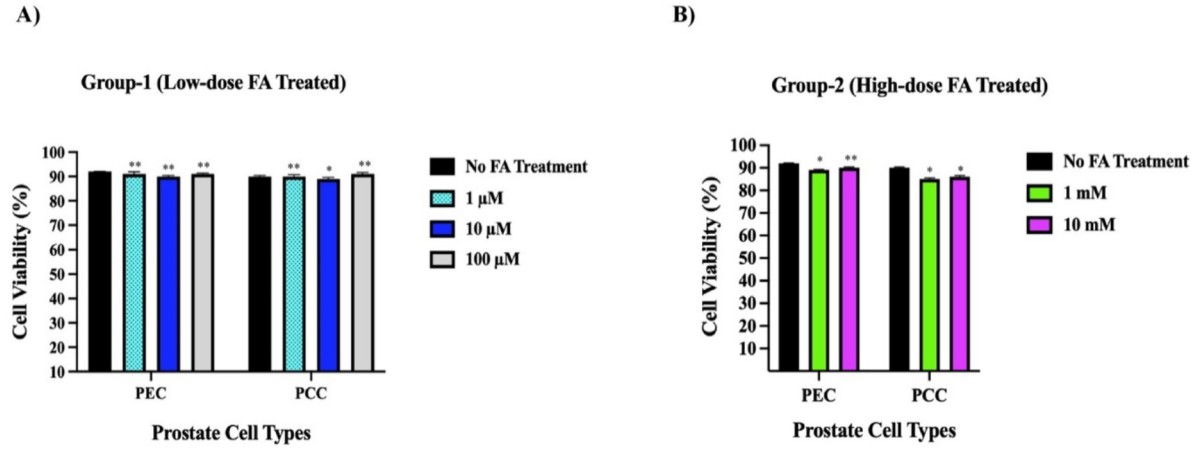

Figure 1. A, B) The effect of variable FA concentrations on the viability of prostate cancer cells
PEC: Prostate epithelial cell, PCC: Prostate cancer cell, FA: Folic acid *p<0.05, **p≥0.05. P values were calculated by comparing untreated and treated PCC

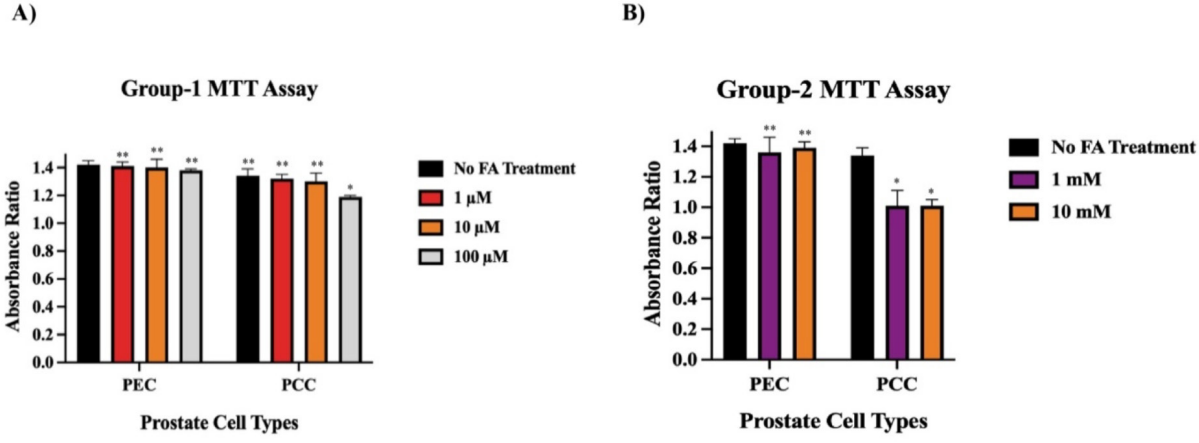

Figure 2. A, B) The results of the MTT assay to estimate prostate cell proliferation after FA treatments
PEC: Prostate epithelial cell, PCC: Prostate cancer cell, FA: Folic acid *p<0.05, **p≥0.05. P values were calculated by comparing untreated and treated PCCs

cancer (32-34). Despite these findings, a few epidemiological studies have shown that FA can prevent prostate cancer at low doses. Therefore, the use of excessive FA may increase the risk of malignancy in the prostate gland (35). The National Cancer Institute of the USA (NCI) describes folate as a protective agent against prostate cancer. Additionally, NCI has also declared FA as a risk factor for prostate cancer when taken at high levels as a supplement (29). Interestingly, for the first time, we demonstrated that excess cellular FA reduced cell proliferation and viability in PCCs.

FRs are overexpressed on the cell surface of solid tumor cells, including ovarian, kidney, lung, brain, endometrial, colorectal, pancreatic, gastric, prostate, testicular, bladder, head and neck, breast, and lung cancer (36). In our study, mRNA expression of *FOLR1* was increased in high-dose FA-treated PCCs compared to controls. In contrast, mRNA expressions of *FOLR2* and *FOLR3* were not significant compared with the controls. It can be suggested that the increasing level of FA concentration causes upregulation of *FOLR1* but not *FOLR2* and *FOLR3* in PCCs. Several factors regulate *FOLR1* expression, such as extracellular folate concentration, intracellular homocysteine concentration, and epigenetic and hormonal regulations (36). Here, we could demonstrate the positive effect of an increase in the level of FA concentration in promoting the expression of *FOLR1* mRNA. Recently, Jia et al. (37) reported that elevated core-fucosylation of FOLR1 can enhance the uptake of folate to the cell to induce epithelial-mesenchymal transition which triggers metastasis and invasion of hepatocellular carcinoma. This finding suggests that FA concentration might not be the only factor to enhance *FOLR1,* but also post-translational modifications of the protein should be considered and PEC-specific glycoproteomic-based studies should be performed.

We identified that mRNA expression of *JAK1* and *STAT3* was upregulated with increasing concentrations of FA compared to controls. However, downregulated *SOCS1* mRNA expression suggests the suppression of this tumor suppressor gene with the elevated level of FA in PCCs. The SOCS family of proteins are negative-feedback inhibitors of signaling induced by cytokines that act via the JAK/STAT pathway (38). Furthermore, SOCS1 acts as a negative regulator of STAT3 (39). These earlier findings confirm our study in which *SOCS1* is downregulated and *STAT3* is upregulated in high-dose FA-treated PCCs.

JAK/STAT pathway, a well-known intracellular signal chain, includes proteins that act on signal transduction. This signaling pathway affects several processes such as cell division, cell death, tumor formation and immunity (40). Additionally, JAK/STAT signaling can change the transcriptional regulation of genes that have a role in cell division (41). Excessive production of STAT proteins has been associated with cancer, in particular aggressive tumor types (42). Groner and von Manstein (43) reported that high-level STAT3 in a cell stimulated *BCL2* and *c-Myc* genes, which are involved in cell division. These findings are inconsistent with our FA-treated PCCs study in which *JAK1 and STAT3* mRNA expressions were upregulated, and cell proliferation was decreased. Furthermore, since SOCS has a role in the inhibition of JAK/STAT signaling (38,42), it can be suggested that decreased level of *SOCS1* is a critical factor to induce JAK/STAT signaling but an unknown mechanism decelerate prostate cancer progression. These findings may pave the way to investigate the effect of high-dose FA on *JAK1* and *STAT3* with further studies.

Study Limitations

The basic limitation of this study is the use of only one type of PCC. More detailed studies should be performed to reveal the precise effect of high-dose FA on prostate cancer.

Conclusion

In conclusion, we demonstrated increased mRNA expression levels of *FOLR1*, *JAK1,* and *STAT3* in PCCs compared to control depending on the increased uptake of FA. These findings emphasize that a higher level of cellular FA might decrease *SOCS1* expression and trigger JAK/STAT signaling by inducing JAK1 and STAT3. FA may play a dual role in prostate carcinogenesis and circulating FA at high concentration might

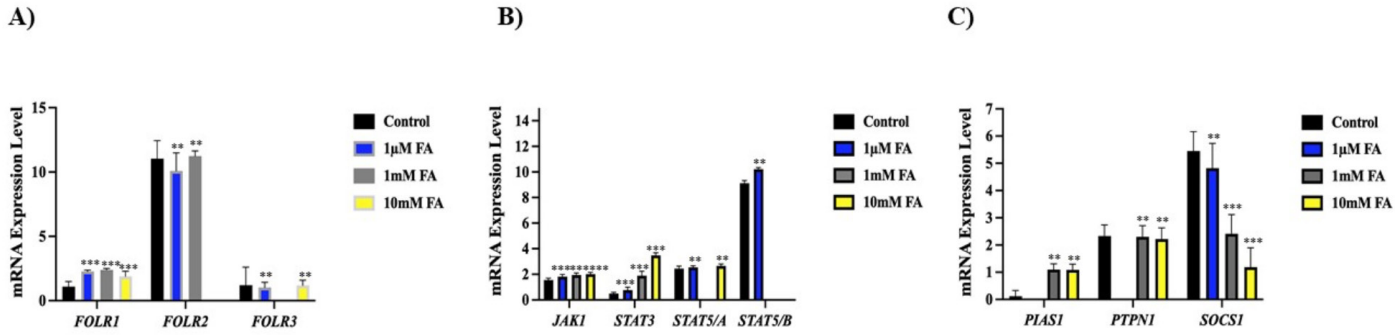

Figure 3. A, B) Gene expression analysis of selected genes
PCC: Prostate cancer cell, FA: Folic acid. Control is the untreated PCC group. **p≥0.05, ***p<0.005. P values were calculated by comparing untreated and treated PCCs. Blank columns represent no obtained data for related genes

enhance prostate cancer progression (44) or high-dose cellular FA may reduce the proliferation of PCCs via dysregulation of JAK/STAT signaling. However, new molecular targets should be identified to define the effect of a higher concentration of FA on the SOCS1/JAK/STAT pathway, which could clarify how PCC proliferation is inhibited.

Ethics

Ethics Committee Approval: This study was approved by the Gülhane Military Medical Academy Ethics Committee (17/11/2014- GATA Ethics Committee decision 2014-Session 46).

Informed Consent: Since it was a study based on a commercial cell line no consent form was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.G., Z.D.Ç., Concept: Ş.G., Z.D.Ç., Design: Ş.G., Z.D.Ç., Data Collection or Processing: Ş.G., Z.D.Ç., H.G., Analysis or Interpretation: Ş.G., Z.D.Ç., H.G., Literature Search: Ş.G., H.G., Writing: Ş.G., H.G.

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Evaluation of ischemic stroke patients associated with COVID-19

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Keywords: COVID-19, stroke, risk factors, comorbidities, D-dimer

ABSTRACT

Aims: Many neurological symptoms and complications, including stroke, may develop during Coronavirus disease-2019 (COVID-19). This study evaluated the risk, timing, prognosis, relationship between stroke and COVID-19, and treatment modalities of stroke due to COVID-19 by examining stroke patients with COVID-19.

Methods: This retrospective cross-sectional study included 12 patients aged ≥18 years with acute ischemic stroke and who were hospitalized with a confirmed diagnosis of COVID-19. In this study, demographic findings, clinical and stroke symptoms, stroke time, comorbid conditions that could pose a risk for stroke, inflammatory markers, D-dimer levels, imaging results, cardiologic evaluations, O_2 need, administered treatments, intensive care support, and prognosis were recorded retrospectively from patient files.

Results: Of the patients, 50% were male and 50% were female. The mean age was 70.6±9.3 (range, 55-84) years. The most common comorbid conditions were hypertension (58.3%) and diabetes mellitus (41.7%). Stroke developed at a median of 10.5 [interquartile range (IQR), 5-19.5] days after symptoms COVID-19. The mean National Institutes of Health Stroke Scale score was 7.8±4.7 (range, 3-18) (1-25). The average D-dimer and IL-6 levels of the patients were measured as 3.7 (IQR, 2.7-7.6) mg/L and 44.1±41.2 (range, 4.0-117) pg/mL, respectively. Most patients (66.7%) required oxygen during their hospitalization.

Conclusions: Patients with a stroke due to COVID-19 infection have several risk factors, particularly diabetes mellitus and hypertension. They had increased D-dimer levels, and most patients had severe disease. These results suggested that COVID-19 triggered or facilitated stroke rather than being an independent cause.

Introduction

On December 31st, 2019, the China Country Office for the World Health Organization (WHO) reported pneumonia cases with an unknown etiology in Wuhan, China. On January 7th, 2020, the agent was defined as a novel coronavirus that was not previously detected in humans (2019-nCoV). Later, the disease was named Coronavirus disease-2019 (COVID-19). Then, the WHO declared a pandemic on March 11th, 2020, upon detecting COVID-19 cases in 113 countries, excluding China and considering the spread and impact of the disease. COVID-19 causes many neurologic symptoms and signs, including stroke additional to the respiratory symptoms. Although the incidence of stroke is not known, the incidence was 0.9% in a study conducted in New York in patients with positive Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) tests (1-4). COVID-19 has a poor course in the elderly and those with hypertension, diabetes mellitus, heart disease, and obesity (5,6). Studies have reported an increased prevalence of neurological disorders in those with a severe COVID-19 infection. In a study conducted on 214 patients in Wuhan, although the overall incidence of stroke was 2.3%, it was 5.7% in patients with severe disease (7).

Several potential mechanisms have been identified for how COVID-19 increases the risk of stroke. These mechanisms are hypercoagulability evidenced by increased levels of D-dimer, cytokine storms indicative of excessive systemic inflammation or severe disease, and cardioembolism resulting from

virus-associated cardiac injury (8-10). The proinflammatory mechanisms during COVID-19 infection lead to increased clotting and disruption in vasomotor activity, which increases the risk for stroke (11). Further, recent studies have reported that COVID-19 acts on angiotensin-converting enzyme functional receptors, which have been implicated in severe cerebrovascular events, including stroke, among patients with risk factors for cerebrovascular diseases (CVD) (e.g., smoking or diabetes mellitus) (12-15). Despite the plethora of studies associating ischemic stroke with COVID-19, it has not yet been fully elucidated whether SARS-CoV-2 has a causal relationship with ischemic stroke.

This study evaluated the risk, timing, prognosis, relationship between stroke and COVID-19, and treatment modalities for stroke due to COVID-19 by examining stroke patients with COVID-19.

Methods

Study design and participants

In this retrospective cross-sectional study, the study population was patients with COVID-19 aged ≥18 years. This work was conducted at the University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Ankara, Türkiye. Medical records of the patients admitted to the COVID-19 clinic from September 2020 to January 2021 were evaluated. Of the patients, twelve with acute ischemic stroke and COVID-19 confirmed by laboratory tests (positive real-time reverse transcription-polymerase chain reaction results via nasopharyngeal swab) were included in the study. Patients with hemorrhagic stroke, cerebral venous thrombosis, negative COVID-19 test, suspected but not an imaging-confirmed acute stroke, and patients under 18 years of age were excluded.

COVID-19 infection was categorized according to the WHO classification (16). Mild COVID-19 was defined as respiratory symptoms without evidence of pneumonia or hypoxia, and moderate or severe infection was defined as the presence of clinical and radiological evidence of pneumonia. In moderate cases, SpO_2 ≥90% in room air, and one of the following was required to define severe disease: respiratory rate >30 breaths/min or SpO_2 <90% in room air (16). The severity of acute respiratory tract infection was defined according to oxygen demand and chest computed tomography (CT) scans. Low-flow oxygen therapy was defined as 1-6 $L.min^{-1}$ through a nasal cannula to keep a SpO_2 level of 90-92% (17). If the oxygen requirement was >6 L/min, it was accepted as a high oxygen demand. Body temperature ≥37.4 °C was considered fever. The stroke classification was based on the Trial of Org 1010172 in Acute Stroke Treatment (TOAST) classification (18).

The presence of new neurological symptoms that were confirmed by neuroimaging results was considered to be recurrent stroke.

Data collection

The study variables included demographic characteristics, COVID-19-related clinical and neurological symptoms, stroke time after the onset of COVID-19 symptoms, comorbid conditions associated with a higher risk of stroke [hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease (CAD), heart failure (HF), atrial fibrillation (AF), CVD, malignancy, and other conditions], inflammatory markers on admission [leucocyte, lymphocyte, neutrophil, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6)]; D-dimer level closest to the stroke time, imaging findings [brain CT/CT angiography (CTA) and/or color Doppler ultrasonography (CDUS) and/or digital subtraction angiography (DSA) and magnetic resonance imaging (MRI) and chest CT] and cardiologic evaluations (transthoracic echocardiography and electrocardiography results); O_2 requirement, treatment, intensive care support, and prognosis (19,20).

Ethics

The study was approved by the Turkish Republic Ministry of Health (protocol no: 2021-03-27T11-00-17) and the University of Health Sciences Türkiye, Medical Ethics Committee (date: 17.06.2021, no: 2021-273). This study was conducted in accordance with the principles of the revised Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version. 22.0 software (IBM Corp., Armonk, NY: USA). The normality of distribution was tested with Shapiro-Wilk for numerical data. In the descriptive analyses, frequencies, and percentages were used for categorical variables, and mean±standard deviation and median [interquartile range (IQR)] values were used for continuous variables according to the distribution.

Results

Demographic characteristics and clinical presentation

Of the 12 patients, 50% were female. The mean age was 70.6±9.3 years (range, 55-84 years) (Table 1). Eight (66.7%) patients were admitted with stroke, and four (33.3%) patients had a stroke during hospitalization. One patient had previous hospitalization due to COVID-19, and his disease was severe. Except for one (8.3%), 91.7% of the patients developed at least one symptom of COVID-19, such as fever, cough, shortness of breath, and gastrointestinal symptoms (Table 2).

Stroke occurred on average 10.5 (IQR, 5-19.5) days after symptoms of COVID-19 and as the first sign of COVID-19 in one patient. All patients had neurological findings due to posterior and/or anterior system involvement (Table 3). The mean stroke

severity was 7.8±4.7 points (range, 3-18) according to the National Institutes of Health Stroke Scale (1-25) score, with variability among patients (Table 3).

Laboratory findings

Except for one (8.3%) patient, 91.7% of patients had varying degrees of involvement on chest CT (Table 2). Among the inflammatory markers, mean CRP was 85.3±84.6 mg/L [(range: 0.6-236) (reference: 0-5 mg/L)] and IL-6 was 44.1±41.2 pg/mL [(range: 4.0-117) (reference: 0-5 pg/mL)]. Mean lymphocyte count was 1.1±0.70x10^3 cells/uL [(range: 0.4-2.7) (reference: 1.3-3.4x10^3 cells/μL.)] (Table 2). The median D-dimer level was 3.7 mg/L [(IQR 2.7-7.6) (reference: 0-0.5 mg/L)] and was high in all patients except two (Table 2).

Imaging

All patients had varying degrees of involvement in the posterior and/or anterior system on brain MRI and/or CT due to acute ischemic stroke (Table 3). We observed a large vessel occlusion (LVO) in 33.3% of the patients (Table 3). A hemorrhagic transformation developed in the infarct area of patient #2. Patient #12 had a subdural hematoma on the opposite side of the infarct area (Table 3).

Risk factors for stroke

Hypertension (58.3%), diabetes mellitus (41.7%), CAD (33.3%), AF (16.7%), prior CVD (16.7%), HF (25%), and malignancy (8.3%) were the most common risk factors for stroke (Table 1, 3).

Etiology of stroke

Two patients (16.7%) had a postulated cause of cardiogenic cause of stroke, AF. According to the TOAST classification, stroke due to large vessel atherosclerosis was present in 33.3%, cardioembolic stroke in 16.7%, lacunar stroke in 8.3%, and undetermined stroke in 41.7% of the patients (Table 3).

Clinical course and treatment

Most patients (66.7%) required oxygen during their hospitalization, and 75% had a high need for oxygen. Seven (58.3%) patients required intensive care during hospitalization. Steroid therapy was admistered to six (50%) patients, and one (8.3%) patient received immune plasma therapy. Low-molecular-weight heparin (LMWH) was not administered in the first stroke of patient #5 because this patient had a diagnosis of myelodysplastic syndrome (MDS), and the simultaneous platelet count was 9000. After the stroke recurred, LMWH treatment was administered considering the platelet count. All other patients received LMWH or intravenous heparin therapy (Table 2).

Thrombectomy was performed in one patient due to the presence of a thrombus formation protruding into the lumen in the middle part of the right common carotid artery (CCA), which impacted the wall in DSA (Table 3). On follow-up angiography, the part protruding into the lumen disappeared, and some thrombi impacted the wall persisted. Right CCA was found to be normal in CDUS performed at the follow-up 3 months later (Table 3).

One-quarter of the patients died (Table 3).

Patients with recurrent stroke

Stroke recurred in two (16.7%) patients 30 days after the first stroke in patient #5, and 24 days in patient #10 (Table 4). Among these patients, patient #5 had a high oxygen requirement during hospitalization (Table 2). D-dimer levels increased in both patients during their first and recurrent strokes. In the brain imaging of both patients, newly developed multiple infarct areas were detected in their recurrent strokes (Table 4). Both patients had underlying risk factors such as malignancy and CVD

Table 1. Baseline patient characteristics (n=12)

Age, years Mean±SD (min-max)	70.6±9.3 (55-84)
Female	n=6 (50%)
Hypertension	(n=7, 58.3%) (#1, 2, 6, 7, 9, 11, 12)
Diabetes mellitus	(n=5, 41.7%) (#1, 4, 7, 8, 11)
Atrial fibrillation	(n=2, 16.7%) (#3, 12)
Coronary artery disease	(n=4, 33.3%) (#2, 7, 11, 12)
Cerebrovascular disease	(n=2, 16.7%) (#5, 7)
Heart failure	(n=3, 25%) (#7, 11, 12)
Myelodysplastic syndrome	(n=1, 8.3%) (#5)
Malignancy	(n=1, 8.3%) (#10)
COPD	(n=2, 16.7%) (#2, 4)
Epilepsy	(n=1, 8.3%) (#11)

COPD: Chronic obstructive pulmonary disease, min-max: Minimum-maximum, SD: Standard deviation, #: Patients number

Table 2. COVID-19 related symptoms, inflammatory markers, D-dimer level, chest CT findings, O_2 requirement, intensive care support, and COVID-19 treatments (n=12)

Patient no.	Symptoms of COVID-19	Leukocytes (4.49-10.9x10^3 cells/µL*)	Neutrophil (2.1-8.89 x10^3 cells/µL*)	Lymphocyte (1.26-3.35 x10^3 cells/µL*)	CRP (0-5 mg/L*)	IL-6 (0-5 pg/mL*)	Procalcitonin (0-0.65 ng/mL*)	D-dimer (0-0.5 mg/L*)	Chest CT results	O_2 requirement	Intensive care support	Treatments of COVID-19
1	-	8.5	6	1.3	10.2	-	0.05	0.35	Bilateral multi-lober GGO	-	-	Steroid, LMWH
2	Cough, dyspnea, diarrhea	7.8	7.2	0.4	142	117	0.13	13.5	Bilateral multi-lober GGO	+, high	+	Steroid, LMWH
3	Anorexia, nausea	5.5	3.1	1.6	13.8	9.26	0.12	2.62	Bilateral peri-pheral multi-lober GGO	+	+	LMWH
4	Fever, cough	13.1	10.4	1.7	13	-	0.09	5.65	Bilateral peri-pheral lower lobes multi-focal GGO	+	+	IVH
5	Fever, dyspnea	2.6	1.9	0.4	236	36	3.23	4.28	Right upper lobe, multi-focal GGO	+, high	-	LMWH treatment according to the platelet count
6	Cough	5.6	2.3	2.7	0.6	3.97	-	0.46	N	-	-	LMWH
7	Cough, dyspnea	6	4.8	0.5	108	28	0.2	3.95	Bilateral patchy multi-lober GGO	+, high	+	Steroid, immune plasma, LMWH
8	Fever, dyspnea, cough	13.2	11.3	1.1	230	57.3	1.49	8.29	Bilateral multi-lober GGO	+, high	+	Steroid, LMWH
9	Cough	7.7	6.4	0.9	22.8	14.2	0.12	3.3	Peri-pheral bilateral GGO	-	-	LMWH
10	Cough	7.3	4.8	1.3	96	-	-	9.9	Right sub-pleural GGO	-	-	LMWH
11	Fever, cough	9.4	8.1	0,8	120	105	0.21	2.76	Bilateral multilober GGO	+, high	+	Steroid, LMWH
12	Dyspnea, cough, hemoptysis	14.1	12.2	0.8	31	26	-	3.54	Bilateral multilober GGO and paraseptal emphysematous changes	+, high	+	Steroid, LMWH

*Reference range.
CRP: C-reactive protein, IL-6: Interleukin-6, IVH: Intravenous heparinization, GGO: Ground-glass opacity, LMWH: Low-molecular-weight heparin, COVID-19: Coronavirus disease-2019, CT: Computed tomography

Table 3. Demographic features, acute ischemic stroke features, cardiac investigations, and clinical outcomes (n=12)

Patient no.	Age, sex	Risk factors for ischemic stroke	Clinical symptoms of ischemic stroke	NIHSS score	CT-MRI Stroke location	DSA -CTA- CDFI	ECG, ECHO	Stroke classification	Treatment for ischemic stroke	Discharged/ death
1	60, M	HT, DM	Left hemiparesis	5	Right cerebral multiple infarct	Right ICA 75% stenosis	SR, N	LVA	Medical therapy	Discharged
2	77, M	HT, CAD	Aphasia, dysphagia, right hemiplegia	10	Left cerebral multiple infarcts, hemorrhagic transformation in left temporal	Right ICA 75% stenosis, left ICA 30% stenosis	NSR, N	LVA	Medical therapy	Death
3	74, F	AF	Stupor, right hemiplegia	18	Large infarct in the left MCA area	Left MCA M1 occlusion	AF, N	Cardioembolism	Medical therapy	Death
4	60, M	DM	Left upper extremity hemiplegia left lower extremity hemiparesis, left hemihypoesthesia	9	Large infarct in the right MCA area	Right CCA thrombus	NSR, not performed	Undetermined	Mechanical thrombectomy, intravenous heparinization	Discharged
5	79, F	CVD (VST)	Left hemianopia, right-sided ataxia	4	Right cerebellar and occipital infarct	N	NSR, N	Undetermined	Medical therapy	Discharged
6	84, F	HT	Right hemiparesis, motor aphasia	3	Left periventricular infarction	25-50% stenosis on the left and 25% on the right in CCA	NSR, not performed	LVA	Medical therapy	Discharged
7	71, M	HT, DM, CAD, CVD, HF	Right hemihypoesthesia, ataxia	4	Left parietal cortex lacunar infarction	Left ICA 25-50% stenosis	SR, 1st AV block, No thrombus	Lacunar stroke	Medical therapy	Discharged
8	69, M	DM	Confusion, right hemiparesis	6	Left frontal infarction	Left MCA M2 distal stenosis	NSR, not performed	LVA	Medical therapy	Discharged
9	63, F	HT	Left hemiparesis left hemianopia	7	Infarct in the right PCA area	Right PCA P1 distal occlusion	NSR, N	Undetermined	Medical therapy	Discharged
10	55, F	Malignancy	Right hemihypoesthesia, right hemianopia, dysarthria	3	Left parietal and occipital Infarcts	N	NSR, not performed	Undetermined	Medical therapy	Discharged
11	74, F	HT, DM, CAD, HF	Confusion left hemiplegia	14	Infarct in right MCA area	N	ST, No thrombus	Undetermined	Medical therapy	Death
12	81, M	HT, CAD, HF, AF	Confusion aphasia, left hemiparesis and left hemianopia	11	Infarct in right PCA area left subdural hematoma	-	AF, No thrombus, biatrial dilatation, MI, TI	Cardioembolism	Medical therapy	Discharged

Medical therapy: Antiaggregant and/or LMWH or IVH.
NIHSS: National Institutes of Health Stroke Scale, HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, AF: Atrial fibrillation, CVD: Cerebrovascular disease, VST: Venous sinus thrombosis, HF: Heart failure, N: Normal, NSR: Normal sinus rhythm, SB: Sinusoidal bradycardia, ST: Sinus tachycardia, MCA: Middle cerebral artery, PCA: Posterior cerebral artery, CCA: Common carotid artery, ICA: Internal carotid artery, MI: Mitral insufficiency, TI: Tricuspid insufficiency, LVA: Large vessel atherosclerosis, M: Male, F: Female, CT: Computed tomography, CTA: CT angiography, CDFI: Color Doppler flow imaging, ECHO: Echocardiography, ECG: Electrocardiogram

(venous sinus thrombosis) (Table 3). LVO was observed after recurrent stroke in patient #10, and both patients were classified as having a stroke of undetermined cause. Patient #5 had a diagnosis of MDS, and, as mentioned above, LMWH was not administered in the first stroke because platelet levels were low.

Discussion

In this study, we examined the demographic characteristics, risk factors, and clinical and laboratory characteristics of patients who developed ischemic stroke associated with SARS-CoV-2 infection. We evaluated the characteristics of stroke patients associated with COVID-19, and the relationship between stroke and COVID-19.

The typical signs and symptoms of COVID-19 infection are fever, cough, and dyspnea. Almost all of our patients developed at least one of these symptoms. While 66.7% of our patients required oxygen during their hospitalization, 75% had a high oxygen required. Intensive care was required in 58.3% of the patients during hospitalization. Studies have reported that SARS-CoV-2 infection causes many neurological signs and symptoms, including stroke, in addition to respiratory ones, and an increased incidence of stroke in patients with severe disease (3,7).

Stroke has emerged as a serious complication of COVID-19. There is a trend toward LVO, multiple infarcts, and involvement of rarely affected vessels in strokes due to COVID-19. However, cerebral vein thrombosis, hemorrhagic infarction, and lacunar infarcts due to small vessel disease are less common (21). In this study, we observed infarcts due to LVO in four patients and lacunar infarction in one patient. The low number of infarcts due to LVO among our patients may be explained by the fact that we did not have a chance to investigate existing occlusion because the pandemic-related conditions restricted us from performing CTA or DSA in some patients.

In the study by Li et al. (22) with 219 patients with COVID-19, 11 patients had a stroke following infection, and the mean age of these patients was 75.7 years. In the same study, it was observed that patients with stroke had risk factors for stroke, such as hypertension, diabetes mellitus, CAD, and prior CVD, and most had severe SARS-CoV-2 infection. In our study, the mean age of the patients was 70.6 years and most patients had severe disease. It was determined that there were risk factors for stroke, such as hypertension and diabetes mellitus, prior CVD, AF, CAD, and malignancy.

In our study, the average D-dimer and IL-6 levels of the patients were high. Hypercoagulability, as evidenced by high D-dimer levels and “cytokine storm” associated with the severity of SARS-CoV-2 infection, may play a role in the pathophysiology of stroke in patients with COVID-19 (8,9). Apart from these factors, the mean age that might cause an increased risk for stroke was high in our patients. Additionally, as mentioned above, vascular risk factors such as hypertension and diabetes mellitus were also commonly observed. More care should be taken in the management of patients with severe COVID-19 and risk factors for stroke, especially in controlling diseases such as hypertension and diabetes mellitus.

In recent studies, the mean time to onset of stroke after symptoms of COVID-19 was similar. In the study of Li et al. (22), this period was 12 days on average. In another study examining six patients with stroke and COVID-19, patients had a stroke on the 10th, 24th, 10th, 2nd, 15th, and 8th days of the onset of COVID-19 symptoms (23). In two other studies in the literature involving large patient groups, the mean time up to the onset of stroke was 10 days (4,24). In our study, the onset of stroke was late, with an average of 10.5 days. This situation is described in the literature as a patient with severe COVID-19 infection possibly developing a prothrombotic state, often complicated by both venous and arterial thromboembolism, following a hyperinflammatory state

Table 4. Analysis of patients with recurrent stroke

Patient no.		Stroke time	Clinical symptoms of ischemic stroke	NIHSS score	CT-MRI stroke location	DSA -CTA-CDFI	D-dimer (mg/L)
5	First stroke	5th day	Left hemianopia, right-sided ataxia	4	Right cerebellar and occipital infarct	N	4.3
	Recurrent stroke	35th day	Left hemianopia, motor aphasia, ataxia	5	Newly developed left frontoparietal and cerebellar multiple infarctions	Not performed	6.3
10	First stroke	21st day	Right hemihypoesthesia, right hemianopia, dysarthria	3	Left parietal and occipital Infarcts	N	9.9
	Recurrent stroke	45th day	Right hemiparesis	5	Newly developed left cerebral and cerebellar multiple infarcts	Left MCA M2 distal occlusion	Not performed

NIHSS: National Institutes of Health Stroke Scale, CT: Computed tomography, CTA: CT angiography, MRI: Magnetic resonance imaging, CDFI: Color Doppler flow imaging

due to a cytokine storm (24). Future studies should focus on the stroke onset time after COVID-19, which may be useful for elucidating the pathophysiology of stroke.

Emergency prophylactic anticoagulation with LMWH is recommended in the literature to prevent a prothrombotic state (25). A study by Shen et al. (26) supported the use of LMWH in patients with COVID-19, especially in older patients with high levels of IL-6, an indicator of hyperinflammatory response, and those with evidence of hypercoagulability (e.g., high D-dimer levels). The international expert panel suggested that LMWH could be started in patients with COVID-19 with suspected cardioembolic stroke or those with low bleeding risk and severe COVID-19 (27).

The Stroke Council of the American Heart Association/ American Stroke Association repeated that all stroke teams should strive to adhere to publish guidelines on patient selection for treatment (28). In an international expert panel review, it was recommended that all eligible patients with stroke should continue to receive intravenous therapy irrespective of their COVID-19 status (27). Mechanical thrombectomy therapy should be offered to all eligible patients regardless of their COVID-19 status (29). However, in a study by Escalard et al. (30), early intravenous thrombolysis and mechanical thrombectomy with successful and rapid recanalization yielded poor outcomes in patients with acute ischemic stroke with COVID-19 due to LVO.

All stroke patients with COVID-19 should be evaluated for prothrombotic and cardioembolic causes, and, accordingly, appropriate secondary prevention should be initiated promptly (29).

Our findings showed that large vessel atherosclerosis, cardioembolic, and lacunar stroke patterns could occur in strokes associated with COVID-19, most patients had severe disease, and the D-dimer level was high, suggesting a hypercoagulable state. This systemic, highly prothrombotic state may facilitate the occurrence of stroke, particularly in patients with cardiovascular risk factors.

Study Limitations

Our study was limited by its retrospective and single-center design and the small number of patients. Additionally, diagnostic tests remained incomplete in some patients due to the pandemic conditions.

Conclusion

These results suggest that COVID-19 triggers stroke rather than being an independent cause and that more care should be taken in the management of patients with risk factors for stroke, and early treatment with LMWH may be beneficial in eligible patients with high D-dimer levels; however, attention should be paid to the risk of hemorrhagic transformation due to LMWH.

Ethics

Ethics Committee Approval: University of Health Sciences Türkiye, Gülhane Faculty of Medicine Ethics Committee (date: 17.06.2021, no: 2021-273).

Informed Consent: This was a retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ü.D., Ö.K., Concept: Ü.D., Ö.K., Design: Ü.D., Ö.K., Data Collection or Processing: Ü.D., Analysis or Interpretation: Ü.D., Ö.K., Literature Search: Ü.D., Writing: Ü.D., Ö.K.

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Predictors of discontinuation of antiretroviral therapy among HIV-infected adults at Hospital Sungai Buloh: A 10-year retrospective cohort study

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Keywords: Antiretroviral therapy, HIV, loss to follow-up, tuberculosis, regime switch

ABSTRACT

Aims: Discontinuation from antiretroviral therapy (ART) lowers the immunological advantages of human immunodeficiency virus (HIV) treatment and increases the problems related to HIV. Therefore, this study determined the predictors of discontinuation among HIV-infected adults at Hospital Sungai Buloh.

Methods: We carried out an institutional-based retrospective cohort study and reviewed the medical records of ART-naïve patients with HIV in Hospital Sungai Buloh, Selangor, Malaysia from January 2007 through December 2016. Discontinuation was defined as not attending the follow-up for 90 days or longer. Simple and multiple logistic regression was used to identify the predictors.

Results: Out of 339 patients, 63 (18.6%) experienced discontinuation. Among them, 63.5% were younger than 39 years, 81.0% were male and 62.0% were of Malay ethnicity. Sexual transmission via homosexual and heterosexual routes was the commonest mode of HIV transmission (76.7%). Patients with Malay ethnicity [adjusted Odds ratio (AOR): 2.5, confidence interval (CI): 1.4-4.5, p=0.002], tuberculosis co-infection (AOR: 2.0, CI: 1.1-3.7, p=0.025) and a history of ART regimen switch (AOR: 5.3, CI: 2.2-13.1, p<0.001) were significantly associated with ART discontinuation.

Conclusions: The proportion of patients who discontinued ART in our observation presents a challenge to the long-term success of the treatment program. There is a need for special attention to patients with HIV who are Malay, had tuberculosis co-infection, and history of switching to other regimens to avoid discontinuation of therapy.

Introduction

The human immunodeficiency virus (HIV) is a major public health issue and affects the whole world. In 2017, over 36.9 million people were living with HIV (PLHIV). Africa is one of the most affected continents with 25.7 million (70%) of PLHIV (1). Asia also had no exception for the HIV epidemic where Malaysia is a country with the highest number of PLHIV.

According to Malaysia Global AIDS Report 2020, a total of 77,903 PLHIV were reported in 2019, with 77,602 (99.6%) aged above 15 years old and 301 (0.4%) aged below 15 years old. There were 3,564 newly infected individuals with HIV in 2019, slightly less than the estimated cases of new HIV infection in 2019 (2). The epidemic is higher in Selangor (30.4%), Kuala Lumpur (12.6%), Johor (9.5%), Sarawak (8.1%), and Penang (8.1%), which contributed to more than 50% of new HIV cases in Malaysia (2).

In the beginning most efforts to combat the AIDS epidemic mainly focused on access to antiretroviral drugs. Antiretroviral therapy (ART) coverage escalated progressively from 7% in 2005 to 59% in 2017. ART has significantly extended the life expectancy of patients with HIV (1,3). However, a major challenge of ART is treatment discontinuation.

ART should be continued to show its benefits, requiring a lifelong commitment. Discontinuation from ART contributed to the slow destruction among patients and lead to poor quality of life and death (4). Besides, it shows a negative effect on the immunological benefit of treatment, increases the complications of HIV, and results in severe consequences such as treatment failure due to poor adherence to ART, drug toxicity, and drug resistance (1,5).

Past studies reported different factors to influence the discontinuation of ART. Socio-demographic factors such as gender (6,7), age (8,9), educational level (10), marital status (11), occupation status (12,13), disclosure status (6,10), distance from the health facility (14), caregiver (15,16) and clinical- and treatment-related factors such as baseline stage (11,17), baseline CD4 count (13), history of opportunistic infection at enrollment (18), baseline functional status (8,15), opportunistic prophylaxis (8) and type of ART regimen after initiation of their medication (13,17).

Previous authors defined discontinuation as the interruptions to ART treatment due to loss to follow-up, defaulting, transferring out, and stopping the medications while remaining in care (19). However, the current study defined ART discontinuation as not attending the scheduled follow-up for more than three months. Therefore, this study determined the factors that influence the discontinuation of ART among the population with HIV in Malaysia.

Methods

This retrospective cohort study included all PLHIV registered in Hospital Sungai Buloh, Malaysia, between January 1, 2007, and December 31, 2016, with the addition of 20 months of follow-up from January 1, 2017, and August 31, 2018. Hospital Sungai Buloh was chosen as the study location because it was a center of excellence for infectious diseases in Malaysia. It is one of the biggest tertiary hospitals under the Ministry of Health Malaysia. Also, this hospital is in the Selangor state, with the highest number of PLHIV in Malaysia.

We reviewed the medical records in the electronic Hospital Information System. The sampling frame was obtained based on an internal database called Malaysian Antiretroviral Treatment Cohort (MATCH). This database was developed by the Infectious Disease Unit of Hospital Sungai Buloh and updated in the first quarter of every year by the unit staff. Using the MATCH database, patient name, registration number, ART initiation date, and the latest status (on treatment or transferred out) were collected.

The patients were included if they were above 15 years old at the time of ART initiation, diagnosed with HIV using enzyme-linked immunosorbent assay and particle agglutination test, and ART-naïve. We excluded the patients transferred from Hospital Sungai Buloh to other hospitals or clinics during the study period.

Between 2006 and 2016, 7090 HIV-infected patients were newly started with ART in Selangor. However, 931 patients were transferred out, and 6159 patients were eligible as the reference population. Among 6159 patients, 385 patients were treated with ART at Hospital Sungai Buloh. Following exclusions, 339 patients were eligible for the analysis.

The study outcome was the discontinuation of ART. Discontinuation was defined as not attending the scheduled follow-up for more than three months. If a patient showed up more than three months after the appointment date, he or she would be considered as having a history of defaulting from follow-up.

The required information was extracted from the MATCH and e-HIS databases, using a data collection form. The information included was patients-related characteristics (age, gender, ethnicity, smoking status, alcohol intake, history of illicit drug use, transmission mode) and clinical-related characteristics (time between first HIV positive test and ART initiation, baseline CD4 cell count, baseline viral load, World Health Organization clinical staging, tuberculosis co-infection, hepatitis B co-infection, hepatitis C co-infection, opportunistic infection, underlying liver disease, renal disease, cardiovascular disease, and diabetes mellitus).

The information for the treatment-related characteristics included the year of ART initiation, the first non-nucleoside reverse transcriptase (NNRTI) background regimen, number of regimen substitutions, regimen switch, and default history. Anemia was defined as a hemoglobin level of less than 11 g/dL.

Universiti Sains Malaysia Research Ethics Committee (ref: USM/JEPeM/18060287) and the National Medical Research and Ethics Committee of the Ministry of Health Malaysia (NMRR; Ref: KKM.NIHSEC. P18-1703(6)) approved the study.

We recorded the data on a password-protected computer with limited access. All data were anonymous; thus, there were no unique identifiers. All the data were kept strictly confidential.

Statistical Analysis

Data entry and analysis were conducted using the Statistical Package for Social Sciences (SPSS) version 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Continuous variables were expressed as a mean and standard deviation or as a median and interquartile range, depending on the normality of distribution. The categorical variables were presented as frequency (n) and percentage (%). Simple and multiple logistic regression analyses were used to determine the predictors of discontinuation of ART among patients with HIV. The results are tabulated as crude and adjusted Odds ratio (OR), 95% confidence interval (CI), and p-value. Statistical significance was set at $p<0.05$.

Results

Baseline findings

A total of 385 patients with HIV were evaluated. However, 46 patients were ineligible to include in this study (7 patients had their ART planned but it was not started, eight were not treatment naïve, 30 were transferred out and one subject had incomplete information. As a result, 339 patients were included in the final analysis and of these, 63 patients (18.6%) had discontinued ART treatment.

Socio-demographic characteristics

The details of the socio-demographics are summarized in Table 1. Most HIV-infected patients who discontinued the ART treatment were in the age group below 30 years old (33.3%) and 81.0% were male. Malay ethnicity constituted 62.0% of all patients who discontinued the ART treatment, followed by Chinese (19.0%) and other ethnicities (19.0%). Other ethnicities consisted of Indians, Sabahan, and Sarawakian, and non-Malaysians include Myanmar, Indonesia, the Philippines, and Nigeria.

The most common HIV transmission mode reported among patients who discontinued the ART was via the homosexual/bisexual route with 17 patients (28.3%) and the heterosexual route with 29 patients (48.3%). Only 14 patients (23.4%) were infected with HIV via the parenteral route including patients who were parenteral drug users, needlestick injury, blood transfusion, and via tattooing.

Of the subjects who discontinued ART, 41.2% had a history of illicit drug use such as heroin and methamphetamine. Information on alcohol intake was largely missing (41.6%). Of

Table 1. Socio-demographic characteristics of HIV-infected patients in Hospital Sungai Buloh (n=339)

Variables	Discontinuation of ART	
	No Frequency (%)	Yes Frequency (%)
Age (years)		
<30	75 (27.2)	21 (33.3)
30-39	104 (37.6)	19 (30.2)
40-49	62 (22.5)	16 (25.4)
≥50	35 (12.7)	7 (11.1)
Gender		
Male	238 (86.2)	51 (81.0)
Female	38 (13.8)	12 (19.0)
Ethnicity		
Malay	108 (39.1)	39 (62.0)
Chinese	115 (41.7)	12 (19.0)
Others*	53 (19.2)	12 (19.0)
Smoking status		
Non-smoker	103 (49.8)	19 (42.2)
Ex-smoker	15 (7.2)	5 (11.1)
Active smoker	89 (43.0)	21 (46.7)
Alcohol intake		
Non-drinker	103 (62.4)	24 (72.7)
Social drinker	53 (32.1)	6 (18.2)
Heavy drinker	9 (5.5)	3 (9.1)
History of illicit drug		
No	184 (82.1)	30 (58.8)
Yes	40 (17.9)	21 (41.2)
Mode of transmission		
Homo/bisexual	117 (45.9)	17 (28.3)
Heterosexual	107 (42.0)	29 (48.3)
Parenteral route	31 (12.1)	14 (23.4)

*Others included Indians, Sabah and Sarawak and non-Malaysians (Myanmar, Indonesia, Philippines and Nigeria).
HIV: Human immunodeficiency virus

those 33 patients who reported the discontinuation of the ART, 24 patients (72.7%) were non-drinkers, six patients (18.2%) were social drinkers and three patients (9.1%) were heavy drinkers. Also, information on smoking status was not available for 87 patients (25.7%). Concerning the smoking status, 42.2% was non-smoker, 46.7% was active smokers and 11.1% was ex-smoker.

Clinical characteristics

Half of the patients who discontinued ART (56.7%) started ART after six months after the first HIV-positive test as in Table 2. A total of 31 patients (53.4%) had less than 200 cells/μL in their baseline CD4 cell count. Information on the baseline viral load was not available for 129 patients (38.1%). Of the remaining 210 patients, 25 patients who discontinued ART treatment (59.5%) had more than 100,000 copies/mL of HIV/RNA as the baseline. There were 14 (26.4%) patients who were anemic.

HIV clinical staging was not available in almost half of the patients (45.7%). However, the patients were categorized into clinically advanced stages, with 15 patients (39.5%) having clinically advanced HIV and 23 patients (60.5%) who did not. Less than half (34.8%) of the patients had experienced at least one episode of opportunistic infection.

A total of 24 patients (38.1%), five patients (8.6%), and 16 patients (25.4%) were diagnosed with tuberculosis, hepatitis B, and hepatitis C co-infection, respectively. As the underlying disease at ART initiation, only a minority, 4 (6.3%), 1 (1.6%), and 4 (6.3%) patients had underlying liver, renal and cardiovascular diseases, respectively. Liver diseases included fatty liver and liver hemangioma. Only 6.3% of the patients had diabetes mellitus.

Treatment characteristics

Among patients with HIV who discontinued ART, 26 (41.3%) patients had initiated ART between 2007 and 2010. The remaining 22 patients (34.9%) and 15 patients (23.8%) were from the years between 2011 and 2013 and 2014 and 2016, respectively. When we stratified according to the year, a greater proportion of patients had ART in the later years. This trend was consistent with the increasing number of patients who started ART at Hospital Sungai Buloh from 2007 to 2016. Since we applied systematic random sampling, more patients were recruited from the later years.

The commonest NNRTI in the first ART was efavirenz (65.6%) and the commonest first NNRTI background was the combination of zidovudine and lamivudine (57.1%). Less than half of the patients had their ART substitution; 15 patients (23.8%) had it once and 14 patients (22.2%) had it more than two. Even lesser for regimen switch, only 12 patients (19.0%) had their regimen switch to a second-line ART. During the study period, 26 patients (41.3%) experienced at least one adverse drug reaction (ADR). Table 3 shows the details of treatment-related characteristics.

Simple logistic regression

There were 25 clinically important variables to be tested for univariable analysis using simple logistic regression. Ethnicity, history of illicit drugs, mode of transmission, baseline viral load, tuberculosis co-infection, hepatitis C and history of ART regime switch were significant factors as the p-value was less than 0.05 (Table 4).

Multiple logistic regression

Ethnicity, tuberculosis co-infection, and history of ART regime switch were associated with the discontinuation of ART. Malay patients (adjusted OR: 2.5, 95% CI: 1.4-4.5, p=0.002), had tuberculosis co-infection (adjusted OR: 2.0, 95% CI: 1.1-3.7, p=0.025) and history of ART regime switch (adjusted OR: 5.3, 95% CI: 2.2-13.1, p<0.001) had higher risk to discontinue the ART as presented in Table 4.

Malay patients had 2.53 higher Odds of discontinuing the ART compared with non-Malay patients after controlling for tuberculosis co-infection and history of ART regime switch. Tuberculosis co-infection proved to be significant in the model. There were 2.01 higher odds of discontinuing ART in subjects with tuberculosis co-infection controlling for ethnicity and history of ART regime switch. The history of ART regime switches was also associated with the discontinuation of ART. Those who switched to the ART regime had 5.31 higher odds of discontinuing the ART compared to those without a switch to the ART regime after controlling for ethnicity and tuberculosis co-infection.

Discussion

Discontinuation occurred in 63 (18.6%) patients. It was not uncommon that a proportion of lost to follow-up patients in Hospital Sungai Buloh returned to the clinic several months or years after defaulting follow-up. Some female patients came back on subsequent pregnancy a few years after defaulting follow-up. Among them, 63.5% were younger than 39 years, 81.0% were male and 62.0% were of Malay ethnicity. Sexual transmission via homosexual and heterosexual routes was the commonest mode of HIV transmission (76.7%).

The current finding was comparably lower compared to the studies conducted in Southwest Ethiopia (22.3%) (20), Nigeria (28%) (21), the United States (45.1%) (22), Guinea-Bissau (51.1%) (23) and a multi-clinic study from the Republic of Congo, Cameroon and Burundi (83%) (24). Access to HIV care facilities (25), variations in measurement, innovation, adoption and implementation of cost-effective retention strategies may explain the differences in the prevalence numbers between the current study and others (1).

Table 2. Clinical-related characteristics of HIV-infected patients in Hospital Sungai Buloh (n=339)

Variables	Discontinuation of ART	
	No Frequency (%)	Yes Frequency (%)
Duration of the first HIV-positive test to ART initiation (months)		
0-6	153 (62.4)	26 (43.3)
>6	92 (37.6)	34 (56.7)
Baseline CD4 cell count (cells/μL)		
0-49	80 (29.7)	16 (27.6)
50-199	74 (27.5)	15 (25.9)
≥200	115 (42.8)	27 (46.5)
Baseline viral load (copies/mL)		
<10 000	25 (14.9)	10 (23.8)
10 000-100 000	64 (38.1)	7 (16.7)
>100 000	79 (47.0)	25 (59.5)
Baseline hemoglobin		
No anemia	192 (75.0)	39 (73.6)
Anemia	64 (25.0)	14 (26.4)
WHO clinical staging		
Class I	96 (63.2)	17 (53.1)
Class II	20 (13.2)	5 (15.6)
Class III	24 (15.8)	7 (21.9)
Class IV	12 (7.8)	3 (9.4)
Clinically advanced HIV		
No	118 (83.7)	23 (16.3)
Yes	71 (82.6)	15 (17.4)
Opportunistic infections		
No	147 (53.2)	31 (49.2)
Yes	129 (46.8)	32 (34.8)
Tuberculosis		
No	215 (77.9)	39 (61.9)
Yes	61 (22.1)	24 (38.1)
Hepatitis B		
No	254 (92.0)	58 (92.1)
Yes	22 (8.0)	5 (7.9)
Hepatitis C		
No	242 (87.7)	47 (74.6)
Yes	34 (12.3)	16 (25.4)
Underlying liver disease		
No	262 (94.9)	59 (93.7)
Yes	14 (5.1)	4 (6.3)
Underlying renal disease		
No	275 (99.6)	62 (98.4)
Yes	1 (0.4)	1 (1.6)
Underlying cardiovascular disease		
No	261 (94.6)	59 (93.7)
Yes	15 (5.4)	4 (6.3)
Diabetes mellitus		
No	261 (94.6)	59 (93.7)
Yes	15 (5.4)	4 (6.3)

HIV: Human immunodeficiency virus, ART: Antiretroviral therapy, WHO: World Health Organization

Table 3. Treatment-related characteristics of HIV-infected patients in Hospital Sungai Buloh (n=339)

Variables	Discontinuation of ART	
	No Frequency (%)	Yes Frequency (%)
Year of ART initiation		
2007-2010	62 (22.5)	26 (41.3)
2011-2013	93 (33.7)	22 (34.9)
2014-2016	121 (48.8)	15 (23.8)
First NNRTI in the first ART		
Efavirenz	208 (75.6)	40 (65.6)
Nevirapine	67 (24.4)	21 (34.4)
First NNRTI background		
ZDV/3TC	146 (52.9)	36 (57.2)
TDF/FTC	84 (30.4)	13 (20.6)
D4T/3TC	43 (15.6)	13 (20.6)
ABC/3TC	3 (1.1)	1 (1.6)
Number of regimen substitution		
0	158 (57.2)	34 (54.0)
1	75 (27.2)	15 (23.8)
≥2	43 (15.6)	14 (22.2)
Regimen switch		
No	264 (95.7)	51 (81.0)
Yes	12 (4.3)	12 (19.0)
Adverse drug reaction		
No	188 (68.1)	37 (58.7)
Yes	88 (31.9)	26 (41.3)

ART: Antiretroviral therapy, NNRTI: Non-nucleoside reverse transcriptase, ZDV/3TC: Zidovudine/lamivudine, TDF/FTC: Tenfovir/emtricitabine: D4T/3TC stavudine/lamivudine, ABC/3TC: Abacavir/lamivudine

Table 4. The associated factors of discontinuation of ART among HIV-infected patients in Hospital Sungai Buloh (n=339)

Variables	Simple logistic regression			Multiple logistic regression		
	b	Crude Odds ratio (95% CI)	p-value	b	Adjusted Odds ratio (95% CI)	p-value
Ethnicity						
Non-Malay	-	1.00	-	-	1.00	-
Malay	0.93	2.53 (1.44, 4.44)	**0.001**	0.93	2.53 (1.41, 4.54)	**0.002**
History of illicit drug						
No	-	1.00	-	-	-	-
Yes	1.17	3.22 (1.67, 6.19)	**<0.001**			
Mode of transmission						
Home/bisexual	-	1.00	-			
Heterosexual	0.62	1.87 (0.97, 3.59)	0.062	-	-	-
Parenteral route	1.13	3.11 (1.38, 6.91)	**0.006**			
Tuberculosis co-infection						
No	-	1.00	-	-	1.00	-
Yes	0.77	2.17 (1.21, 3.88)	**0.009**	0.70	2.01 (1.09, 3.72)	**0.025**
Hepatitis C						
No	-	1.00	-	-	-	-
Yes	- 0.89	0.41 (0.21, 0.81)	**0.010**			
Regime switch						
No	-	1.00	-	-	1.00	-
Yes	1.64	5.18 (2.20, 12.17)	**<0.001**	1.67	5.31 (2.16, 13.07)	**<0.001**

b regression coefficient; CI: Confidence interval, Forward LR and Backward LR variable selection were applied; Multicollinearity and interaction term were checked and not found; Assumptions were met.
ART: Antiretroviral therapy, HIV: Human immunodeficiency virus

Discontinuation of ART is a major predictor of the success of HIV treatment. So this study aimed to determine the factors associated with the discontinuation of ART among patients with HIV in Malaysia. The current study found that the Malay ethnicity had tuberculosis co-infection and had a history of ART regimen switch and had a higher risk for discontinuation of ART.

Malay ethnicity was associated with the discontinuation of ART. The result was not surprising since, among Malaysian citizens, the Malays were the predominant ethnic group in Peninsular Malaysia. Furthermore, stigma and discrimination regarding HIV as a 'humiliating disease' among Malaysian citizens may contribute to the discontinuation of the treatment among Malay citizens. Patients with HIV were often stigmatized by society and even by their family members. Particularly, before the ART is initiated, their HIV status was easy to keep secret. However, dose-taking of ART or such treatments that need to be followed according to the schedule could expose their illness to other individuals and thus, create personal or family humiliation.

HIV-infected patients with tuberculosis co-infection were significantly associated with discontinuation of ART, similar to the previous study (20). The intimate linkage between HIV and tuberculosis speeds up the progression of HIV disease to the advanced stage rapidly and thereby disallowing patients from regular treatment intake (26). Additionally, the double stigma related to tuberculosis/HIV co-infection and the double burden of having to take multiple pills for both conditions (pill effect) could be a compounding factor for discontinuation within this cohort.

We also found regimen switches as one of the significant factors for the discontinuation of ART among HIV-infected patients. During the lifelong treatment of HIV, certain patients commonly experience switches in ART regimens. The current study reported that only 12 patients had their regimen changed to a second-line ART, which was attributable to the higher percentage of patients on ART who have suppressed the viral load. Moreover, the availability and accessibility of second-line ART such as raltegravir are limited, and not freely provided by the Malaysian government. Other reasons for ART regimen change include drug toxicity, anticipated drug-drug interactions, viral suppression, increasing drug availability, simplification of a current regimen, treatment failure, tuberculosis treatment, and pregnancy were reported in previous studies (27,28).

Study Limitations

The study was conducted at one of the biggest hospitals under the Ministry of Health Malaysia. Hence, the results can be translated to the other hospitals in Malaysia, as all facilities under the Ministry of Health share the same system and support. All patients included in this study were treatment naïve, therefore the results were not confounded by previous ART.

Considering the retrospective study design of the current study, there were missing data for a few of the variables of interest such as clinical staging and lifestyle factors such as smoking and alcohol intake. Some of the information such as employment status and underlying disease were collected only at baseline during ART initiation and not after enrollment into the study. This could cause misclassification bias.

Conclusion

The proportion of patients who discontinue ART presents a challenge to the long-term success of the treatment program. There is a need for special attention to patients with HIV who are Malay, had tuberculosis co-infection, and with a history of regimen, switches to avoid discontinuation of therapy. The ID team in hospitals, comprised of doctors, nurses, and other allied staff, should take the initiative to lower the rate of ART discontinuation and avoid the circumstances that cause ART discontinuation. They can assist by identifying the patients who have ART discontinuation predictors.

Ethics

Ethics Committee Approval: Universiti Sains Malaysia Research Ethics Committee (ref: USM/JEPeM/18060287) and the National Medical Research and Ethics Committee of the Ministry of Health Malaysia (NMRR; Ref: KKM.NIHSEC. P18-1703(6)) approved the study.

Informed Consent: This was a retrospective cohort study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.H.S-A., B.N., K.C.S., Concept: A.H.S-A., B.N., S.C.L., K.C.S., Design: A.H.S-A., B.N., K.C.S., Data Collection and Processing: S.C.L., K.C.S., W.A.W-N-A., Analysis and Interpretation: A.H.S-A., B.N., S.C.L., W.A.W-N-A., Literature Search: A.H.S-A., S.C.L., W.A.W-N-A., Writing: S.C.L., W.A.W-N-A.,

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Volume-controlled ventilation versus pressure-controlled ventilation and recruitment maneuvers in video-assisted thoracoscopic surgery

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ABSTRACT

Aims: Various ventilation strategies can be applied to prevent lung injury during one-lung ventilation (OLV). We compared intraoperative ventilation strategies in terms of haemodynamic and respiratory parameters in video-assisted thoracoscopic surgery (VATS).

Methods: Sixty VATS patients, with American Society of Anesthesiologists score of I-III, receiving volume-controlled ventilation (VCV) (Group V) (n=30) or pressure-controlled ventilation (PCV) and recruitment maneuver (RM) (Group P) (n=30) were included in this prospective study. Mean arterial pressure (MAP), peripheral oxygen saturation (SpO_2), tidal volume (TV), airway pressures, compliance, and arterial blood gas values were recorded. In Group P, RM was applied after the 15th minute of OLV. The clinical efficacy and safety of VCV and PCV during VATS were evaluated.

Results: The MAP and PaO_2 were similar between groups throughout the follow-up ($p>0.05$). The peak inspiratory pressure (PIP) and Pplateau in Group V were higher than those in Group P ($p<0.05$). In Group P, there was an increase in TV, airway pressures, and compliance values at the 1st, 2nd, and 3rd minutes of RM ($p<0.05$). No significant change was observed in SpO_2, PaO_2, airway pressures, and compliance in Group P at post-RM 15th min ($p>0.05$).

Conclusions: The ventilation modes did not have clinical superiority over each other. Nonetheless, lower PIP and Pplateau values found during PCV were considered advantage. In Group P, the RM applied during OLV increased compliance and TV. However, extensive research is needed to develop RM models that will ensure improvements in respiratory parameters will last longer.

Introduction

Video-assisted thoracoscopic surgery (VATS) is used widely in thoracic surgery. The advantages of VATS over open techniques include less postoperative pain, better respiratory functions, and shorter hospital stay (1,2). VATS is usually performed with the help of one-lung ventilation (OLV). OLV is a specific application performed to facilitate manipulations and protect other pulmonary structures. However, serious complications can develop, such as hypoxemia (PaO_2 <80 mmHg, SpO_2 <90%) and acute lung injury (ALI) (3,4).

Currently, various "lung-protective ventilation (LPV) strategies" are recommended to prevent ALI and hypoxemia due

to OLV (5). Nonetheless, randomized controlled trials (RCTs) investigating both intraoperative ventilation strategies, and the selection of volume-controlled ventilation (VCV) or pressure-controlled ventilation (PCV) mode are not sufficient to provide evidence. Reviewing the literature, the use of intraoperative PCV can provide better oxygenation, lower airway pressures, and more effective CO_2 elimination. The PCV applied with recruitment maneuvers (RM) would improve oxygenation (6). However, it was found that PCV and VCV modes showed the same performance in terms of intraoperative oxygenation and postoperative complications during OLV in the other study (7).

Despite different strategies, the incidence of postoperative pulmonary complications may still be high (8). Since the optimum intraoperative ventilation strategy effective in preventing lung injury in thoracic surgery patients has not yet been clarified yet, studies on this subject are still ongoing.

We hypothesized that PCV, especially along with RM, could be a more effective ventilation strategy than VCV in patients undergoing VATS. We compared the effects of VCV and PCV applications on hemodynamics and oxygenation in patients who underwent VATS. We also evaluated the effects of the RM in the PCV mode.

Methods

This single-center study with prospective enrollment was conducted on patients in the American Society of Anesthesiologists I-III risk class and administered OLV for elective VATS admitted to a tertiary care center between June 2015 and August 2015. Wedge resection or biopsy with VATS was planned. The study protocol agreed with the ethics committee approval, and informed consent was obtained for all participants. All procedureswere in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patients with a diagnosis of chronic obstructive pulmonary disease, bronchiectasis, asthma, central airway obstruction, tuberculosis, bullous lungs, and those who had FEV_1 values of <80% and FEV_1/FVC <70% were excluded. Intracranial pathologies or cardiovascular diseases (advanced heart failure or coronary heart disease) were also among the exclusion criteria. The flowchart of study recruitment is shown in Figure 1. A total of 60 patients with a body-mass index between 18 and 30 kg/m^2 were divided into two groups. Thirty patients underwent VCV and were classified as Group V. Another 30 patients underwent PCV and RM and were classified as Group P. All patients who underwent PCV received RM as one of the LPV strategies. After sedative premedication with 2 mg of iv midazolam andpreoxygenation, general anesthesia was performed. Anesthesia was induced with 2.5 mg/kg of propofol, 1.5 µg/kg of fentanyl, and 0.7 mg/kg of rocuronium. Radial arterial catheterization was performed in all the patients. Endobronchial intubation was carried out with a left-sided double-lumen tube (DLT), without fiberoptic bronchoscopy (FOB). The DLT placement was confirmed conventionally by the inspection, auscultation, and peak inspiratory pressure (PIP) monitoring. After the patient waspositioned in the lateral decubitus position (LDP), a clinical re-evaluation was performed to check whether there was any displacement in the DLT. In case of unexpected hypoxemia, an increase in PIP value, or a decrease in tidal volume (TV), it was planned to check and correct the position of the DLT via FOB. However, none of the patients required a FOB throughout the operation. The maintenance of anesthesia was provided with sevoflurane at 1 minimum alveolar concentration, remifentanil (0.01-0.20 µg/kg/min), and rocuronium. A mixture of 80% O_2 and 20% air was used for ventilation. The inspiratory fresh gas flow rate was 4 L/min. The fluid administration was managed with balanced crystalloids at a dose of 4-6 mL/kg/hr, considering the fasting period of the patient and the risk group of the operation.

The respiratory rate was adjusted to keep the end-tidal CO_2 ($EtCO_2$) value in the range of 35 - 45 mmHg. In LDP, at the end of 15 min of TLV, arterial blood gas (ABG) sample was taken, OLV was initiated and positive end-expiratory pressure (PEEP) was adjusted to 5 cmH_2O. In Group V, TV was determined according to the ideal body weight, as 8 mg.kg^{-1} during TLV and 6 mL.kg^{-1} during OLV. In Group P, ventilation was performed to provide the same TV as VCV and with a maximum PIP of 35 mmHg during TLV and OLV. In this group, ABG analysis was performed after the 15th minute of OLV, and RM was applied subsequently. The RM protocol was as follows: PIP/PEEP values were gradually increased and applied for 1 min each with 3 minutes as 30/10, 35/15, 40/20 cmH_2O. After 10 breaths, PIP and PEEP were reduced to baseline values (9).

In the perioperative period, electrocardiography, systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), peripheral oxygen saturation (SpO_2), heart rate (HR), respiratory rate, invasive arterial pressures, and ABG were monitored. During the intraoperative period TV, $EtCO_2$, PIP, plateau pressure (Pplateau), and compliance measurements were also monitored. Measurement times were identified as pre-induction (T1), induction 3rd min (T2), intubation 1st min (T3), TLV 5th min (T4), TLV 10th min (T5), TLV 15th min (T6), OLV 5th min (T7), OLV 10th minute (T8), OLV 15th minute (T9), OLV 30th min (T10), extubation 1st minute (TE), recovery (TR). ABG analyses were performed on T1, T6, T9, and T10. At the end of the operation, ABG and vital signs were recorded during the TE and TR periods. After extubation, all the patients with Modified Aldrete Score ≥9, were transferred to the surgical intensive care unit for follow-up as a routine procedure of the clinic.

Statistical Analysis

To test the statistical significance of a difference of at least 10 (kPa) in terms of arterial oxygen saturation at a 90% power and 5% error level, between at least two of the groups in the sample width calculations, it was anticipated to include at least 26 cases in each group (10). Descriptive analyses of the study were expressed as the mean and standard deviation for numerical data, and frequency and percentages for categorical data. In comparisons between Group P and Group V, which are independent groups of the study, the chi-square test was completed for categorical data and the Mann-Whitney U test was completed for numerical data. To determine the temporal changes in each group, the Friedman non-parametric variance analysis was performed in general group comparisons. In cases where a difference was found in general group comparisons, the Wilcoxon signed-ranks test was used in post-hoc evaluations to determine from which measurement time this difference originated. All the statistical analyses of the study were interpreted by performing them in a two-way hypothesis structure and at the 5% type-1 error level. Statistical analyses of the study were done using Statistical Package for the Social Sciences (SPSS) 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0., Armonk, NY: IBM Corp.) software.

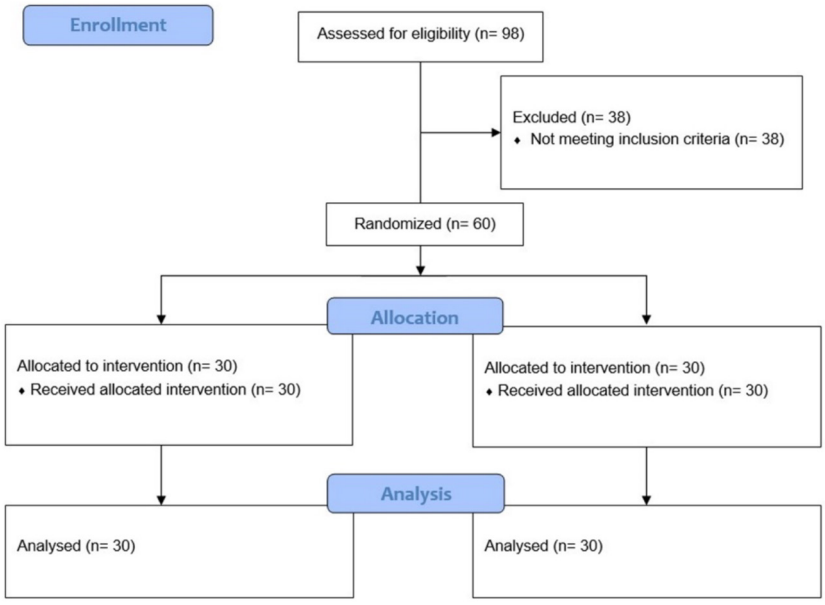

Figure 1. Flowchart of study recruitment

Table 1. Demographic and clinical characteristics of the patients

	Group P (n=30)	Group V (n=30)	p
Age (year), mean±SD	46.1±15.1	41.9±15	0.267
Body mass index (kg.m^{-2}), mean±SD	24.9±4.2	25.4±4.6	0.437
Ideal body weight (kg), mean±SD	64.3±12.4	67.5±12.3	0.180
Gender, n (%)			
Male	20 (66.7)	23 (76.7)	0.390
Female	10 (33.3)	7 (23.3)	
Pulmonary function tests, mean±SD			
FEV_1 (%)	90.6±11.6	88.3±9.4	0.291
FVC (%)	79.6±16.2	76.3±18.5	0.586
FEV_1/FVC (%)	76.7±10.1	75.6±10.7	0.552
Operation side, n (%)			
Right	19 (63.3)	13 (43.3)	0.121
Left	11 (36.7)	17 (56.7)	

SD: Standard deviation, FEV_1: Forced expiratory volume in 1 second, FVC: Forced vital capacity

Results

The study included 60 patients (17 female and 43 male) aged between 18 and 65 years (Table 1). There was no statistically significant difference in demographic parameters, operation side, and basal pulmonary functions (FEV_1, FVC, and FEV_1/FVC) between the groups (Table 1).

No statistically significant difference was found in the MAP values measured from the T1 to TR period between groups. A significant decrease in MAP was observed during the operation and TR period compared to T1 within both groups. MAP changes in T2, T3, T4, T7, T8, TE, and TR were statistically significant compared to the previous measurement time in both groups. Additionally, a statistically significant increase was observed in the transition from TLV to OLV. The changes in MAP values are shown in Figure 2A.

HR changes over time are shown in Table 2. Patients in Group P had a significantly lower HR at T4 than Group V. HR reduced significantly in Group P at T4 and beyond, compared with T1. In Group P, HR was significantly lower at T4 and T6 compared to the preceding measurement. In Group V, HR was significantly higher in T3 and lower in TR than in T1.While there was no difference in HR in the transition from TLV to OLV in Group P, HR decreased in Group V. In both groups, HR was significantly higher in TE and lower in TR than the preceding measurement.

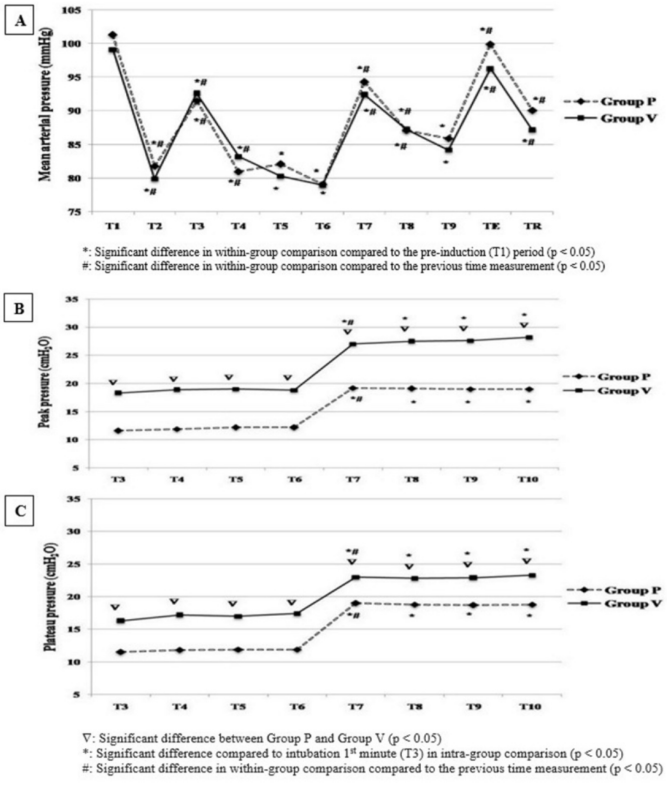

Figure 2. A) Mean arterial pressure. B) Peak inspiratory pressure. C) Plateau pressure

Measurement times: pre-induction (T1), induction 3rd min (T2), intubation 1st min (T3), TLV 5th min (T4), TLV 10th min (T5), TLV 15th min (T6), OLV 5th min (T7), OLV 10th minute (T8), OLV 15th minute (T9), OLV 30th min (T10), extubation 1st minute (TE), recovery (TR)

The changes in SpO_2 are shown in Table 2. T5 and T6 SpO_2 values were lower in Group V. In both groups, compared with the T1, SpO_2 increased during the operation. SpO_2 decreased in TE compared to T10 in Group P. The decrease in SpO_2 in TR compared with the TE was also significant. The decrease in SpO_2 during the transition from TLV to OLV in both groups was not significant. No significant change was observed in SpO_2 in Group P 15 min after RM (OLV 30th min; T10) than before RM (OLV 15th min; T9).

The differences in PaO_2 are shown in Table 2. No statistically significant difference was detected in the PaO_2 from the T1 period to the TR period between groups. A significant increase was observed in PaO_2 at all measurements compared with T1. In both groups, a statistically significant decrease was observed in PaO_2 with the transition from TLV to OLV, and the decrease in PaO_2 in the TR period was significant compared with the TE. In Group P, no significant change was observed in PaO_2 at 15 min (T10) after RM than before RM (T9).

The PIP and Pplateau in Group V were significantly higher at all periods compared to Group P ($p<0.05$). There was an increase in PIP and Pplateau during OLV compared to T3 and in the transition from TLV to OLV in groups ($p<0.05$). At the 15th min (T10) after RM, no significant change was observed in PIP and Pplateau compared to pre-RM in Group P (T9) ($p>0.05$). Changes in PIP and the Pplateau are shown in Figures 2B, 2C.

There was no difference in compliance at any measurement time in Groups P and V. Compliance decreased following the transition from TLV (Group P: 47.2±13.9; Group V: 41.2±14.1 $mL.cmH_2O^{-1}$) to OLV (Group P: 31.3±13.6; Group V: 27.7±8.2 $mL.cmH_2O^{-1}$) in both groups ($p<0.05$). There was no significant change in compliance at 15 min (T10) after RM (30.1±7.3 $mL.cmH_2O^{-1}$) than before RM (T9) (29.1±9.7 $ml.cmH_2O^{-1}$) in Group P.

The results of RM in Group P are summarized in Table 3. When the effects of RM at in the 1st, 2nd, and 3rd minutes were compared with T9 (pre-RM), there was no difference in MAP, SpO_2, and HR values, while TV, PIP, Pplateau, and compliance values increased. The changes in all parameters measured at the 1st, 2nd, and 3rd minutes of RM were similar. No hemodynamic complications, intraoperative hypoxemia, or post-operative ALI findings were observed during the study. We did not observe a barotrauma, such as pneumothorax, related to RM or OLV.

Discussion

We aimed in this study to compare the effects of VCV and PCV on hemodynamics and oxygenation in patients undergoing VATS. Briefly, VCV and PCV modes showed no

Table 2. Heart rate, SpO_2, and PaO_2 values

	Periods	Group P (n=30)	Group V (n=30)	p
	T1	87.1±15	85.3±12.6	0.807
	T2	85.0±15.2	87.8±13.4	0.248
	T3	87.1±14.8	90.4±11.5*	0.240
	T4	81.2±13.4*†	88.5±12.9	**0.016‡**
Heart rate, mean±SD	T5	80.3±12.5*	86.1±12.3	0.053
(beats.min^{-1})	T6	78.5±11.6*†	82.8±16.3	0.086
	T7	77.3±17.1*	80.7±10.2†	0.604
	T8	76.3±12.5*	80.5±12.6	0.231
	T9	77.2±11.8*	81.1±12.3	0.173
	TE	85.4±14†	87.7±12.4†	0.158
	TR	75.0±13.8*†	80.8±14.7*†	0.190
	T1	96.5±2.0	96.3±2.6	0.875
	T2	99.5±0.9*†	99.1±2.1*†	0.173
	T3	99.5±0.6*	99.1±1.2*	0.246
	T4	99.4±0.7*	99.1±0.9*	0.130
	T5	99.5±0.8*	99.1±1.9*	**0.042‡**
SpO_2 (%), mean±SD	T6	99.4±0.9*	98.6±1.9*	**0.023‡**
	T7	98.0±2.8*	97.4±2.8*	0.129
	T8	97.8±2.3*	97.7±2.4*	0.670
	T9	98.1±2.3*	97.7±2.3*	0.400
	T10	97.1±1.2*	97.2±1.5*	0.521
	TE	95.7±1.3†	98.8±2.0*	0.745
	TR	95.1±6.4*	94.8±2.8*†	0.125
	T1	89.3±21.2	87.8±15.9	0.859
	T6	292.7±68.0*†	263.7±83.3*†	0.198
PaO_2 (mmHg), mean±SD	T9	144.4±64.8*†	143.3±53.8*†	0.842
	T10	143.8±63.7*	142.2±55.1*	0.576
	TE	155.9±80.1*	153.3±67.2*	0.906
	TR	107.5±29.3*†	103.4±28.1*†	0.663

*: Significant difference in within-group comparison compared to the pre-induction (T1) period ($p<0.05$).
†: Significant difference in within-group comparison compared to the previous time measurement ($p<0.05$).
‡: Significant difference between Group P and Group V ($p<0.05$).
Measurement times: pre-induction (T1), induction 3rd min (T2), intubation 1st min (T3), TLV 5th min (T4), TLV 10th min (T5), TLV 15th min (T6), OLV 5th min (T7), OLV 10th minute (T8), OLV 15th minute (T9), OLV 30th min (T10), extubation 1st minute (TE), recovery (TR).
SD: Standard deviation, SpO_2: Peripheral oxygen saturation, PaO_2: Partial pressure of arterial oxygen

Table 3. Measurements in Group P before and after the recruitment maneuvers (mean±SD)

	T9	RM (1st min)	RM (2nd min)	RM (3rd min)	p
MAP (mmHg)	85.9±1.6	84.0±10.7	85.8±11.4	83.6±10.2	0.129
SpO_2 (%)	98.1±2.3	98.0±2.3	97.8±2.5	97.8±2.6	0.073
HR (beat.min^{-1})	77.2±11.8	76.8±11.2	79.2±12.7	78.4±11.8	0.169
TV (mL)	422±66.8	471.7±7.6*	495.7±88.9*	476.3±101.1*	**<0.001**
PIP (cmH_2O)	19.0±3.5	26.7±4.3*	31.7±3.8*	36.6±3.6*	**<0.001**
Pplateau (cmH_2O)	18.7±3.5	26.4±4.3*	31.1±4.0*	36.4±3.6*	**<0.001**
Compliance (mL.cmH_2O^{-1})	29.1±9.7	31.1±10.2*	31.5±9.3*	29.9±9.3*	**0.005**

*: Significant difference when compared to OLV 15th min (T9) ($p<0.05$).
SD: Standard deviation, T9: OLV 15th minute, HR: Heart rate, MAP: Mean arterial pressure, PIP: Peak inspiratory pressure, Pplateau: Plateau pressure, RM: Recruitment maneuver, SpO_2: Peripheral oxygen saturation, TV: Tidal volume, OLV: One-lung ventilation

significant clinical differences. There was also no significant difference in oxygenation during and after RM. Hypoxemia, the first of the undesirable effects in the OLV process, is mainly due to intrapulmonary shunt (4). In our study, conducted by providing proper monitoring for gas exchange, it was observed that oxygenation decreased slightly in both groups during the transition from TLV to OLV.

It is safe to set FiO_2 at the lowest possible level to provide SpO_2≥92-94% during OLV (11). The use of inhalation anesthetics should be preferred in thoracic surgery (12). Sevoflurane was shown to provide better oxygenation, lower driving pressure, and less pro-inflammatory response compared to propofol, especially during lung resections (13). In our study, we used sevoflurane, adjusted FiO_2 to 0.8, and observed no critical decrease or increase in oxygenation.

The choice of the ideal TV and PEEP in OLV is still controversial. Many researchers chose to use low TV and moderate PEEP, as we did in the current study. A low TV (5-6 $mL.kg^{-1}$) and 5 cmH_2O PEEP combination provided sufficient oxygenation while decreasing the incidence of ALI perioperatively in a previous study (14). In the study by Ferrando et al. (15), during OLV in thoracic surgery, the effects of "individualized" PEEP and "standard" PEEP (5 cmH_2O) were compared. Arterial oxygenation was found superior with individualized PEEP, and the effects of alveolar RM on lung function were better preserved (15). Although moderate PEEP is conventionally and widely used for OLV, the future trend seems to be to routinely implement individualized PEEP.

As soon as OLV begins, PIP and the Pplateau may increase by about 50% (16). Airway pressures should be controlled during OLV, and Pplateau <25 mmHg, and PIP <35 mmHg should be targeted (17). We observed an optimal increase in airway pressures and a decrease in compliance, as expected, with the transition from TLV to OLV in both groups. We also observed lower PIP and Pplateau in the PCV group than in the VCV group during OLV. The lower airway pressures found in PCV mode may have been due to the decreasing flow pattern.

Intraoperative PCV can provide better oxygenation, lower airway pressures, and more effective CO_2 elimination. A lower TV target, long inspiration time, appropriate PEEP, and PCV applied with RM would improve oxygenation (6). According to a guideline on the management of mechanical ventilation in lobectomy patients, PCV should be preferred (18).

Another study found that PCV and VCV modes had the same performance in terms of intraoperative oxygenation and postoperative complications during OLV (7). VCV or PCV mode is recommended for lung-protective OLV. However, it is emphasized that PCV can be chosen in cases where the risk of ALI increases, such as after bullous lung, pneumonectomy, and lung transplantation (17). Consistent with our findings, Pardos et al. (19) compared VCV and PCV modes in OLV and found no difference in oxygenation but lower airway pressures in PCV mode.

Concerning the effects of VCV and PCV on hemodynamics in VATS patients, no statistically significant difference was observed in HR and MAP values (20). We detected an increase in blood pressure with the transition from TLV to OLV in both groups. A decrease in HR was observed with the transition to OLV in the VCV group. When a 5-minute HR of TLV was compared, was lower in the PCV group. However, PCV or VCV did not make a significant clinical difference in hemodynamics.

RMs are breathing maneuvers that are performed with hyperinflation in different durations and pressures to reopen atelectatic areas. While opening closed alveoli with RM, they should also be kept open. Currently, there is no consensus on the routine use and benefits of RM in anesthetized patients with healthy lungs. Miura et al. (21) reported that only the patients with lower compliance responded to alveolar RM.

The dead space volume decreases with RM, oxygenation improves, and a balanced ventilation/perfusion is achieved (14,22). Tusman et al. (22) found that arterial oxygenation increased after RM in OLV, and that application of 40 cmH_2O of inflation pressure for 40 seconds was effective. In our study, there was no significant difference between the SpO_2 at the 1st, 2nd, and 3rd min during RM in the PCV group. This finding can be explained by the sufficient but relatively high FiO_2 we used. When the 15th minute after RM was compared with pre-RM, similar SpO_2 and PaO_2 values suggested that RM did not affect oxygenation. During the RM, due to high PIP and PEEP application, an effective increase was observed in airway pressures, TV, and compliance.

When OLV 15th and 30th min values were compared, we did not find any between-group and within-group differences in hemodynamics, oxygenation, and respiratory parameters. It showed that the effects in the first 3 min did not persist. RM can lead to some complications, such as barotrauma, hypoxemia, and hypotension (23). However, we did not record any RM-related complications. There was no significant hemodynamic difference between the RM and pre-RM period. The values of MAP and HR at 1, 2, and 3 min of RM were not different compared with pre-RM. RM did not also cause hypotension or any deterioration in hemodynamics.

Recently, the "open-lung approach (OLA)" ventilator strategy (low TV, RM, and reductive PEEP titration) has been put on the agenda. A study on the effects of OLA during OLV recommended individualized PEEP adjustment (24). Besides, Slinger (25) reported that the benefit of the OLA is low in VATS patients compared to routine OLV applications. As the research for LPV in thoracic surgery is still in progress, current studies support the combined use of low TV, RM, and PEEP as the three main components of LPV (26).

Study Limitations

Our study has several limitations. Firstly, routine bronchoscopic control of the DLT placement was not available. In general left-sided DLT, which can be easily used without the need for routine FOB, is preferred. We also used the left-sided DLT uneventfully, without the need for a FOB. Secondly, we used widely accepted, fixed (5 cmH_2O) PEEP intraoperatively. Thirdly, we did not perform a follow-up for long-term postoperative pulmonary complications.

Conclusion

In conclusion, this study showed that the VCV and PCV modes did not have clinical superiority over each other regarding hemodynamics and oxygenation in VATS patients. This finding suggests that both modes are effective and can be used safely in patients who are respiratory and hemodynamically stable preoperatively. However, the lower peak and plateau pressure in the PCV group may be an advantage in preventing ALI. In the PCV group, RM applied in the OLV period effectively provided higher compliance and TV. Nevertheless, future RCTs should be conducted to improve these parameters to have a stable effect on oxygenation and to optimize RMs.

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Ethics

Ethics Committee Approval: Ethics approval for the study was obtained from Kecioren Training and Research Hospital Clinical Research Ethics Committee (date: 27.05.2015, reference number: 859/2015).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices - Analysis or Interpretation - Writing: E.S., H.S., M.T., F.U., A.A., Concept - Design: E.S., H.S., F.U., Data Collection and Processing: E.S., Literature Search: E.S., H.S.

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Endoscopic resection of intracranial dermoid and epidermoid tumors from a minimally invasive perspective

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ABSTRACT

Aims: Intracranial dermoid and epidermoid tumors (IDETs), benign, congenital lesions, are traditionally treated using open surgery. However, total resection (TR) can be difficult due to adhesions to surrounding structures. While endoscopic surgery has emerged as a less invasive approach, its success may differ across centers. This study evaluated the outcomes of IDET endoscopic surgery cases and compared them with the literature.

Methods: We retrospectively reviewed patients who underwent an operation for epidermoid and dermoid tumors using an endoscopic approach from 2010 to 2020. Age, sex, tumor location, tumor size, surgical approach, resection rates, intraoperative and postoperative complications, length of hospital stay and recurrence rates of the cases were evaluated.

Results: The study included 18 patients (mean±standard deviation age: 39.6±15.5 years, age range 15-68 years, male sex: n=12, 66.6%). The tumor locations were 2 temporal (11.1%), 2 frontal (11.1%), and 1 occipital (5.5%) (all intraparenchymal); 2 frontobasal (11.1%), 3 suprasellar (16.6%), 2 lateral orbital (11.1%), 1 intraorbital (5.5%), 3 cerebellopontine angle (16.6%), and 1 located in the lateral ventricle (5.5%); and 1 located in the third ventricle (5.5%). TR, near-TR and subtotal resection were performed in 7 (38.8%), 5 (27.7%), and 6 (33.3%) cases, respectively. No postoperative complication was recorded except for one patient who develpped rhinorrhea and pneumocephaly. No intraoperative complication was recorded. The mean length of hospital stay was 7.9 days (range, 5-28 days).

Conclusions: IDETs can be treated by endoscopic approaches, without the need for large incisions and craniotomy in open approaches. Decreased hospital stay seems to be the primary advantage of endoscopic surgery.

Introduction

Intracranial dermoid and epidermoid tumors (IDETs) are benign congenital lesions that constitute approximately 1% of all intracranial lesions (1). In the third to fifth stages of embryogenesis, they occur because of embryological sequestration of the neural tube during ectoderm development (2,3). These lesions can be found at many intracranial locations, often in the cerebellopontine angle (CPA) (4,5). They can rarely be located in the spinal canal.

IDETs are generally treated with standard craniotomy using microscopic methods (6). The traditional open approach requires a large craniotomy and a wide corticotomy. However, total resection (TR) is not possible in most cases due to tumor enlargement toward the subarachnoid area and capsular invasion of the lesions extending into the neural and vascular structures (2,6,7). Therefore, morbidity and mortality rates may be high in patients for whom TR is aimed (6,7). A large

craniotomy, wide corticotomy, and significant brain retraction increase the possibility of intraoperative and postoperative complications. Hence, the need for more minimally invasive surgical methods has emerged.

Recently, endoscopic methods have also been used for the treatment of IDET. Some authors have argued that endoscopic interventions have some advantages over microscopic surgeries because they are minimally invasive (8-11).

In this study, we aimed to compare IDET cases treated with endoscopic methods with similar studies in the literature and contribute to the literature in terms of treatment effectiveness.

Methods

Patients

Between 2010 and 2020, patients who underwent an operation for IDET with endoscopic interventions were retrospectively reviewed. Cases operated in our clinic due to dermoid or epidermoid tumors were included in the study. Patients who did not undergo surgery were excluded from the study. Resection rates, difficulties encountered with the endoscopic technique in the intraoperative period, intraoperative and postoperative complications, length of hospital stay, and recurrence rate were evaluated. The Gülhane Training and Research Hospital Local Ethics Committee approved the study protocol (date: 05.12.2020, no: 322).

Radiological evaluation

All patients underwent preoperative magnetic resonance imaging (MRI) that included diffusion sequence because IDETs are distinguished from other cystic lesions because of diffusion restrictions. Computed tomography (CT) and CT angiography orders that were performed to evaluate the relationship between vascular structures and suprasellar and CPA localized tumors were also reviewed. Before the operation, a navigation MRI was also performed.

The tumor resection rate was evaluated according to the intraoperative findings and MRI obtained in the postoperative 3rd month. Tumor resection was classified as TR (TR; complete evacuation of the capsule contents and total excision of the capsule), near-TR (NTR; complete evacuation of the capsule content but the presence of capsule remnant, and subtotal resection (STR) (STR: presence of the remnant of the intracapsular content and capsule).

Age, sex, tumor location and size, surgical approach, resection rates, postoperative complications, histopathological diagnosis, and follow-up periods of the cases were recorded (Table 1).

Surgery

After the induction of general anesthesia, a skull clump was attached and positioned according to the lesion site and surgical procedure. In brief, following positioning, an intraoperative navigation system (Stealth Station, Medtronic, USA, for all patients in this study) is used during surgery. An 11 mm diameter, 5 cm long thoracoport (Tyco Healthcare Group LP, for all patients in this study) is used as the endoscopic port in the endoscopic tubular transcortical (ETTC) approach. Rigid endoscopes (Karl Storz, GmbH & Co. KG, Tuttlingen, Germany, for all patients in this study) with 0°, 30°, and 70° angles, 4 mm diameter, and 18 cm length are used.

The endoscopic endonasal (EE) approach is chosen for midline frontobasal and suprasellar tumors, ETTC approach is chosen for intraparenchymal (frontal, temporal, occipital), lateral orbital, intraorbital and ventricular tumors (lateral and third ventricle), and endoscopic non-tubular posterior fossa (ENTPF) approach is chosen for CPA tumors. In the EE approach, the right lower quadrant of the abdomen or lateral left thigh is used as the source of grafts for the perinasal region and fascia.

A nasoseptal (Hadad) flap is prepared for reconstruction from the appropriate side (12). The sphenoid sinus ostia are identified, and the base of the sellae, tuberculum sella, and part of the planum sphenoidale is opened to reach the dura (Figure 1G-L). For frontobasal localization, the ethmoid bulla is reached after uncinectomy. Anterior and posterior ethmoidectomies are performed, and the anterior and posterior ethmoid arteries are coagulated and cut. After the superior and posterior parts of the nasal septum were resected, the cribriform plate and fovea ethmoidalis are resected, and the dura is exposed (Figure 2G-H). The dura is opened, and the tumor contents are evacuated via endoscopic vision.

In the ETTC approach, depending on the localization of the lesion, a skin incision of approximately 4 cm or a small “horseshoe” flap that would allow a craniotomy of approximately 3x3 cm is made. The dura opens in an “X” shape. After a 1 cm corticotomy, a thoracoport is placed with navigation-guided parenchymal dissection. The lesion was reached by advancing through the port and the tumor contents were evacuated under endoscopic vision (Figure 3G-L, Figure 4G-I). For tumors in the third ventricle, the tumor is visualized from the lateral ventricle with an endoscope using the transforaminal approach. The tumor contents were evacuated via endoscopic vision.

ENTPF approaches (CPA localized cases), after the linear incision, a retrosigmoid suboccipital small craniectomy is performed. The dura is opened in an “X” shape. The cerebellomedullary cistern is opened, some cerebrospinal fluid (CSF) is drained, and cerebellar retraction is achieved. The tumor capsule is accessed, and the tumor contents are evacuated via endoscopic vision.

The tumor was excised using biopsy forceps, curetted, and aspirated. After the contents are completely removed by blunt dissection and copious washing, if the tumor capsule allows resection, it is dissected from the surrounding structures and

removed. In cases of aseptic meningitis, the tumor site is washed abundantly with physiological saline. After controlling the bleeding, the closing phase is started.

In the EE approaches, an "inlay" graft is placed in the subdural space of the autologous fascia lata as the first layer for reconstruction after resection. The second layer, fascia lata, or synthetic dura "onlay" graft is laid on the bone defect. As the third layer, a previously prepared nasoseptal flap is placed on the onlay graft. Lumbar drainage is applied to all cases before extubation and 8-10 cc drainage per hour was planned. Following the initiation of oral intake, acetazolamide treatment (3x250 mg/day) is started.

The bone flap is fixed to the cranium after suturing the dura mater except for the EE approach. The skin and subcutaneous tissue are sutured. In the first 24 h after the surgery, CT imaging is performed to rule out possible complications (hematoma and pneumocephaly).

Statistical Analysis

Parameters such as age, gender, symptoms at admission, comorbidities and other quantitative parameters were analyzed. Numerical variables of patient data were expressed as mean±standard error and minimum (lowest)-maximum (highest values). Categorized variables were explained as the number of patients (n) and percentage (%) with descriptive statistics.

Results

Patient population

The study included 18 patients (mean±standard deviation age: 39.6±15.5 years, age range 15-68 years, male sex: n=12, 66.6%). The distribution of the cases according to the location was as follows: 2 temporal (11.1%), 2 frontal (11.1%), 1 occipital (5.5%) (all intraparenchymal); 2 (11.1%) midline frontobasal, 3 (16.6%) suprasellar, 2 (11.1%) lateral orbital, 1 intraorbital (5.5%), 3 (16.6%) CPA, 1 (5.5%) lateral ventricle and 1 (5.5%) third ventricle.

Surgical approach

EE approach (2 midline frontobasal, 3 suprasellar positions) was performed in 5 cases [2 (40%) TR, 1 (20%) NTR, and 2 (40%) STR]. Rhinorrhea and pneumocephalus developed in a patient with a dermoid tumor, despite lumbar drainage. The defect was repaired using the EE method on the sixth postoperative day. As the pneumocephaly continued and the general condition of the patient deteriorated, reconstruction was performed using a pericranial flap with the bifrontal craniotomy approach. The patient was discharged on the 28th day of hospitalization.

The ETTC approach (5 parenchymal, 1 intraorbital, 2 lateral orbital, 2 ventricular positions) was performed in 10 cases [5 (50%)

Table 1. Age, sex, tumor location and size, surgical approach, resection rates, postoperative complications, pathological diagnosis and follow-up periods of the cases

Case	Age/sex	Location	Tumor size (cm)	Approach	Resection	Complication	Pathology	Follow-up (month)
1	27/M	Midline frontobasal	6x3.5x4.8	EE	TR	Rhinorrhea, pneumocephaly	D	60
2	36/M	Suprasellar	3.1x3x3.7	EE	STR	-	E	8
3	55/M	Temporal	2.8x1.7x3	ETTC	TR	-	E	16
4	30/F	Intraorbital	3x1.2x1.8	ETTC	STR	-	E	60
5	44/M	Lateral orbital	2.9x1.7x2	ETTC	TR	-	D	9
6	25/M	Frontal	3.5x2.5x3	ETTC	TR	-	E	60
7	64/M	Lateral orbital	2x2.3x2.5	ETTC	NTR	-	E	20
8	21/M	Cerebellopontine angle	4.5x2.4x4.3	ENTPF	STR	-	E	60
9	33/F	Lateral ventricle	3.5x2.5x2	ETTC	NTR	-	D	48
10	31/M	Suprasellar	3.5x2.5x3	EE	STR	-	D	31
11	22/M	Frontal	4.5x4.5x4	ETTC	NTR	-	E	23
12	68/M	Third ventricle	2.8x2.5x2.6	ETTC	NTR	-	D	11
13	57/M	Occipital	3.5x3x2	ETTC	TR	-	E	60
14	43/M	Temporal	4x3.5x4	ETTC	TR	-	E	60
15	29/F	Midline frontobasal	4x4x3.5	EE	NTR	-	D	48
16	36/F	Cerebellopontine angle	4x4.5x3.5	ENTPF	STR	-	D	40
17	15/F	Cerebellopontine angle	2.5x1.8x2	ENTPF	STR	-	E	7
18	29/F	Suprasellar	2x2.5x3	EE	TR	-	E	50

EE: Endoscopic endonasal, ETTC: Endoscopic tubular transcortical, ENTPF: Endoscopic non-tubular posterior fossa, TR: Total resection (complete excision of tumor contents and capsule), NTR: Near total resection (complete resection of the tumor content and leaving the capsule remnants in places), STR: Subtotal resection (remaining of the capsule and less than 10% tumor content), E: Epidermoid, D: Dermoid, M: Male, F: Female

TR, 4 (40%) NTR, and 1 (10%) STR]. None of the patients developed intraoperative or postoperative complications due to the operation.

The ENTPF approach was applied in 3 cases (localized in the CPA). STR was performed in all of them. No patient developed intraoperative or postoperative complications due to the operation.

No patient-developed complications, such as stroke, septic, and aseptic meningitis, cranial nerve or other neurological injuries, or wound complications.

Seven (38.8%) TR, 5 (27.7%) NTR, and 6 (33.3%) STR were performed (Figures 1-4). In 6 patients who underwent STR, the capsule and a portion of the tumor were left because the tumor capsule adhered to critical neurovascular structures.

In the histopathological evaluation, 11 (61.1%) cases were epidermoid tumors and 7 (38.8%) were dermoid tumors.

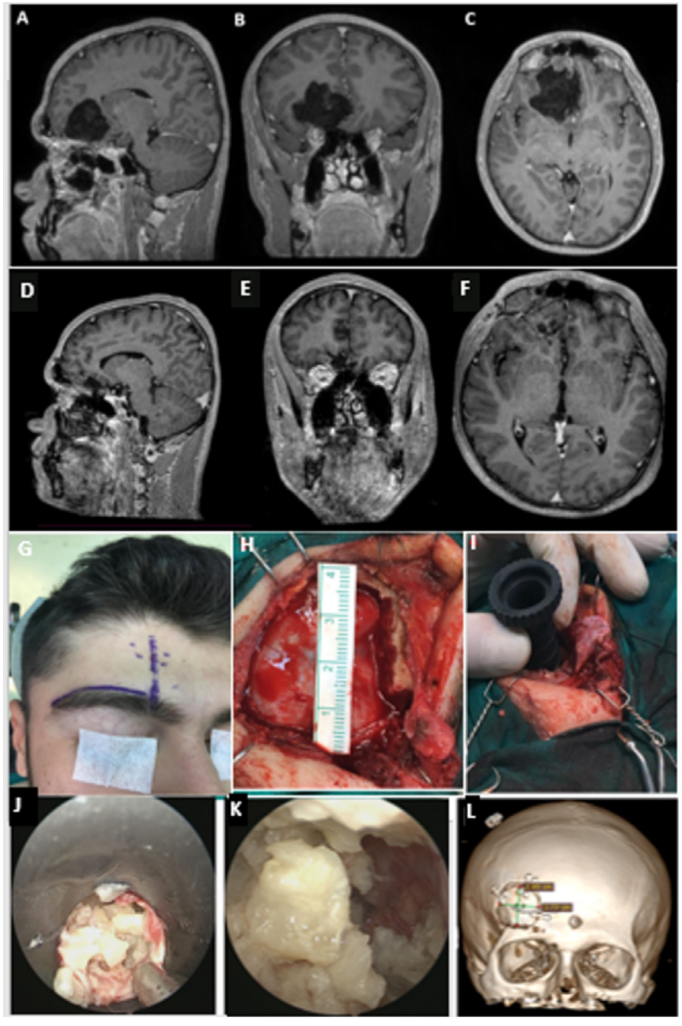

Figure 1. Case #11 Pre-intra-postoperative images of a frontal located epidermoid tumor case operated with ETTC approach. **A-C)** Preoperative cranial T1 contrast MRI. **D-F)** Postoperative 3rd month T1 contrast MRI. **G)** Incision plan and supraorbital nerve line. **H)** After craniotomy. **I)** Placement of the port after corticotomy. **J)** Reaching the tumor tissue. **K)** View of the intraparenchymal area during resection of the tumor. **L)** Three-dimensional view after surgery and craniotomy borders

MRI: Magnetic resonance imaging, ETTC: Endoscopic tubular transcortical

Postoperative period

Lumbar drainage was followed for an average of 5 days (range, 3-8 days). When all the cases were evaluated, the mean length of hospital stay was 7.9 days (range: 5 to 28 days). The reason for a longer hospital stay was the reoperation of a patient with a frontobasal tumor due to rhinorrhea and pneumocephalus. The patients were followed up at the 3rd, 6th, and 12th months in the first year. The mean follow-up period was 37.1 months (range: 7 to 60 months). Reoperation was not required in any patient.

Discussion

IDETs are slow-growing, benign, congenital lesions (4,5). They are usually found in the CPA, the fourth ventricle, and parasellar regions, and are less frequently found in the cerebral hemispheres and brainstem (4,5,13).

The classical surgical treatment of these tumors involves evacuation of the tumor contents under microscopic visualization and then excision of the tumor capsule if the parenchyma tissue allows capsule excision (6). TR of IDET cannot often be possible (14-16). New surgical techniques have been sought to reduce operative morbidity and achieve better surgical results.

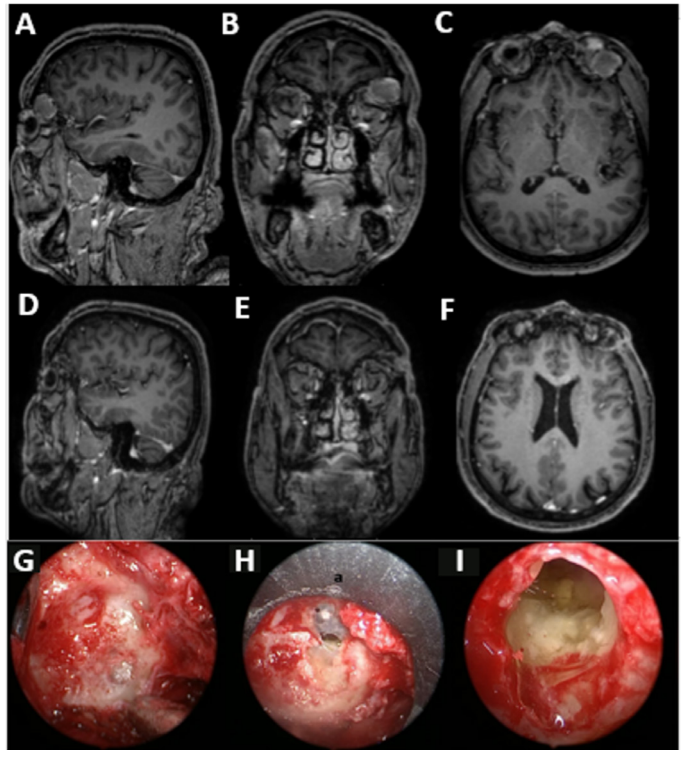

Figure 2. Case #7 Pre-intra-postoperative images of a lateral orbital epidermoid tumor case operated with ETTC approach. **A-C)** Preoperative cranial T1 contrast MRI. **D-F)** Postoperative 3rd-month cranial T1 contrast MRI. **G)** Before craniotomy. **H)** Appearance of tumor after craniotomy (a. port inserted through craniotomy defect). **I)** Stage of tumor resection

MRI: Magnetic resonance imaging, ETTC: Endoscopic tubular transcortical

Endoscopic methods have been used for treating IDET recently owing to their advantages such as panoramic vision, close view, clear detail, and ideal illumination of the surgical area. Additionally, resection of epidermoid and dermoid tumors can be achieved by making less brain retraction, minor craniotomy, and dural incision (17-19). With angled endoscopes (30-45-70 degrees), tumor tissues hidden behind important neurovascular structures under the microscope can be resected without any retraction (13,18,20). As a result, fewer complications occur after surgery. Postoperative complications developed in only 1 of 18 patients who underwent surgery in our study.

Tubular retractors have been used in various cranial approaches, such as intracranial hematomas, cranial tumors, colloid cysts, arteriovenous malformations, and cavernous malformations (9,21). Tubular retractors provide the ability to visualize the tumor and surrounding structures during the operation, as well as to create and maintain a safe corridor into the tumor. The use of a thoracoport also prevents accidental enlargement of the small corticotomy and iatrogenic damage to the surrounding tissues (9,21). Tubular retractors minimized retractor-related force injuries through the radial force distribution. Additionally, with tubular retractor systems, deep white matter tracts can be divided and preserved instead of being cut using the blunt tip and progressive dilation technique (9,21). Thoracoport has been especially used in endoscopic surgery of deeply located tumors because it allows dynamic mobilization and facilitates bimanual operation (9,21). In transcortical approaches since non-tubular retractors do not apply equal force to the surrounding parenchyma, the stress on the brain tissue may cause increased local cerebral tissue pressure and impaired regional cerebral blood flow. It has even been reported that this may cause focal neurological deficits, seizures, or cognitive disorders (9,21). Additionally, non-tubular retractors may cause damage to the surrounding parenchyma by applying high pressure during withdrawal. We also used a thoracoport in our cases with intraventricular, intraparenchymal, and intra-lateral orbital localizations and did not need a large craniotomy.

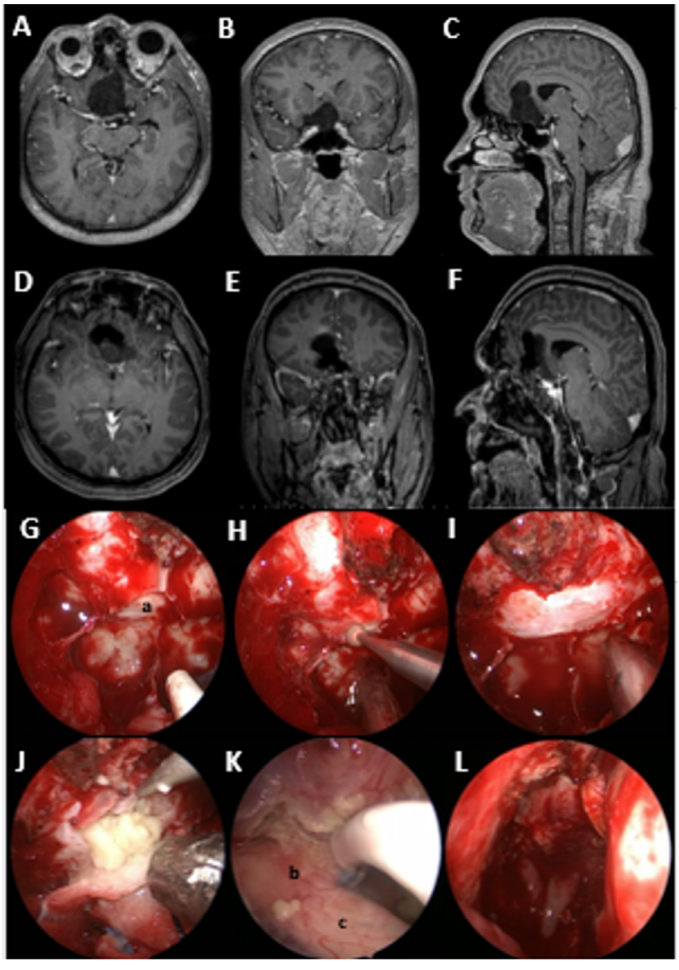

Figure 3. Case #2 Pre-postoperative images of a suprasellar epidermoid tumor case operated by the EE approach. **A-C)** Preoperative cranial T1 contrast MRI, **D-F)** Postoperative day 0 cranial T1 contrast MRI. **G)** Sphenoid sinus (a. Floor of the sella). **H)** Drilling of the floor of the sella. **I)** Before opening the dura. **J)** Evacuation of tumor contents. **K)** Tumor lodge after resection (b. Optic chiasm, c. Left optic nerve), **L.** Resection post-reconstruction phase and laying of the nasoseptal flap

MRI: Magnetic resonance imaging, EE: Endoscopic endonasal

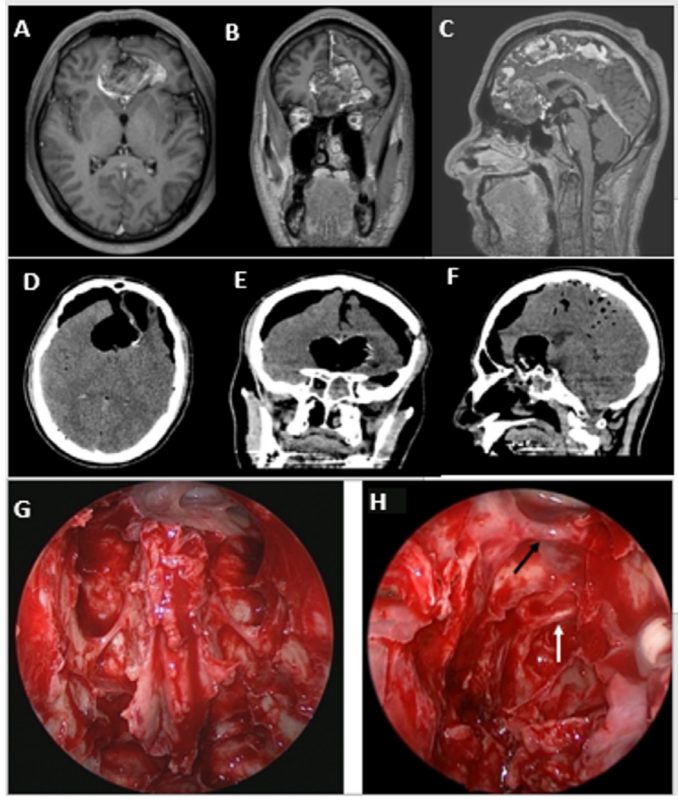

Figure 4. Case #1 A midline frontobasal dermoid tumor case operated by the EE approach. **A-C)** Preoperative cranial T1 contrast MRI, **D-F)** Postoperative cranial tomography. **G)** Identification of the cribriform plate. **H)** View of the anterior (black arrow) and posterior ethmoidal (white arrow) arteries

MRI: Magnetic resonance imaging, EE: Endoscopic endonasal

Generally, resection rates range from 0% to 97% in IDETs surgically treated using the microscopic method (14,15,19,20,22). Yaşargil et al. (6) reported a TR rate of 95% in the epidermoid tumor series that they treated with the microscopic operation. Velho et al. (22) reported a rate of 15.3% in their series containing 234 IDET cases that they treated with microscopic methods. Singh et al. (23) reported a TR rate of 91.6% and an STR rate of 8.3% in their series of 48 cases, which they treated with combined endoscopic and microscopic techniques. In that study, the cases were first treated with a microscopic technique, and then the surgical area was controlled with an endoscope. Residual tumor was detected in 38 patients (79%) after endoscopic control, therefore, the authors stated that the endoscopic method is more beneficial. Similarly, other studies have shown that endoscopic procedures are at least as effective as microscopic procedures in removing these tumors (18,24,25). In our study, resection results were also correlated with endoscopic studies in the literature: 7 (38.8%) total, 5 (27.7%) NTR, and 6 (33.3%) STRs were performed.

When IDET cases located in the CPA were evaluated, Gopakumar et al. (26) reported TR in a bilateral epidermoid tumor using endoscopic methods. Additionally, Peng et al. (18) reported 83% TR in six cases localized in the pontocerebellar angle and treated with endoscopic interventions. In our study, capsular resection could not be performed in all three cases of tumors localized in the CPA because of the adhesion of the tumor capsule to critical neurovascular structures, and the tumor resection remained at the subtotal level. In our study, capsular resection could not be performed in all three cases of tumors located in the CPA due to the adhesion of the tumor capsule to critical neurovascular structures, and the tumor resection remained at a subtotal level. Insisting on resection in these cases may cause cranial nerve damage or vascular injury.

When the resection of intraventricular IDET is examined, Paz et al. (24) reported STR of an epidermoid tumor localized in the third ventricle with suprasellar extension who underwent endoscopic resection. In our study, NTR was achieved in both cases located in the lateral and third ventricles. Because of its proximity to critical neurovascular structures, lesions of this region are difficult to remove to a large extent. However, tumor tissues were largely resected in 2 of our cases.

Recently, EE approaches have also been used in the treatment of non-pituitary lesions of the midline ventral skull base (25,27-29). These approaches provide a direct surgical route to tumor access by minimizing neurovascular manipulation and eliminating brain retraction, without the need for a skin incision (27,28,30). In a study including 21 cases (suprasellar, prepontine, parasellar, anterior-middle cranial fossa localized) with epidermoid and dermoid tumors treated with the EE approach, 8 (38.1%) patients had a total, 9 (42.9%) patients had gross total, and 4 (42.9%) patients had reported STR (30). When the literature was reviewed, other studies reporting successful resection rates for the EE resection of dermoid and epidermoid tumors localized in the suprasellar region, and anterior and middle cranial fossa were also found (18,25,31). In our study, TR was performed in 2 of the 5 cases with midline frontobasal and suprasellar localization, with the EE route, and NTR in 1 and STR in 2 patients.

Another advantage of endoscopic surgery is its short hospital stay. It has been reported that 86% of the patients in the TR group were followed in the intensive care unit for more than 10 days in 234 intracranial epidermoid tumor cases operated with microscopic methods (22). Also, the hospitalization period in an epidermoid tumor case operated with microscopic methods was reported 7 days (32). The length of hospital stay after EE resection of IDET ranges from 3 to 5 days in many studies (17,33,34). In one of our cases with a frontobasal location, the hospitalization duration was prolonged because of postoperative rhinorrhea and pneumocephalus, and the patient was discharged on the 28th day of hospitalization. Therefore, the mean length of hospital stay in our series was prolonged to 7.9 days (range, 5-28 days).

Another problem in the surgery of IDET is postoperative recurrence. Generally, the recurrence rates after the resection of IDET range from 1% to 27% (6,22,35,36). Singh et al. (23) did not report recurrence in 48 intracranial epidermoid tumors using the combined endoscopic and microscopic technique. Vaz-Guimaraes et al. (30) reported recurrence in two cases (9.5%) who underwent inadequate resection due to the suprasellar extension among 21 patients (15 primary, 6 relapses). Reoperation of these tumors is difficult due to their adhesion to the surrounding critical neurovascular structures after the first surgery (6,37). Therefore, these tumors should be reoperated when the patient becomes symptomatic again. Reoperation is usually done for decompression purposes. It is not easy to predict the time of recurrence. Although dermoid tumors are easier to resect than epidermoid tumors because of their dark consistency, these tumors may recur more aggressively than epidermoid tumors (6,20,37). The rate of postoperative morbidity in reoperated patients is higher than in patients who undergo primary surgery (6,7). The mean follow-up period in our cases was 37.1 months (range, 7 to 60 months). During the follow-up period, none of the patients who underwent near total and STRs required reoperation.

The mortality rate was reported as 5% in 43 patients with IDET who were operated with the microscopic methods (37). It has been reported in the literature that a patient with a tumor located in the parasellar region, who was operated with microscopic methods, died from hydrocephalus and other complications in the postoperative period (38). No mortality was encountered in the postoperative period in any of our cases. Although it was located at critical locations, mortality did not develop in any of our cases during the postoperative period.

Endoscopic methods have both advantages and disadvantages. First, the images obtained with the endoscope are two-dimensional. Another disadvantage of the endoscope is the long learning curve of the endoscopic surgical technique. That is, the skill of using the endoscope is a time-consuming process.

Complications such as aseptic meningitis, bacterial meningitis, anosmia, hypopituitarism, and cranial nerve paralysis have been reported in addition to rhinorrhoea in EE approaches (30). In the epidermoid and dermoid tumor series of 21 cases (15 primary and 6 relapses) treated with the EE route, CSF leakage was reported in 5 cases, bacterial infection was reported in 4 cases, transient 6th cranial nerve palsy was reported in 2 cases, and hydrocephalus requiring ventriculoperitoneal shunt was reported in 4 cases (30). In our study, postoperative complications, which were rhinorrhea and pneumocephalus, occurred in only 1 of 5 patients (midline frontobasal tumor) who underwent EE surgery. Complications such as aseptic meningitis, bacterial meningitis, focal neurological deficit, cranial nerve paralysis, seizures, hearing loss (especially in cases with CPA localization), wound infection, deep vein thrombosis and pulmonary embolism, and hydrocephalus requiring postoperative shunt have been reported in non-endonasal approaches (6,14,15,22,30). In our study, the capsule contents and subarachnoid spaces were washed with plenty of saline during the operation, and aseptic meningitis did not develop in any patient.

Study Limitations

The strength of our study is limited by its retrospective data collection and small sample size. Additionally, there are no long-term follow-up data available.

Conclusion

To the best of our knowledge, this is the first study from Türkiye to report the outcomes after endoscopic resection of IDETs. The success with endoscopic resection of epidermoid and dermoid tumors in various parts of the intracranial region, including the ventral skull base, appears similar to open resection using microscopic methods.

Ethics

Ethics Committee Approval: The Gülhane Training and Research Hospital Local Ethics Committee approved the study protocol (date: 05.12.2020, no: 322).

Informed Consent: This was a retrospective study, and descriptive data (identity information, face picture) were not used. Therefore, patient consent was not obtained for this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.D., M.O.D., Concept: A.D., A.M.K., Design: A.D., A.M.K., S.K., Data Collection or Processing: A.D., Ş.K., Analysis or Interpretation: M.O.D., A.M.K., Ş.K., Literature Search: A.D., M.O.D., Writing: A.D., M.İ.Ö.

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

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Serum asporin levels in maintenance hemodialysis patients without osteoarthritis

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ABSTRACT

Aims: Several human and experimental studies have shown that small leucine-rich proteoglycans might play a significant role in inflammation and fibrosis in various renal diseases. However, as far as we know, no study has reported asporin levels in patients with advanced renal disease. The primary aim of this study was to determine serum asporin levels in hemodialysis (HD) patients without symptomatic osteoarthritis.

Methods: This single-center, cross-sectional study prospectively enrolled maintenance HD patients and healthy control subjects. Subjects with clinically clear osteoarthritis were excluded. Serum asporin level was measured via Human ASPN (Asporin) ELISA Kit (Elabscience Biotechnology Inc. Houston, Texas, USA) in fasting blood samples.

Results: The study included 25 (mean age: 43.3±13.5 years, 60% were females) patients and 29 control subjects (mean age: 38.0±8.8 years, 37.9% were females). Patients and controls were similar in age and sex. Serum asporin levels were significantly higher in HD patients compared with the controls 2.4 (0.9-4.8) ng/mL vs. 0.3 (0.2-0.6) ng/mL, respectively, $p<0.001$). Asporin levels were not correlated with age ($r=0.344$, $p=0.092$) and the duration of HD ($r=0.385$, $p=0.077$). Among HD patients, asporin level was not significantly correlated with C-reactive protein, parathyroid hormone, calcium, or phosphorus levels.

Conclusions: This study showed that serum asporin levels were significantly elevated in patients undergoing HD. Further studies must elucidate the possible origins of increased asporin in these patients.

Introduction

Small leucine-rich proteoglycans (SLRPs) are non-collagen constituents of extracellular matrix (ECM) that play important roles in cell proliferation and function (1,2). Asporin is a member of the SLRP family. Since its discovery in 2001, it has been increasingly shown to be involved in the pathogenesis of various disease processes, including osteoarthritis (OA), cardiovascular disease, and cancer (3-6). The pathophysiologic roles of SLRPs stem from the fact that they can interact with several extracellular receptors, such as transforming growth factor-β1 (TGF-β1), fibronectin, and bone morphogenic protein-4 (7,8). Despite several studies suggesting the association of aspartic acid repeat polymorphism of the asporin gene with OA risk, some meta-analyses have not reported similar findings (9,10). However, D-repeat polymorphism was associated with an increased risk of OA only in male patients (11).

The plausible explanations supporting the role of asporin in the pathogenesis of OA and the heterogeneity of data in the meta-analysis suggest that larger and more detailed studies are needed to clarify this issue. Asporin can inhibit the binding of TGF-β1 to its receptor and consequently impairs TGF-β1 driven chondrogenesis (7). Due to the chondrogenesis and osteogenesis-blocking effects of TGF-β1, asporin can induce OA when expressed in increased amounts (3). Moreover, asporin can bind collagen and help its mineralization (12).

Musculoskeletal disorders are prevalent in the hemodialysis (HD) population. In a cross-sectional study of HD patients, Hage et al. (13) reported that 54% of the patients had OA changes at least in one skeletal area. The most frequently affected area was the spine. The relatively advanced age of end-stage kidney disease patients might in part account for this increased prevalence of OA. However, renal osteodystrophy is a heterogeneous syndrome involving several distinct bone pathologies, namely, osteoporosis, osteomalacia, osteitis fibrosa, and adynamic bone disease (14). Some studies reported changes in serum asporin levels in some disease states other than OA (15). Asporin has been mentioned in the literature as a promising extracellular tissue-specific protein for pharmacogenomic approaches in bone and joint diseases, but there is little published data, and its level is increased in common bone and joint diseases (5,16).

Chronic inflammation and fibrosis are common features of chronic kidney disease (CKD). ECM deposition is central to the evolution of kidney disease as it can lead to impaired matrix composition and ultimately scar formation. It also acts as a network for different molecular mediators, such as enzymes, growth factors, and cytokines (17). Among the various ECM components, SLRPs seem to play a crucial role in renal inflammation and fibrogenesis and can lead to loss of organ function (18). It has become increasingly known that SLRPs can induce anti-inflammatory responses as well as act as “classic damage-associated molecular patterns” in renal inflammation and fibrosis (19). Several human and experimental studies have shown that SLRP might play a significant role in inflammation and fibrosis in various renal diseases (20). These studies also unveiled the dual role of SLRPs in renal disease: pro- or anti-inflammatory, depending on the context (19). Several studies reported increased serum SLRP levels; however, data regarding asporin levels and SLRP levels in human disease are not sufficient.

As far as we know, no study has reported asporin levels in patients with advanced kidney disease. Understanding changes in the serum levels of asporin in advanced renal disease is considered important because several disease markers show altered levels in patients with kidney disease independent of their disease associations. Moreover, several markers are either metabolized in or excreted from the kidney. Thus, changes in renal function might affect the serum levels of the markers being studied. In the asporin example, prevalent bone and cartilage changes in the setting of CKD might be another reason for altered serum levels. Hence, for the first time, we evaluated serum asporin levels in maintenance HD patients in a cross-sectional case-control study.

Methods

This was a cross-sectional study that compared serum asporin levels in HD patients and gender- and age-matched control subjects. The association of the asporin level with the variables of calcium-phosphorus metabolism was also studied in HD patients.

The study was conducted at Medicana International Ankara Hospital, Türkiye. Outpatients who were in the chronic HD program due to end-stage renal disease, who were followed up/evaluated for transplantation in the nephrology and renal transplantation outpatient clinic, and who volunteered to participate in the study were consecutively included. Control subjects were the volunteers from the check-up clinics. Exclusion criteria were as follows: known symptomatic OA, patients who are not on a regular HD program, peritoneal dialysis patients, and patients ≤18 years of age. All study participants gave written informed consent. The Medicana International Ankara Hospital’s Ethics Committee approved the study protocol (BŞH-2022/11). All study procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Age, sex, body weight, and body height were recorded. Midweek predialysis blood samples were used to measure serum asporin and calcium and phosphorus metabolism parameters, including calcium, phosphorus, 25(OH) vitamin D, and parathormone (PTH). Control subjects also provided blood samples.

Asporin was measured via Human ASPN (Asporin) sandwich ELISA Kit (Elabscience Biotechnology Inc. Houston, Texas, USA). The micro ELISA plate in the kit was pre-coated with a human ASPN, and then Avidin-Horseradish Peroxidase (HRP) conjugate was added to each microplate well and incubated. After washing away the free components, the substrate solution was added to each well. Only wells that included ASPN, detection antibody, and Avidin-HRP conjugate appeared blue. stop solution was added to end the enzyme-substrate reaction. The optical density was determined by spectrophotometry at 450 nm±2 nm. ASPN level in samples was calculated by comparing their optical density against the standard curve.

Statistical Analysis

IBM Statistical Package for the Social Sciences Statistics for Windows, Version 25.0. (IBM Corp., Armonk, NY: USA) was used for statistical calculations and to prepare boxplot graphics. Continuous variables were expressed as mean±standard deviation or median (minimum-maximum), whereas categorical variables were reported as numbers and percentages. The chi-square test was used to compare categorical variables between HD patients and controls. To detect whether the distribution of continuous variables is normal or not, we used the Shapiro-Wilk test, histogram, and Q-Q plots. To compare the groups in terms of age, body mass index, and PTH level, the independent t-test was performed. Serum phosphorus, albumin, corrected calcium, and asporin levels were compared between the two groups by the Mann-Whitney U test. The statistical significance level was set as $p<0.05$.

Results

The study included 25 maintenance HD patients and 29 control subjects. The mean age of patients vs. controls was similar (43.3±13.5 years vs. 38.0±8.8 years, p=0.102). The median duration of HD was 30 months (9-75 months). In the patient group, 10 had hypertension, two had hypertension + hypothyroidism, one had diabetes mellitus, one had hypertension + hyperlipidemia, one had Alport syndrome, one had coronary artery disease + hypertension, and one had hypertriglyceridemia. Eight HD patients did not have additional comorbidities. The two groups were also comparable in terms of sex. Mean serum phosphorus and PTH levels were significantly higher in HD patients compared with controls (Table 1).

Asporin level was significantly higher in HD patients [2.4 (0.9-4.8) ng/mL] relative to the control group [0.3 (0.2-0.6) ng/mL] (Table 1, Figure 1). In HD patients, the median asporin level was 2.5 (0.9-4.3) ng/mL in females, and 2.4 (1.0-4.8) ng/mL in male patients (p=0.523). Among HD patients, serum asporin levels showed no significant correlation with C-reactive protein (CRP), PTH, calcium, or phosphorus levels (Table 2). No correlation was detected between age and asporin level. However, there was a strong positive correlation between serum asporin level and dialysis duration (Table 2).

Discussion

In this study, we demonstrated that HD patients had significantly higher serum asporin levels compared with control subjects. Serum asporin level significantly increased in parallel with the duration of dialysis. To the best of our knowledge, this is the first study reporting serum asporin levels in patients with CKD.

Dialysis patients have many comorbid diseases, including musculoskeletal diseases (21). Renal osteodystrophy, now known as the mineral and bone disorder of CKD, affects almost all HD patients (22). The strong correlation between the dialysis vintage and the serum asporin level in the current study may point to a more severe underlying renal osteodystrophy in patients with a longer dialysis duration. Although we excluded patients with clinically manifest OA from the study, asymptomatic or unreported OA might have been missed in some patients. Thus, increased serum asporin levels might reflect the underlying asymptomatic OA changes, particularly in the spine. However, many disease markers are altered in HD patients due to changes in their metabolism of renal excretion, which might have also contributed to elevated serum asporin levels in our patients (23).

Experimental studies have revealed the anti-inflammatory and anti-fibrotic roles of several SLRPs. Particularly biglycan, an SLRP, interacts with interleukin-1-beta and nicotinamide adenine dinucleotide phosphate oxidase (NOX2) (19). Another SLRP, decorin, neutralizes the fibrotic effects of TGF-β1 and connective tissue growth factor (24). However, no such data exist for asporin regarding its effects on inflammation and renal fibrosis. CKD is considered a condition with increased inflammation (25). Increased asporin levels in HD patients thus might be among the reasons for inflammation or represent a compensatory response. However, there was no significant correlation between asporin and CRP levels in our patients.

Although not studied for serum asporin levels, there are data on the association of elevated serum levels of some SLRPs, i.e., decorin and biglycan and with albuminuria in lupus nephritis patients. In a murine study, serum biglycan level showed a correlation with the emergence of albuminuria (26). Another experimental study showed that in the renal ischemia

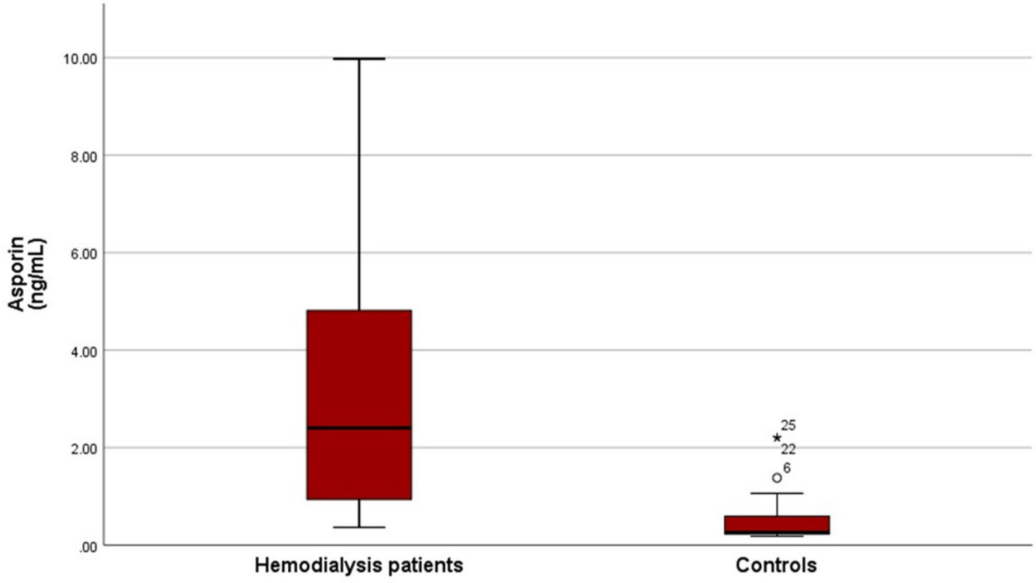

Figure 1. Boxplot diagram of serum asporin levels in hemodialysis patients and control subjects

Table 1. Age, sex distribution, calcium-phosphorus metabolism parameters and serum asporin levels in patients and controls

	Patients (n=25)	Controls (n=29)	p value
Age (years), mean±SD	43.3±13.5	38.0±8.8	0.102
Sex			
Female, n (%)	10 (40.0)	18 (62.1)	0.179
Male, n (%)	15 (60.0)	11 (37.9)	
Comorbidities			
Diabetes mellitus, n (%)	1 (4)	2 (6.9)	
Hypertension, n (%)	2 (8)	2 (6.9)	1.000
Ischemic heart disease, n (%)	1 (4)	1 (3.5)	
Duration of hemodialysis (months), median (min-max)	36 (6-300)	-	-
Body mass index (kg/m^2), mean±SD	25.2±4.2	25.8±4.0	0.653
Phosphorus (mg/dL), median (min-max)	4.4 (3.3-5.8)	3.4 (3.1-3.7)	**0.014**
Albumin (g/dL), median (min-max)	4.4 (4.2-4.7)	4.4 (4.3-4.5)	0.480
Corrected calcium (mg/dL), median (min-max)	8.7 (8.4-9.5)	9.1 (9.0-9.4)	0.090
Parathyroid hormone (pg/mL), mean±SD	499.1±432.6	43.9±14.9	**<0.001**
Asporin (ng/mL), median (range)	2.4 (0.9-4.8)	0.3 (0.2-0.6)	**<0.001**
C-reactive protein (mg/dL), median (min-max)	3.1 (2-23.1)	-	-
25(OH) vitamin D (ng/mL), median (min-max)	11.0 (6.5-17.5)	21 (11.3-37.1)	**0.002**

*Median (min-max).
NA: Not applicable, min-max: Minimum-maximum, SD: Standard deviation

Table 2. Pearson correlation analysis between asporin and age, dialysis duration and calcium-phosphorus metabolism parameters

		Age	BMI	Phosphorus	Calcium	PTH	25(OH) vitamin D	Duration of HD
Asporin level	r	0.344	-0.408	0.118	0.273	-0.077	0.133	0.610
	p value	0.092	0.074	0.575	0.187	0.713	0.546	**0.003**

BMI: Body mass index, HD: Hemodialysis, PTH: Parathormone

perfusion injury model, serum biglycan level correlated with kidney injury (27). However, despite some experimental data, human studies are lacking. Furthermore, we still do not know the exact effect of glomerular filtration rate on serum SLRP levels, particularly on asporin.

Study Limitations

Some limitations of this study should be acknowledged. First, we did not perform a comprehensive evaluation of bone and joint lesions of renal osteodystrophy in the study population. Second, although we excluded clinically clear OA, asymptomatic patients with OA might have been included, resulting in elevated serum asporin levels. However, despite these limitations, this is the first study to report significantly increased serum asporin levels in maintenance HD patients.

Conclusion

In conclusion, this study showed significantly elevated serum asporin levels relative to healthy control subjects in patients receiving maintenance HD. To evaluate the association of asporin with renal osteodystrophy lesions, further studies involving groups of HD patients with and without OA are needed.

Ethics

Ethics Committee Approval: The Medicana International Ankara Hospital's Ethics Committee approved the study protocol (BŞH-2022/11).

Informed Consent: All study participants gave written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Relationships between C-reactive protein, systemic immune inflammation index, and inflammatory markers related to hemograms in children diagnosed with acute otitis media

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Keywords: Otitis media, C-reactive protein, neutrophil-lymphocyte ratio, systemic immune-inflammation index, inflammatory markers

ABSTRACT

Aims: The relationships between C-reactive protein (CRP), the systemic immune inflammation index (SII), and inflammatory markers derived from the hemogram are unknown in children with acute otitis media (AOM) infections. This study investigated the correlations between CRP, SII, and inflammatory markers in pediatric patients with AOM.

Methods: This retrospective study included pediatric cases diagnosed with AOM at the Pediatric Emergency Service of the Gülhane Training and Research Hospital between November 2016 and January 2019. The SII (neutrophil x platelet/lymphocyte), neutrophil-to-lymphocyte ratio (NLR), neutrophil-to-monocyte ratio (NMR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), mean platelet volume (MPV)-to-lymphocyte ratio (MPVLR), and lymphocyte-to-CRP ratio (LCR) values of the patients were analyzed. Correlations between CRP level, hemogram parameters and inflammatory markers were studied.

Results: Among the 252 cases, the median age was 2.0 (1-5) years, and 140 (55.6%) were male. There were no significant differences between the boys and the girls in terms of age, CRP, hemogram parameters, or related inflammatory markers ($p>0.05$). Significant correlations were found between CRP and SII ($r=0.375$, $p=0.001$), leukocyte count ($r=0.300$, $p=0.001$), neutrophil count ($r=0.459$, $p=0.001$), lymphocyte count ($r=-0.174$, $p=0.006$), NLR ($r=0.407$, $p=0.001$), NMR ($r=0.257$, $p=0.001$), LMR ($r=-0.393$, $p=0.001$), PLR ($r=0.137$, $p=0.03$), MPVLR ($r=0.173$, $p=0.006$), and LCR ($r=-0.881$, $p=0.001$). There were no correlations between CRP or SII and PLT or MPV.

Conclusions: High CRP, SII, and inflammatory markers calculated using the routine hemogram parameters correlate in pediatric patients with AOM.

Introduction

Acute otitis media (AOM) is a common childhood disease caused by middle ear inflammation and accompanied by middle ear effusion with acute signs and symptoms (1). AOM may present with an earache in older children and non-specific symptoms such as fever, irritability, or pulling or rubbing of the ear in younger children (1).

Middle ear inflammation begins with the early and intense bacterial colonization of the nasopharynx, and early-onset AOM is caused by acute ongoing inflammation in the middle ear due to continued exposure to infective agents (2). Viral infections of the epithelia of the nasopharynx and Eustachian tube underlie AOM. After viruses initiate the inflammatory process in the nasopharynx, bacteria and viruses induce middle ear inflammation, and otopathogen bacteria colonizing the nasopharynx begin causing damage (3).

Various markers are used to evaluate inflammation. One of these markers is C-reactive protein (CRP) which is widely

used in acute and chronic inflammatory conditions. CRP is an important marker in determining the cause of infection, and bacterial infections can increase its level significantly (4).

The distributions and counts of inflammatory and proinflammatory cells such as neutrophils, lymphocytes, and platelets (PLT) in the peripheral blood are altered by the release of inflammatory mediators (5,6). Parameters such as the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the systemic immune inflammation index (SII) can be calculated using routine values in the hemogram, which can be used as inflammatory markers (7,8). As was emphasized in many recent studies, inflammatory markers such as NLR, PLR, and SII have both prognostic and diagnostic value in some diseases (9-11).

Although many studies are conducted on children with various infectious diseases regarding inflammatory markers such as CRP, NLR, and SII (4,9-11), there are no studies on these inflammatory markers in children with AOM.

In this study, we investigated the relationship between hemogram parameters, CRP, SII, and inflammatory markers related to hemogram parameters in AOM cases.

Methods

The patient group of this retrospective study included pediatric outpatients diagnosed with AOM between November 2016 and January 2019 at the Emergency Service of the Gülhane Training and Research Hospital (Ankara, Türkiye). Findings of the moderate-to-severe bulging of the tympanic membrane, the mild bulging of the tympanic membrane, and recent (<48 h) onset of ear pain or intense erythema of the tympanic membrane, air-fluid level behind the tympanic membrane, perforation of the tympanic membrane, and/or discharge in the ear canal not caused by otitis externa were considered AOM (12). The inclusion criteria for patients were as follows: the completion of relevant laboratory tests, being aged between 1 month and 18 years, not having used any antiplatelet drugs in the last 30 days and having no hematological or chronic diseases. We excluded subjects with missing relevant clinical or laboratory tests, and those with other acute or chronic infectious and hematological diseases accompanying acute AOM.

Laboratory test results were retrieved retrospectively from the hospital's information system. The SII (neutrophil x platelet/lymphocyte), NLR, neutrophil-to-monocyte ratio (NMR), lymphocyte-to-monocyte ratio (LMR), PLR, mean platelet volume (MPV) -to-lymphocyte ratio (MPVLR), and lymphocyte-to-CRP ratio (LCR) values of the patients were calculated from their hemogram results and analyzed. The patients were classified according to their CRP levels, where serum CRP results were <5.0 mg/L in Group 1 and >5.0 mg/L in Group 2. Correlation analyses of the CRP levels, complete blood parameters, and related inflammatory markers of the patients were performed.

The University of Health Sciences Türkiye, Gülhane Training and Research Hospital Local Ethics Committee approved the study (date: 26.02.2019, decision no: 66).

We obtained complete blood counts (CBC) measured with an automated device (Beckman Coulter UniCel® DxH 800 Cellular Analysis System Hematology Analyzer, Miami, FL, USA). CRP values were also obtained using an automated device (Beckman Coulter AU680® analyzer, Miami, FL, USA). The DxH 800 is a fully automated analyzer that provides a CBC, white blood cell (WBC) differential, and reticulocyte percentage and count and has an improved reportable range using advanced signal-to-noise algorithms.

Statistical Analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) software (ver.22, IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to evaluate whether the data were normally distributed. The non-normally distributed numeric data were compared using the Mann-Whitney U test, and the results are summarized in terms of medians and interquartile ranges. The categorical data are presented with n and % values. We analyzed the correlations of CRP levels, hemogram parameters, and related inflammatory markers using Pearson's correlation test or Spearman's correlation test. Differences were considered statistically significant at $p<0.05$.

Results

The study included 252 patients with acute AOM. The age range was 1 month and 18 years (median: 2.0 years). Most patients were in the 2-4 age group (n=73, 29%). Males comprised 55.6% (n=140) of the sample. There were no significant differences between the female and male patients in terms of their age, CRP, WBC, neutrophil count, lymphocyte count, PLT, or MPV values ($p>0.05$). Table 1 shows the demographic characteristics, CRP, WBC, neutrophil counts, lymphocyte counts, PLT values, MPV values, and inflammatory markers.

There were no significant differences between the female and male patients in terms of their inflammatory markers (NLR, NMR, LMR, PLR, LCR, MPVLR, SII) ($p>0.05$). Table 2 shows the results of inflammatory markers in different age groups by sex.

We classified the patients using a cut-off for CRP level. Subjects with a CRP <5.0 mg/L formed Group 1, and subjects with a CRP >5.0 mg/L formed Group 2 (Table 3). Age, PLT, and MPV values were similar in both groups. Female sex ratio was higher in Group 2 than in Group 1 ($p<0.001$). Group 1 had significantly lower median values of WBC (8800/μL vs. 11550/μL; $p<0.001$), neutrophil (3600/μL vs. 6550/μL; $p<0.001$), NLR (0.9 vs. 2.4; $p<0.001$), NMR (4.7 vs. 6.0; $p<0.001$), PLR (85.9 vs. 112.9; $p=0.005$), MPVLR (2.1 vs. 2.9; $p=0.002$), and SII (323.0 vs. 710.2; $p<0.001$) compared to Group 2.

Moreover, Group 1 had significantly higher lymphocyte (3990/μL vs. 2800/μL; p=0.002), LMR (5.1 vs. 2.7; p<0.001), and LCR (2.1 vs. 0.1; p<0.001) values compared with Group 2.

CRP positively correlated with WBC (r=0.300, p=0.001), neutrophil count (r=0.459, p=0.001), NLR (r=0.407, p=0.001), NMR (r=0.257, p=0.001), PLR (r=0.137, p=0.03), MPVLR (r=0.173, p=0.006), and SII (r=0.375, p=0.001), and negatively correlated with lymphocyte counts (r=-0.174, p=0.006), LMR (r=-0.393, p=0.001), and LCR (r=-0.881, p=0.001). Similarly, SII positively correlated with WBC (r=0.445, p=0.001), neutrophil count (r=0.837, p=0.001), NLR (r=0.948, p=0.001), NMR (r=0.763, p=0.001), PLR (r=0.780, p=0.001), and MPVLR (r=0.600, p=0.001), and negatively correlated with lymphocyte counts (r=-0.629, p=0.001), LMR (r=-0.760, p=0.001), and LCR (r=-0.564, p=0.001) (Table 4). However, there was no correlation between the CRP and SII values of the patients and their PLT or MPV values (p>0.05).

Discussion

To the best of our knowledge, there is no study on the relationships of CRP, SII, and inflammatory markers related to hemogram parameters in children with AOM.

We performed the current study to emphasize the value of CRP, NLR, NMR, LMR, PLR, LCR, MPVLR, and SII in pediatric AOM infections as inflammatory biomarkers as fast, simple, easily accessible and useful in early diagnosis. We provided evidence of correlations between CRP and SII values and inflammatory markers related to CBC parameters in our study.

Biomarkers used in the differential diagnosis of infections in clinical practice are diverse, and the most commonly used acute-phase reactants are CRP, leukocyte counts, and platelet counts (13). As an inflammatory marker, CRP is a non-specific acute phase protein with highly accurate and easily reproducible results. Although leukocyte counts alone are not specific for the diagnosis of bacterial infections, they are widely used together with CRP to diagnose patients with acute bacterial infections and predict their prognosis (14).

Recently, NLR has been widely used in almost all medical disorders as a reliable and easily accessible marker of inflammation against various infectious and non-infectious conditions (15). NLR is considered an excellent indicator of inflammation, reflecting the harmony between innate and adaptive immune responses (15). Normal range values need to be defined to identify whether an NLR value is high. To this end, several studies have investigated normal values of NLR in healthy adult populations (16,17). Generally, NLR values greater than 5 are considered pathological in adults (15). Although different NLR values have been reported in different infections, a few studies in children have reported different

Table 1. Demographic characteristics, CRP, WBC, neutrophil, lymphocyte, PLT values, and inflammatory markers of the sample

	Female	Male	Total
N (%)	112 (44.4)	140 (55.6)	252 (100)
Age (years), median (IQR)	2.0 (1-5)	2.0 (1-6)	2.0 (1-5)
1-12 months, n (%)	18 (7.1)	16 (6.4%)	34 (13.5)
13-24 months, n (%)	28 (11.1)	42 (16.7)	70 (27.8)
2-4 years, n (%)	33 (13.1)	40 (15.9)	73 (29.0)
5-9 years, n (%)	24 (9.5)	32 (12.7)	56 (22.2)
10-18 years, n (%)	9 (3.6)	10 (3.9)	19 (7.5)
CRP (mg/L), median (IQR)	11.2 (4.7-34.1)	9.8 (3.2-5-29.0)	10.1 (4.0-31.6)
WBC (/μL), median (IQR)	11360 (8252-15325)	10300 (7710-13250)	10700 (8100-14150)
Neutrophil (/μL), median (IQR)	6450 (3600-9625)	4980 (3600-7600)	5600 (3600-9000)
Lymphocyte (/μL), median (IQR)	3025 (2025-4865)	3240 (1900-4900)	3200 (1900-4900)
PLT (/μL), median (IQR)	349000 (263500-412500)	313000 (254500-374000)	328000 (258000-394500)
MPV (/fL), median (IQR)	7.8 (7.1-8.8)	8.0 (7.1-8.9)	7.9 (7.1-8.9)
NLR, median (IQR)	2.2 (1-4.0)	1.5 (0.8-3.1)	1.8 (0.8-3.6)
NMR, median (IQR)	5.9 (3.8-9.3)	5.3 (3.9-8.3)	5.6 (3.9-8.8)
LMR, median (IQR)	3.0 (2.0-5.0)	3.5 (2.0-5.5)	3.2 (2.0-5.3)
PLR, median (IQR)	103.4 (69.4-160.1)	97.8 (70.3-150.3)	100.8 (70.3-152.4)
MPVLR, median (IQR)	2.6 (1.6-4.1)	2.4 (1.6-4.2)	2.5 (1.6-4.2)
LCR, median (IQR)	0.3 (0.1-1.0)	0.4 (0.1-1.5)	0.3 (0.1-1.2)
SII, median (IQR)	679.1 (303.5-1481.1)	525.4 (270.0-899.8)	570.0 (278.6-1033.6)

CRP: C-reactive protein, WBC: White blood cell, PLT: Platelet, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, MPVLR: Mean platelet volume-to-lymphocyte ratio, LCR: Lymphocyte-to-CRP ratio, SII: Systemic immune inflammation index

values of the normal range of NLR values in children according to age (18). It has been shown that NLR can be a useful inflammatory parameter for some diseases (19,20). High NLR values have been associated with severe inflammation and may indicate a worsening prognosis of the examined disease (15). NLR was investigated as an inflammation biomarker in association with bacterial infections and sepsis compared with CRP, and it was shown that both the NLR and significant lymphopenia were better predictors of bacteremia than CRP levels and total WBC counts (21).

In a study in which PLR was also studied as an inflammatory marker, it was determined that hemodialysis had a significant relationship with NLR, PLR, and CRP in patients with end-stage renal disease (22). In the novel Coronavirus disease-2019 (COVID-19), higher NLR and PLR and lower LMR values were observed in patients compared to healthy individuals (23). Higher PLR values were associated with an increased risk of serious illness in COVID-19 patients (23).

SII allows the simultaneous evaluation of platelets, lymphocytes, and neutrophils, which are markers of the hemostatic system involved in the inflammatory process (24). In COVID-19, SII values were found to be significantly higher in patients in intensive care units (ICU) compared with non-ICU patients (11). A statistically significant relationship was shown

Table 2. Comparison of inflammatory markers by age groups and sex

Parameter	Age	Female	Male	p value
NLR	1-12 months	0.69 (0.30-1.92)	0.75 (0.53-1.59)	0.91
	13-24 months	1.63 (0.64-2.61)	1.22 (0.76-2.42)	0.83
	2-4 years	3.00 (1.16-4.92)	1.51 (0.78-3.89)	0.14
	5-9 years	3.64 (2.19-7.69)	2.56 (1.43-5.04)	0.09
	10-18 years	3.00 (1.93-9.00)	2.21 (1.26-9.51)	1.62
NMR	1-12 months	3.29 (2.48-5.06)	3.57 (2.06-5.37)	0.87
	13-24 months	4.74 (3.25-6.55)	4.63 (3.58-6.61)	0.97
	2-4 years	6.66 (4.69-10.63)	5.17 (3.93-8.68)	0.13
	5-9 years	8.16 (5.97-10.68)	7.04 (5.26-9.49)	0.37
	10-18 years	6.10 (5.73-10.14)	7.37 (6.31-10.89)	0.56
LMR	1-12 months	5.28 (3.94-7.49)	4.86 (3.07-5.59)	0.35
	13-24 months	3.66 (2.24-5.41)	4.00 (2.14-5.83)	0.79
	2-4 years	2.71 (2.07-4.19)	3.24 (2.08-5.75)	0.48
	5-9 years	2.34 (1.23-3.40)	2.28 (1.80-4.44)	0.28
	10-18 years	2.00 (1.18-3.89)	3.04 (1.22-4.71)	0.41
PLR	1-12 months	59.68 (51.07-78.95)	51.09 (41.54-76.73)	0.11
	13-24 months	85.94 (58.31-127.93)	87.67 (70.03-130.29)	0.51
	2-4 years	137.27 (77.06-189.05)	96.84 (66.41-135.68)	0.08
	5-9 years	162.40 (114.07-210.64)	138.89 (99.41-156.25)	0.21
	10-18 years	113.91 (98.80-187.35)	160.39 (100.84-222.06)	0.56
LCR	1-12 months	1.24 (0.17-3.72)	1.35 (0.23-2.31)	0.91
	13-24 months	0.27 (0.06-0.94)	0.42 (0.16-1.59)	0.12
	2-4 years	0.35 (0.04-0.86)	0.45 (0.05-1.37)	0.10
	5-9 years	0.19 (0.06-0.26)	0.26 (0.09-0.77)	0.15
	10-18 years	0.09 (0.04-0.36)	0.23 (0.08-4.95)	0.22
MPVLR	1-12 months	1.30 (1.07-1.59)	1.18 (0.96-1.60)	0.51
	13-24 months	1.84 (1.29-2.63)	2.26 (1.71-3.13)	0.19
	2-4 years	2.92 (2.17-4.45)	2.29 (1.59-4.52)	0.33
	5-9 years	3.85 (2.94-6.17)	4.07 (2.24-5.40)	0.47
	10-18 years	4.82 (3.19-6.66)	4.77 (2.76-7.31)	0.87
SII	1-12 months	176.11 (127.56-622.79)	308.47 (165.93-554.10)	0.77
	13-24 months	431.88 (275.99-870.68)	414.09 (267.44-751.47)	0.73
	2-4 years	683.64 (432.32-1852.41)	474.17 (246.56-934.34)	0.069
	5-9 years	1024.28 (679.29-2321.97)	751.12 (463.85-1554.73)	0.10
	10-18 years	787.50 (534.23-1822.50)	698.39 (446.38-2690.61)	0.98

Data are presented as median (interquartile range).
NLR: Neutrophil-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, MPVLR: Mean platelet volume-to-lymphocyte ratio, LCR: Lymphocyte-to-CRP ratio, SII: Systemic immune inflammation index

between SII and sepsis, and the mean SII value was significantly higher in patients with sepsis (25).

Various studies have investigated inflammatory biomarkers to determine inflammatory processes, and these biomarkers have been evaluated along with the diagnosis and prognosis of AOM, including its bacterial and viral forms. Concentrations of interleukin (IL) 1β and lactate dehydrogenase in nasopharyngeal secretions were associated with an increased risk of developing AOM after a viral upper respiratory tract infection (26). While increased serum IL-10 concentrations were found in pneumococcal AOM (27), a biomarker risk score including serum cytokines was developed to aid in the diagnosis of AOM caused by non-typeable Haemophilus influenzae and predict its prognosis (28).

In our study, consistent with the literature, WBC, neutrophil count, NLR, NMR, PLR, MPVLR, and SII values were found to be higher in the group that we considered to have a high CRP value compared to the group with a low CRP value, while

Table 3. Demographic characteristics, complete blood count parameters, and inflammatory markers by CRP

	Group 1 (CRP <5 mg/L)	Group 2 (CRP >5 mg/L)	p value
N (%)	91 (36.1)	161 (63.9)	
Sex (female), n (%)	28 (30.8)	84 (50.2)	**<0.001**
CRP (mg/L), median (IQR)	1.89 (0.6-3.27)	24.6 (11.45-5-46.68)	
Age (years), median (IQR)	2.00 (1-4)	2.00 (1-5)	0.09
WBC (/μL), median (IQR)	8800 (6600-12100)	11550 (8850-14845)	**<0.001**
Neutrophil (/μL), median (IQR)	3600 (2530-5960)	6550 (4725-9700)	**<0.001**
Lymphocyte (/μL), median (IQR)	3990 (2300-6070)	2800 (1800-4400)	**0.002**
PLT (/μL), median (IQR)	342000 (265000-395000)	318000 (241000-397000)	0.25
MPV (/fL), median (IQR)	7.9 (7.2-8.7)	8.0 (7.1-8.9)	0.78
NLR, median (IQR)	0.85 (0.55-1.89)	2.35 (1.31-4.13)	**<0.001**
NMR, median (IQR)	4.68 (3.01-7.40)	6.02 (4.31-9.17)	**<0.001**
LMR, median (IQR)	5.14 (3.17-7.35)	2.69 (1.83-4.09)	**<0.001**
PLR, median (IQR)	85.85 (59.02-132.41)	112.87 (77.28-156.78)	**0.005**
MPVLR, median (IQR)	2.13 (1.34-3.42)	2.90 (1.72-4.29)	**0.002**
LCR, median (IQR)	2.06 (0.93-7.02)	0.12 (0.05-0.26)	**<0.001**
SII, median (IQR)	322.97 (163.44-665.17)	710.18 (420.15-1424.96)	**<0.001**

CRP: C-reactive protein, WBC: White blood cell, PLT: Platelet, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, MPVLR: Mean platelet volume-to-lymphocyte ratio, LCR: Lymphocyte-to-CRP ratio, SII: Systemic immune inflammation index

Table 4. Correlation analysis of CRP levels and SII scores with complete blood count parameters and inflammatory markers

	CRP	SII
Parameter	r (P)	r (P)
WBC count	**0.300 (0.001)**	**0.445 (0.001)**
Neutrophil count	**0.459 (0.001)**	**0.837 (0.001)**
Lymphocyte count	**-0.174 (0.006)**	**-0.629 (0.001)**
PLT count	-0.101 (0.11)	0.090 (0.155)
MPV	0.034 (0.59)	-0.035 (0.582)
NLR	**0.407 (0.001)**	**0.948 (0.001)**
NMR	**0.257 (0.001)**	**0.763 (0.001)**
LMR	**-0.393 (0.001)**	**-0.760 (0.001)**
PLR	**0.137 (0.03)**	**0.780 (0.001)**
MPVLR	**0.173 (0.006)**	**0.600 (0.001)**
LCR	**-0.881 (0.001)**	**-0.564 (0.001)**
SII	**0.375 (0.001)**	

CRP: C-reactive protein, WBC: White blood cell, PLT: Platelet, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, NMR: Neutrophil-to-monocyte ratio, LMR: Lymphocyte-to-monocyte ratio, PLR: Platelet-to-lymphocyte ratio, MPVLR: Mean platelet volume-to-lymphocyte ratio, LCR: Lymphocyte-to-CRP ratio, SII: Systemic immune inflammation index

lymphocyte, LMR, and LCR values were found to be lower in the former than the latter. Additionally, we found that CRP was positively correlated with WBC, neutrophil count, NLR, NMR, PLR, MPVLR, and SII and negatively correlated with lymphocyte count, LMR, and LCR values. These results showed that as inflammation increases, CRP elevation is seen as an indicator of this increase, and the increase in inflammation is associated with high neutrophil counts and decreased lymphocyte counts.

Although differences in CRP levels and NLR were found between men and women in viral upper respiratory tract infections in different age groups (29), there was no difference between the sexes in terms of CRP, NLR, or other inflammatory parameters in our study, and this result suggested that sex is not a significant predictor of inflammation in AOM infection cases.

In comparison to previous studies, our study is the first original study conducted by comparing the clinical use of NLR and other inflammatory markers related to hemogram parameters to the use of CRP levels in pediatric AOM infections.

Study Limitations

Some limitations of our study were that 1) the small sample size of our study may have been insufficient in the evaluation of CRP, SII, and inflammatory markers related to hemogram parameters in children with AOM infections; 2) the study was a single-center, retrospective study, and detailed demographic data could not be obtained; 3) the follow-up examination and laboratory test results of these children diagnosed with AOM were not known because they were not followed up; 4) the tests for the etiological causes of children with AOM were limited; 5) there may be variables that affect the results of CBC values such as types of CBC analysis devices, analysis-related errors, arterial blood pressure, body mass index, serum lipids, seasonal differences, and time between venipuncture and measurement, but since the study was retrospective, we did not have the opportunity to intervene with these variables, and 6) a healthy control group was not included in the study for comparison to patients with AOM.

Conclusion

In conclusion, inflammatory markers related to hemogram parameters and SII scores may aid decision-making in treatment and disease severity in children with AOM. Further studies of these inflammatory markers in patients with AOM are needed to demonstrate whether the changes in their values persist later.

Ethics

Ethics Committee Approval: The University of Health Sciences Türkiye, Gülhane Training and Research Hospital Local Ethics Committee approved the study (date: 26.02.2019, decision no: 66).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.T., Concept: H.Ö.Ş., Design: A.B., A.T., Data Collection or Processing: A.B., Analysis or Interpretation: A.B., Literature Search: A.B., Writing: A.B.

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The evaluation of eosinophil-to-lymphocyte, eosinophil-to-neutrophil, and neutrophil-to-lymphocyte ratios in adults with allergic/non-allergic rhinitis

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ABSTRACT

Aims: This study investigated the usefulness of inflammatory parameters such as eosinophil-to-lymphocyte ratio (ELR), eosinophil-to-neutrophil ratio (ENR), and neutrophil-to-lymphocyte ratios (NLR) as markers for the diagnosis of patients with allergic rhinitis (AR) and non-AR (NAR).

Methods: This retrospective study included patients admitted to the allergy and immunology outpatient clinic with symptoms of chronic rhinitis. Subjects were divided into AR and NAR groups based on the skin prick test, serum total/allergen-specific immunoglobulin E level, and complete blood count. Subjects who underwent a medical check-up were included as healthy controls. ELR, ENR, and NLR were calculated using the results from complete blood counts.

Results: The study included 121 patients with AR [mean±standard deviation (SD) age: 30.6±7.5 years, female: 68.6%], 101 patients with NAR (mean±SD age: 31.6±10.3 years, female: 72.3%), and 116 control subjects (mean±SD age: 31.1±9.8 years, female: 62.9%). Mean age and sex ratios were similar across the groups. Patients with AR had significantly higher serum eosinophil counts, ELR, and ENR than healthy controls (mean±SD 0.22±0.17 vs. 0.15±0.12; 0.10±0.10 vs. 0.08±0.06; and 0.06±0.04 vs. 0.04±0.04, respectively, $p<0.05$). ENR >0.0684 showed 31.4% sensitivity and 82.9% specificity to predict AR. ELR was higher in NAR patients compared with the healthy controls (mean±SD 0.10±0.10 vs. 0.08±0.06, $p<0.05$). There was no difference in NLR across the three groups.

Conclusions: Our study showed that serum eosinophil counts, ELR, and ENR values were higher in AR patients compared to healthy controls, while ELR values were higher in NAR patients compared to healthy controls.

Introduction

Chronic rhinitis is an inflammation of the nasal mucosa characterized by symptoms of nasal congestion, rhinorrhea (anterior or posterior), sneezing, and nasal/ocular itching. Patients with chronic rhinitis have two nasal symptoms that last at least 12 weeks per year and at least 1 h per day (1). Worldwide, chronic rhinitis is becoming more common, with an estimated 30% prevalence rate across the entire population. This trend is on the rise throughout the world (1). Chronic rhinitis is a clinical manifestation with a high economic burden that can lead to sleep disturbance, fatigue, irritability, decreased school/work performance, and deterioration in the quality of life (2).

Generally, chronic rhinitis is categorized into four main groups according to etiology: allergic, non-allergic, infectious, and mixed (2,3). The diagnosis of allergic rhinitis (AR) is diagnosed according to the symptoms that occur after inhalation of at least one aeroallergens and positive skin prick test (SPT) and/or serum allergen-specific IgE (sIgE) tests revealing allergic sensitization (4). Non-AR (NAR) is the absence of any signs of infection and systemic aeroallergens sensitization (sIgE and/or a positive SPT), often as a diagnosis of exclusion (3).

The pathophysiology of NAR is unclear, although it is generally considered non-IgE. In NAR, there is an excessive response to non-allergic environmental triggers such as physical

(cold air, weather changes) or chemical (perfume, odorants, etc.) stimuli. It is thought that neurogenic mechanisms such as nociceptive dysfunction and autonomic nervous dysregulation may be responsible for this response (5). AR is an IgE-mediated type 1 hypersensitivity reaction to aeroallergens. Mast cells, lymphocytes, eosinophils, neutrophils, and various cytokines and mediators released from these cells are involved in the mucosal inflammation observed in the AR (1).

Several studies have shown that eosinophil-to-lymphocyte ratio (ELR), eosinophil-to-neutrophil ratio (ENR), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) are correlated with chronic inflammatory diseases such as atopic dermatitis, asthma, and sinonasal polyps (6-9). However, there are few studies in the literature investigating these inflammatory markers in both AR and NAR patients.

Rapid, minimally invasive, and inexpensive parameters must enable high identification of patients with AR and NAR. In this study, we investigated whether easily accessible ELR, ENR, and NLR could be used as novel inflammatory markers in the diagnosis of AR and NAR.

Methods

Study groups and classifications

In this study, we retrospectively analyzed the medical records of patients (aged 18 years and over) admitted to the University of Health Sciences Erzurum Region Training and Research Hospital Allergy and Immunology Department between February 2017, and March 2019. Patients with chronic rhinitis symptoms were divided into two groups: patients with a positive SPT and/or serum sIgE test were classified as AR (n=121), while patients with a negative SPT and serum sIgE test were classified as NAR (n=101). Participants who underwent medical a check-up during the study period acted as the control group (n=116). Age, gender, complete blood count parameters, aeroallergens sensitivity, serum total/sIgE and SPT results of the participants were recorded. Also, aged <18 years, patients with systemic/chronic diseases, acute/chronic infections, obesity, pregnancy, and patients receiving systemic corticosteroids, anti-inflammatory or anticoagulant drugs were excluded from the study.

Laboratory

Complete blood count parameters, serum total IgE, SPT and allergen-sIgE tests were performed simultaneously for each patient. ELR, ENR, NLR and PLR were calculated for each patient. Complete blood count parameters were studied on a Sysmex XN-10 (Sysmex Comp., Kobe, Japan) device for each participant in the study. Serum total IgE and sIgE tests were analyzed by chemiluminescence immunometric system (Immulite 2000 Siemens, Erlangen, Germany) for each patient with chronic rhinitis. The serum sIgE panel contained grass mix *(Dactylis glomerata, Lolium temulentum, Phleum pratense, Poa pratensis, Panicum miliaceum),* trees-mix *(Acer negundo, Betula pendula, Quercus macrolepsis, Ulmus laevis, Juglans orientis)*, mite-mix *(Dermatophagoides pteronyssinus, Dermatophagoides farinae, Lepidoglyphus destructor, Euroglyphus maynei, Tyrophagus putrescentiae, Blomia tropicalis, Glycphagus domesticus, Dermatophagoides microcereas)* and mold-mix *(Cladosporium herbarum, Aspergillus fumigatus, Penicillium notatum, Candida albicans, Alternaria tenuis)*. sIgE ≥0.35 kU/L is defined as a positive result.

All patients underwent SPTs with the positive control (histamine), negative control (physiological saline), house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), grasses mix, cereal pollen mix, tree mix, weed mix, Alternaria alternata, cockroaches, cat dander, and dog dander (Lofarma Allergeni, Milan, Italy). We performed SPTs using the same antigens in all patients. About 15 to 20 min after the application of the inhalant prick panel on the forearm, aeroallergens with a wheal ≥3 mm diameter were recorded as positive. The same observer evaluated the SPTs.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM Corp., Armonk, NY, USA) software package. Descriptive statistics were presented as frequencies, percentages, means, and standard deviations (SD). Categorical variables were compared using the chi-square test. The normality of numerical variables was tested using the Kolmogorov-Smirnov test. The Mann-Whitney U test or Kruskal-Wallis test with Tamhane post-hoc comparisons was used to compare numerical variables between the groups. Receiver operating characteristic (ROC) analysis was performed for numerical variables to reveal cut-off points in differentiating patients with AR from the control group. The area under the curve (AUC), sensitivity, and specificity values were calculated. The level of statistical significance was set at $p<0.05$.

Results

The study included 121 patients with AR (mean±SD age: 30.6±7.5 years, female: 68.6%), 101 patients with NAR (mean±SD age: 31.6±10.3 years, female: 72.3%), and 116 control subjects (mean±SD age: 31.1±9.8 years, female: 62.9%) (Table 1). Mean age and sex ratios were similar across the groups. The most common inhalant allergen sensitivity in AR patients was pollens (n=79, 65.3%), followed by house dust mites (n=64, 52.9%).

There were no significant differences in the mean age or sex of the groups. Eosinophils, ELR, and ENR values were significantly different between the groups. Measured only in the AR and NAR groups, serum total IgE values were significantly higher in the AR group (Table 1).

We performed ROC analyses using eosinophil count, ELR, and ENR variables. The highest AUC was observed for the eosinophils variable (Figure 1, Table 2). Cut-off studies have revealed that an eosinophil count >0.325 (x10^3/mL) could predict AR with a sensitivity of 20.7% and a specificity of 88.9% (likelihood ratio=1.86), while an ENR >0.0684 could predict AR with a sensitivity of 31.4% and a specificity of 82.9% (likelihood ratio=1.84).

Discussion

This study showed that serum eosinophil count, ENR, and ELR were higher in AR patients compared to healthy controls and ELR were higher in NAR patients compared to healthy controls. Additionally, total serum IgE levels were higher in the AR group than in the NAR group.

Mast cells, T-helper 2 lymphocytes, eosinophils, and neutrophils play a major role in AR (1). Previous studies have shown that eosinophilia is associated with allergen sensitization and can be used as a sensitization marker (10-12). Liu et al. (12) observed higher eosinophil counts in the AR group than in the control group. In our study, eosinophil count and ELR were higher in patients with AR compared to the control group. Similarly, Yenigun et al. (13) reported that both eosinophil count and ELR were higher in children with AR and that ELR could be used together with SPTs to diagnose AR. However, in the same study, the eosinophil count and ELR in patients with nonsensitized rhinitis were similar to those in the control group. Meanwhile, in our study, ELR was higher in patients with NAR than in controls. However, their study group included pediatric patients and used only SPT for the diagnosis of AR and did not use sIgE tests.

The prognostic significance of ELR was higher in patients with nasal polyps with chronic rhinosinusitis who had disease recurrence after endoscopic sinus surgery (14). Besides, in pediatric patients with atopic dermatitis, ELR and ENR were higher than in the control group (6). Likewise, in our study, ENR was higher in patients with AR than in the control group. Previous studies have shown increased ENR in nasal secretion in allergic conditions (15,16). Furthermore, blood eosinophil counts correlated with eosinophil counts in sputum and bronchoalveolar lavage (17). Zhang et al. (7) reported a correlation between blood ENR and sputum eosinophil count, and a cut-off >0.05 for blood ENR had a sensitivity of 89.6% and a specificity of 77.0% in predicting eosinophilic asthma. Lee et al. (16) found that the ENR level in nasal secretion was higher than 0.1 in patients with AR. In this study, an ENR cut-off >0.0684 could predict AR with a sensitivity of 31.4% and a specificity of 82.9%. To our knowledge, this is the first report of a blood ENR cut-off in patients with AR. Considering all these data, we thought that ENR levels in nasal secretion and blood ENR might correlate in patients with AR.

NLR is a valuable inflammatory marker (6,8,14). However, studies on the correlation between NLR and AR have reported conflicting results (18,19). In our study, there was no difference between both the AR and NAR groups and the healthy control group in terms of NLR. Ha et al. (18) showed higher eosinophil counts and lower NLR in children with AR compared to the control group. In the same study, there was no difference in both eosinophil count and NLR in children with NAR compared with the control group. Their results for patients with NAR were similar to those of our study. In this study, although the number of patients and healthy controls was similar to that in our study, only SPT was used to demonstrate allergen sensitization, and no information was given about SPT results. Dogru et al. (19) found that NLR was higher in children with AR compared with the control group. However, different from the current work, that

Table 1. Comparison of demographic and laboratory findings of patients with AR, NAR and healthy controls

	AR (n=121)	NAR (n=101)	Controls (n=116)	Test	p
Sex, female, n (%)	83 (68.6)	73 (72.3)	73 (62.9)	2.220*	0.330
Age, years, mean±SD	30.6±7.5	31.6±10.3	31.1±9.8	0.189#	0.910
Platelets (x10^3/mL), mean±SD	283.68±63.30	287.10±54.42	276.22±54.69	3.916#	0.141
Leukocytes (x10^3/mL), mean±SD	7.57±1.78	7.29±1.28	7.33±1.48	1.096#	0.578
Neutrophils (x10^3/mL), mean±SD	4.40±1.51	4.35±1.06	4.42±1.33	0.121#	0.941
Lymphocytes (x10^3/mL), mean±SD	2.24±0.68	2.03±0.55	2.12±0.58	4.468#	0.107
Eosinophils (x10^3/mL), mean±SD	0.22±0.17^a	0.21±0.25^{a,b}	0.15±0.12^b	11.326#	**0.003**
ELR, mean±SD	0.10±0.10^a	0.10±0.10^a	0.08±0.06^b	8.209#	**0.017**
ENR, mean±SD	0.06±0.04^a	0.05±0.08^{a,b}	0.04±0.04^b	10.745#	**0.005**
NLR, mean±SD	2.17±1.15	2.35±1.01	2.27±0.98	4.543#	0.103
PLR, mean±SD	136.51±44.37	150.76±46.90	141.40±52.31	5.649#	0.059
Total IgE (U/mL), mean±SD	192.53±251.29	56.59±63.51		6.840**	**<0.001**

Different superscript letters in the cells denote categories whose column proportions do not differ significantly from each other at the 0.05 level, *chi-square test value, #Kruskal-Wallis test value, **Mann-Whitney U test value.
ELR: Eosinophil-to-lymphocyte ratio, ENR: Eosinophil-to-neutrophil ratio, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SD: Standard deviation, AR: Allergic rhinitis, NAR: Non-AR

study included chronic inflammatory atopic diseases (eczema and asthma). Additionally, the sensitivity of house dust mites in patients with AR was 52.9% in our study, whereas it was higher in their study (85.1%). In the literature, neutrophil dominance has been reported in nasal cytology in patients with house dust mite-sensitive AR (20). However, it may also be present in peripheral blood, similar to nasal cytology in patients with AR. Considering all these data, further studies may investigate the clinical significance of NLR in AR and NAR patients.

We observed no significant differences in PLR between the groups. Several studies have investigated PLR as an inflammatory marker (21,22). NLR and PLR were higher in children with atopic dermatitis compared to the control group, and both markers reflected the severity of the disease in these patients (8).

We also found that the mean serum total IgE level was higher in patients with AR than in patients with NAR. Serum total IgE measurement is considered unuseful in the diagnosis of AR and is primarily associated with SPT/sIgE (1). Min et al. (10) found higher serum total IgE in patients with AR compared with patients with NAR in a study conducted on adult patients. Since NAR is a group of non-IgE-mediated inflammatory diseases, we think that serum total IgE levels may be useful in the differential diagnosis of AR.

Study Limitations

This study has several potential limitations. Firstly, we could not exclude patients with local AR to diagnose NAR. However, in most previous clinical studies, the definition of NAR is considered chronic rhinitis with negative SPT and/or sIgE results. In our study, we defined patients who had chronic rhinitis but who had negative tests as NAR. Nasal sIgE and nasal allergen provocation tests are recommended for diagnosing local AR. However, routine use of these tests is challenging for both patients and clinicians. Another limitation of this study was that it was retrospective. However, similar studies in the literature investigating complete blood count parameters are mostly retrospective.

Conclusion

In conclusion, we suggest that SPT, allergen sIgE, ELR, and ENR could be used to diagnose AR in adult patients. Moreover, increased serum total IgE levels in patients with

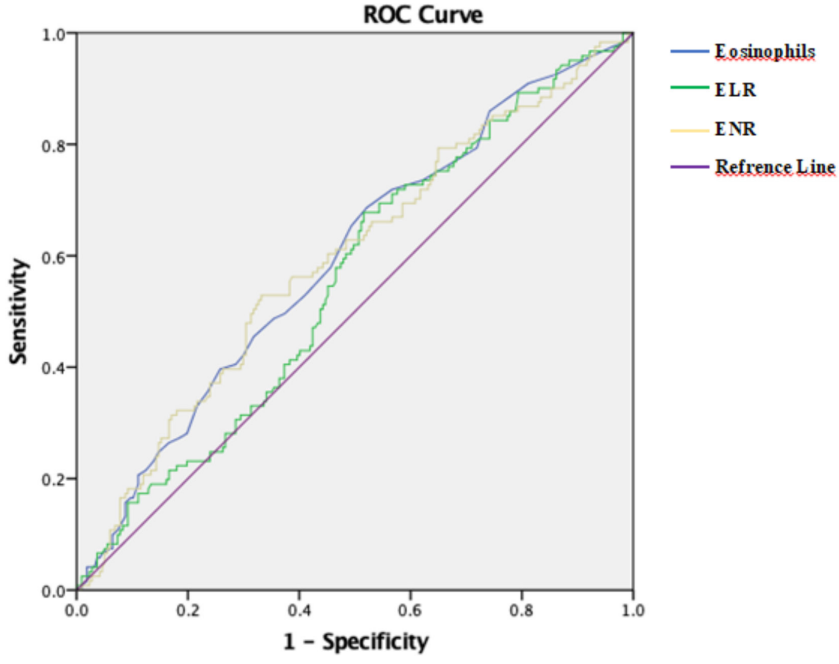

Figure 1. ROC curves for the eosinophils (x10³/mL), ELR, and ENR variables
ELR: Eosinophil-to-lymphocyte ratio, ENR: Eosinophil-to-neutrophil ratio, ROC: Receiver operating characteristic

Table 2. ROC analysis results for the eosinophils (x10³/mL), ELR, and ENR variables

	AUC	p	95% confidence interval	
			Lower bound	Upper bound
Eosinophils (x10³/mL)	0.594	**0.004**	0.532	0.657
ELR	0.556	0.085	0.494	0.619
ENR	0.593	**0.004**	0.530	0.657

AUC: Area under the curve, ELR: Eosinophil-to-lymphocyte ratio, ENR: Eosinophil-to-neutrophil ratio, ROC: Receiver operating characteristic

AR compared with patients with NAR may also be used in the differential diagnosis. Further studies are needed to investigate inflammatory markers, including ELR, ENR, and NLR, which can be measured with a complete blood count and nasal cytology.

Ethics

Ethics Committee Approval: This study was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Erzurum Ataturk University Faculty of Medicine (approval number: 04, date: 13.03.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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The effect of current infection control procedures and application times on the dimensional stability of dental impression materials

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Keywords: Disinfection, dental impression, dimensional stability, SARS-CoV-2

ABSTRACT

Aims: In this study, the effects of different disinfection and sterilization methods and their application duration on the dimensional stability of impression materials were evaluated.

Methods: Two impression materials, condensation (CS) and addition silicone (VPS), disinfectants with 5.25% sodium hypochlorite (NaOCl) immersion, 3% hydrogen peroxide immersion and steam autoclave were selected. Disc-shaped samples (n=112) were obtained in 7 subgroups of each material (n=8). Sixteen untreated samples served as controls. Dimensional change was measured with a digital micrometer in the reference lines on the sample.

Results: The highest mean percentage of dimensional change for the 50 min autoclave was 0.10±0.03% for CS and 0.10±0.02% for VPS. The dimensional change in CS did not differ for hydrogen peroxide. Compared with the controls, dimensional change was significant in 20 min NaOCl and 50 min autoclave ($p<0.05$). Both impression materials in the autoclave showed statistically significant dimensional changes regardless of the time. The difference in application duration significantly affected the dimensional stability of the impression materials regardless of the procedure ($p<0.001$). Extended application duration did not affect the dimensional stability in the hydrogen peroxide for CS, NaOCl and autoclave for VPS.

Conclusions: Chemical disinfection and autoclave sterilization caused statistically significant but clinically acceptable dimensional changes in CS and VPS impression materials used in this study.

Introduction

Impression is a critical step in prosthetic rehabilitation procedures, such as fixed partial dentures, removable dentures, and implant-supported dentures (1). Microorganisms in the body fluids like saliva and blood cause contamination of the impressions used in prosthetic treatments. Immediately after removing the impression from the mouth, it should be washed under tap water. This process partially eliminates bacteria and viruses but does not eliminate the potential for infection by itself (2). Therefore, disinfection of impressions is a mandatory practice. The American Dental Association (ADA) recommends the disinfection of impressions immediately after removal from the mouth to prevent cross-infection between the patient, dentist, assistant staff, and laboratory staff (3).

In December 2019, a coronavirus, Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) was identified as a cause of pneumonia in humans (4). The World Health Organization Coronavirus disease-2019 (COVID-19) guidelines were published following the announcement of the pandemic, which included preventive measures and infection control procedures in addition to the possible case definitions in this guideline (5,6). The transmission by droplets and aerosols creates a high risk, especially in dental practice, in terms of the spread of the disease due to cross-infection, and disinfection/sterilization of materials and materials is critical (7).

Various disinfection methods are used in the disinfection of impression materials. The use of chemical methods are most common as they can be applied by spray or immersion methods.

Disinfectants such as glutaraldehyde, sodium hypochlorite (NaOCl), iodophors, phenols, chlorine compounds, and hydrogen peroxide are also used at different concentrations and times. Methods such as microwave, ultraviolet (UV) light radiation, steam autoclave, s ozone, and electrolyzed oxidizing water are other methods applied as infection control protocols (8).

Both NaOCl and hydrogen peroxide are widely used in dental practice as low-cost and effective surface disinfectants. NaOCl is a water-soluble disinfectant. When dissolved hydrochloric acid and oxygen atoms are released, resulting in effective and broad-spectrum antimicrobial results with its oxidizing effect (9,10). Hydrogen peroxide, on the other hand, affects bacterial spores, viruses, and fungi with its enhanced oxidative effect (11).

Disinfectant solutions should be prepared and used according to the manufacturer's recommendations. The United States Environmental Protection Agency (US-EPA) has recommended disinfectants against SARS-CoV-2 and the effective application duration (12,13). According to the US-EPA, hydrogen peroxide should be applied for 5 min and NaOCl for 1-5 minutes to be effective against SARS-CoV-2.

Unlike disinfection, sterilization ensures the elimination of all microorganisms. Although there is no universally accepted sterilization method, the autoclave has been considered the most effective method (14). It also eliminates bacteria, viruses, and spores that are difficult to eliminate with chemical disinfectants (15).

Silicone-based impression materials, which are elastomeric impression materials, are of two types: condensation (CS) type silicones (C type silicone) and addition type silicone (A type silicone/vinyl polysiloxane). Despite the high elasticity of C-type silicones that were developed earlier, dimensional shrinkage occurs because of the subsequent evaporation of ethyl alcohol (16). In contrast, no by-product is formed, and the dimensional accuracy and stability of the A-type silicones are high (17).

Many studies have evaluated the dimensional stability of different impression materials using different chemical disinfection methods (8,10). However, studies on the autoclave procedure during the pandemic particularly relate to protective equipment sterilization. A few studies have evaluated the effects of autoclaves on the dimensional change of dental impression materials. The results of these studies show differences according to variables such as disinfection method, duration, type of impression material, and disinfectant concentration (18,19).

The aim of this study was to investigate the effect of different infection control methods, which have been published as effective on SARS-CoV-2, on the dimensional stability of silicone-based impression materials at varying application durations. The null hypothesis was that different infection control procedures and application durations could show no effect on the dimensional stability of the impression materials tested.

Methods

Two different elastomeric impression materials and three different disinfection procedures were used. Impression materials were CS-type silicone (Zetaplus, Zhermack, Italy) and addition-type silicone (VPS) (Panasil Putty Fast, Kettenbach GmbH, Germany) (Table 1). They were subjected to 5.25% NaOCl (Chloraxid, Cerkamed, Poland), 3% hydrogen peroxide (Oxivir CE Plus, Diversey, Inc, Fort Mill, NC, USA), and a steam autoclave. Application durations were 10 and 20 min of immersion for disinfectant solutions and 40 and 50 min for autoclave. For each impression material, 56 samples were produced and randomly divided into 7 groups (n=8), totaling 112 samples. Controls were formed by taking random samples not subject to any disinfection procedure. Table 2 displays the groups and procedures. Samples were standardized in accordance with specification number 19 of ADA and the ISO 4823:2000 protocol (20). According to this protocol, a stainless-steel mold was formed, and three parallel horizontal 20-µm-wide lines and two vertical 75-µm-wide lines were prepared on the inner surface of the mold (Figure 1). Before producing the samples, the mold was washed twice with ultrasonic deionized water to prevent possible surface contamination.

Mixing of the impression materials was performed in accordance with the manufacturer's recommendation. Samples were prepared manually mixing the base and catalyst, and the impression material was applied to the standard cavity in the mold. A glass plate covered with a thin layer of polyethylene was placed on the mold, and a 1-kilogram weight was placed on the plate. By exposing the sample to a constant force, the pressure applied by the dentist to the impression tray in the clinical practice was simulated. For the polymerization reaction, the sample assembly was immersed in a 35 °C water bath to simulate the existing temperature with the mouth open. The residence time in the water bath was determined as the polymerization time according to the manufacturer's recommendations (3:30 min for CS, 2:00 min for VPS), and an additional 2 min was added to ensure complete polymerization. The polymerized samples were carefully removed from the water and separated from the mold. This process was repeated until 56 pieces of each impression material were obtained. The samples were washed under tap water for 15 seconds and dried with compressed air spray. For each impression material, 7 subgroups were created and 14 groups were numbered. The first 16 samples formed the control group and no disinfection procedure was applied to these samples. NaOCl immersion, hydrogen peroxide immersion, and autoclave procedures were applied to the remaining samples for the times indicated in Table 2. Subsequently, the samples were washed once more under tap water for 15 seconds, and the remaining water was removed with a compressed air spray.

Immediately after the disinfection and sterilization procedures, measurement was performed to calculate the

dimensional change. Linear measurements were made with a digital micrometer using the reference distance on the models. This reference distance was the vertical line (A) in the middle between the two horizontal lines (Figure 1). All measurements were performed 30 min after sample fabrication. After the measurements in the mold, the test samples were separately measured, and the percentage dimensional change was calculated for each using the ISO 4823:2000 formula:

$$\Delta L = \left(\frac{L1 - L2}{L1}\right) X\ 100$$

L1 indicates the measurement of the distance on the mold, and L2 indicates the measurement of the distance on the samples.

Statistical Analysis

Statistical analyses were performed using PSPP (GNU pspp 0.10.4-g50f7b7) and Microsoft Excel programs. The Shapiro-Wilk test was used to test the normality of distribution. To evaluate the effects of the three different infection control procedures on the dimensional stability of impression materials a Two-Way ANOVA test (post-hoc: Bonferroni) was performed. $p<0.05$ was considered significant.

Results

The dimensional change after disinfection occurred in the form of shrinkage in all samples (Table 3). There was a significant difference between CS and VPS regarding the dimensional changes in all disinfection procedures (Figure 2). The highest mean dimensional change (%) occurred in the 50 min autoclave group (0.10±0.03% for CS and 0.10±0.02% for VPS), while the lowest dimensional changes occurred in the control group (0.06±0.02% for CS and 0.02±0.01% for VPS). Different application durations significantly affected the dimensional stability of the impression materials regardless of the disinfection procedure ($p<0.001$).

Regardless of the application duration, the dimensional change of CS did not differ significantly for hydrogen peroxide treatments compared to the control group. Additionally, the dimensional change was statistically significant in all autoclave groups and 20 min NaOCl group compared to the control group

Table 1. Impression materials used and their properties

Impression material	Type	ISO 4823	Mixing technique	Operation time (23 °C) (min:sec)	Hardening time in the mouth (35 °C) (min:sec)	Brand
Panasil putty fast	Addition type silicone	Tip 0, putty	Manual, 1:1 scale (base and catalyst)	2:00	2:00	Kettenbach GmbH, Germany
Zetaplus	Condensation type silicone	Tip 0, putty	Manual (base and catalyst)	1:15	3:30	Zhermack, Italy

Table 2. Experimental groups for each measurement item

Group	Method and time	Number of samples (n)
Control	-	8
3% hydrogen peroxide	10 min immersion	8
3% hydrogen peroxide	20 min immersion	8
5.25% sodium hypochlorite	10 min immersion	8
5.25% sodium hypochlorite	20 min immersion	8
Autoclave	40 min at 134 °C	8
Autoclave	50 min at 134 °C	8

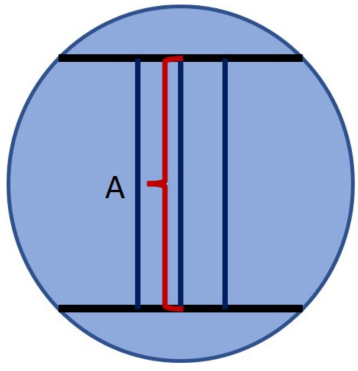

Figure 1. Schematic view of the test setup. Distance "A" (the vertical line in the middle between the two horizontal lines): 25 mm

(p<0.05). Disinfection with NaOCl did not affect the dimensions of the VPS groups, while there was a significant difference in the dimensions in both the 40 and 50 min periods in the autoclave process (Table 3). The autoclave method caused dimensional changes in both impression materials regardless of the application duration.

Two-Way ANOVA was used to evaluate the effect of the disinfection method and application duration on dimensional changes within impression material groups. In the CS group, longer application duration significantly increased the dimensional change (p=0.0001). The prolongation of the time in the VPS groups did not affect the dimensional change in the NaOCl and autoclave groups, while there was a significant difference between the 10 min and 20 min application durations in the hydrogen peroxide group (p=0.02).

When the dimensional changes in impression materials were compared with each other regarding the disinfection method, dimensional changes of CS were significantly higher compared with the VPS in disinfection with NaOCl (p<0.05). There was no significant difference between the dimensional changes of the two impression materials by the autoclave method.

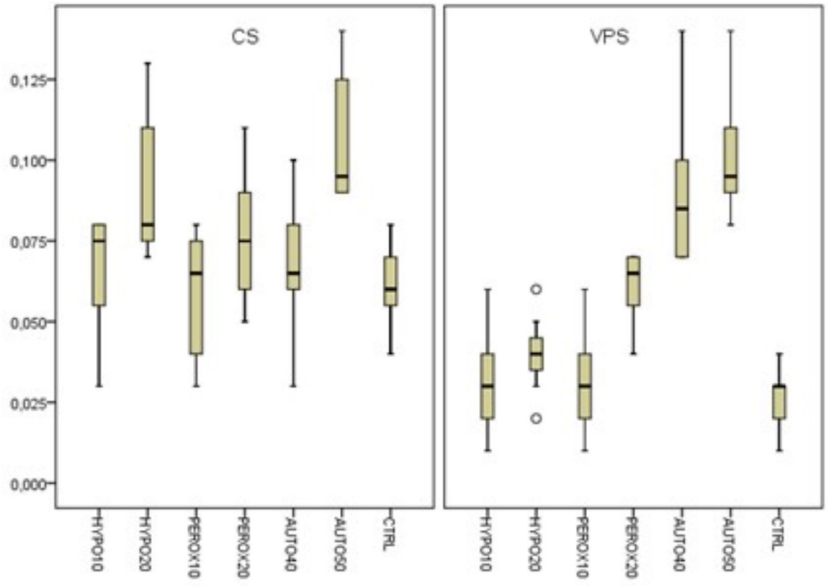

Figure 2. Average dimensional change (%) for the tested procedures
CS: Condensation silicone, VPS: Vinyl polysiloxane

Table 3. Descriptive and statistical analysis

	Dimensional change (%) Mean±SD		p	p**
CS	T_1	T_2		
Hypochlorite	0.07±0.02[1.a]	0.09±0.02[a.2]	0.49	
Peroxide	0.06±0.02[3]	0.08±0.02		**0.0001***
Autoclave	0.07±0.02[b]	0.11±0.02[b]	**0.02**	
Control	0.06±0.01			
VPS				
Hypochlorite	0.03±0.02[1]	0.04±0.01[2]		
Peroxide	0.03±0.02[3.c]	0.06±0.01[c]	**0.02**	**0.001***
Autoclave	0.09±0.02	0.1±0.02		
Control	0.03±0.01			
	p=0.005⁺	**p=0.0001⁺**		

Within any column means with the same superscript numbers are significantly different (p<0.05).
Within any line means with the same superscript letters are significantly different (p<0.05).
⁺Significance between CS and VPS groups.
*Significance within CS and VPS groups.
**Two-Way ANOVA (post-hoc: Bonferroni).
CS: Condensation silicone, VPS: Vinyl polysiloxane, SD: Standard deviation, T_1: 10 min for hypochlorite and peroxide, 40 min for autoclave; T_2: 20 min for hypochlorite and peroxide, 50 min for autoclave

Discussion

Current literature indicates that SARS-CoV-2, the cause of COVID-19, remains as an aerosol for 3 hours and on plastic and steel surfaces for 2-3 days (21). SARS-CoV-2 is a virus with an outer lipid envelope making it more susceptible to disinfectants. Studies on Beta coronaviruses, including SARS-CoV-2, show that these viruses are sensitive to UV light and high temperatures (30 min, 56 °C) (22). The dimensional stability of both impression materials tested in the current study was affected by infection control procedures and different application durations. Hence, the null hypothesis was rejected.

This *in vitro* study was conducted by choosing two previously used disinfection procedures that had been reported effective in SARS-CoV-2 (14). The findings are considered valuable concerning protection from high contamination risk in oral and dental health services. The results showed that the dimensional stabilities of CS and VPS were most affected by the autoclave method, while the prolonged exposure time only affected the dimensional change of CS. According to ANSI/ADA specifications, dimensional changes of less than 0.5% are acceptable (23). No material in this study showed the percentage of dimensional change above this value.

CS is obtained by cross-linking polycondensation reaction, which releases alcohol that contributes to the shrinkage of the impression. This shrinkage increases with the prolongation of time after removal from the mouth. However, VPS has the added benefit of no polymerization shrinkage since no by-products are released (24). This study also confirmed that VPS remained more dimensionally stable than CS due to its chemical structure.

The immersion of elastomers in liquids for a longer duration can lead to dimensional changes due to their hydrophilic nature (9). Moreover, NaOCl is a highly reactive element and may adhere to constituents of the impression material (14). CS and VPS are known as hydrophobic within the elastomeric impression materials. This study explored the causes of why the dimensional changes in CS were significant in the hypochlorite group. However, the previous work stated that the interaction between the NaOCl and the impression material might create a kind of sealing or reduce the dimensional change over time (25). This finding can be considered a beneficial effect of disinfection by immersion in 5.25% NaOCl for 10 min on VPS and could explain the results of our study.

Wetness duration on the applied surface, in other words, the evaporation time of the product from the surface to neutralize viruses and pathogens for any disinfectant, is also extremely important. The evaporation of many disinfectants before the required wet time causes the contaminated surface to be not disinfected at the desired level. This creates the need to apply the product to the surface multiple times to achieve the targeted effectiveness (26). Similarly, immersion of impression materials in different disinfectants in chemical disinfection processes is more effective than spraying their surfaces (8). This may be due to the guaranteed disinfection of all impression surfaces, as well as to the longer exposure time to the disinfectant by immersion rather than spraying (27). However, spray disinfection is preferred, especially for hydrophilic impression materials (28). The solution immersion method will promote the water absorption phenomenon in hydrophilic impression materials, and chemical interactions may occur between the impression material and the disinfectant, especially in long-term applications. The immersion method can be safely preferred for disinfecting hydrophobic elastomeric impression materials such as CS and VPS that were used in the current work. The immersion time of these materials can be longer, as was reported previously (29).

A previous study reported that direct disinfection after water washing reduces microbial contamination but does not change the dimensions of the impression and recommended a 2-step disinfection procedure (water washing + disinfection) (10).

The 10-minute immersion method in 5.25% NaOCl, also chosen in this study, is a disinfection method frequently used in dental practice. It has been reported in previous studies that NaOCl, which has virucidal, fungicidal and bactericidal properties, provides adequate disinfection at this concentration and time (30). Additionally, a previous study has suggested that a 10-minute immersion time in different disinfectants is appropriate (31). The times used in our study are below the ADA-recommended maximum immersion time of 30 min (3). Silva and Salvador reported that immersion of CS in 1% NaOCl for 10 and 20 min did not cause dimensional changes in the material (32). Also, another stud showed no significant expansion in VPS when disinfected by immersion in different chemicals for 10 min or 1 h (33). In the current work, 5.25% NaOCl caused a significant dimensional change in CS in 10 and 20-minute applications, and dimensional stability was not affected in VPS compared to the control group at either application duration. Many studies have reported that the VPS impression material does not undergo dimensional changes when disinfected with NaOCl (34,35). Additionally, the findings here showed that a longer application duration in all disinfection methods in the CS group significantly increased the dimensional change and a significant difference was observed between the 10-minute and 20-minute application durations only in the hydrogen peroxide group in the VPS group.

Disinfection with hydrogen peroxide has been less investigated in the literature. However, a published study highlighted that it is effective in CS and reduces microbial spread (36). This study also found no significant difference in the dimensional change regardless of the disinfection time of CS with hydrogen peroxide. The current findings suggest that hydrogen peroxide might be a disinfectant option for silicone impression materials, while hypochlorite is more suitable for VPS.

Previous authors have emphasized that CS and VPS can be sterilized in an autoclave with a metal standard impression tray at 132 °C (37) without significant dimensional change or at 134 °C (38) with less than 0.5% dimensional change. During the sterilization of the VPS in the autoclave, the dimensional change was significant when measured immediately after the autoclave, but there was no change in 24-hour measurement. Gothwal et al. (39) showed that CS and VPS elastomeric impression materials can withstand higher sterilization temperatures by steam autoclaving at 134 °C for 30 min, without significantly affecting their elastic recovery. Conversely, in this study, possibly due to increased application durations, the autoclave sterilization showed the highest dimensional change values for both impression items, and this finding is similar to the study by Martins et al. (14). However, information on the effects of autoclave sterilization on the dimensional change of the impression is insufficient. As a result, since SARS-CoV-2 is sensitive to high temperatures, it can be predicted that the autoclave method may be successful against the virus, but it may change the dimensions of the measure.

When the dimensional changes of impression materials were compared with each other, CS showed more dimensional changes upon disinfection with NaOCl and hydrogen peroxide. Similar results were obtained with the autoclave method. In this context, current data also confirm that VPS is more dimensionally stable.

Study Limitations

This study has some limitations. The materials tested were not exposed to some conditions that could affect the size of the impression, including moisture in the oral environment, saliva, and removal of the impression from the mouth. Therefore, there may be a disadvantage in fully simulating the clinical environment. Additionally, making the measurements only on a flat surface without assessing three-dimensional dimensional changes can be considered another limitation. The polymerization stages of impression materials were created in the test setup similar to the clinical environment, and the influencing factors were minimized.

In summary, the effect of disinfection or sterilization on the impression varies with factors such as the method used, time, disinfectant concentration, and the type of impression material. Generally, disinfection can affect not only the dimensional stability but also the surface properties of the plaster model obtained from the impression. Therefore, there is a need for future studies that can contribute to the data of this study and evaluate other different clinical parameters.

Conclusions

The percentages of dimensional change in impression materials were clinically acceptable; VPS was more dimensionally stable than CS. Since NaOCl does not affect the dimensional change of VPS, it is preferred over hydrogen peroxide in the disinfection of this material at the recommended times. Hydrogen peroxide did not cause significant dimensional changes in CS. Autoclave sterilization significantly affected the dimensional changes in both impression materials. The prolongation of the application duration correlated with the dimensional changes in CS.

Ethics

Ethics Committee Approval and Informed Consent: This was an experimental study that used *in vitro* environment.

Peer-review: Externally and internally peer-reviewed.

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

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Comment on "Caffeine intake and bone mineral density in postmenopausal women"

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Keywords: Caffeine, postmenopausal osteoporosis, femur, fracture, women

Dear Editor,

Reading the manuscript by Adıgüzel and Köroğlu (1) titled "Caffeine intake and bone mineral density in post-menopausal women", I realized that the average consumption of caffeine in their population was 229.7±119.5 mg, and more than one-third of their patients were classified "high consumers". The authors identified that the mean femoral neck T-score was significantly lower among high caffeine consumers, with the amount of daily caffeine intake showing a negative and moderate correlation with femoral neck T-scores.

Emokpae and Brown (2) have defined caffeine as a stimulant in many beverages affecting the reproductive system, interrupting fertilization and implantation, with several other studies mentioning its adverse effects, and as a compound without a universally accepted "safe dose" for humans. They also mentioned high-caffeine energy drinks being increasingly popular, and the withdrawal symptoms after cessation of caffeine, such as headaches, nausea, and irritability.

Berman et al. (3) concluded that caffeine exerts various biological effects that may adversely affect bone mineral density, possibly by competitive inhibition of adenosine A2 receptors and inhibition of vitamin D receptor activity, increasing osteoporosis and fracture risks in older adults, especially among post-menopausal Caucasian females consuming 2 or more cups of coffee daily.

Adıgüzel and Köroğlu (1) have suggested that the major strength of their study was the assessment of caffeine consumption by an experienced dietician, making their data more valuable. It has been shown that countries consuming the highest amount of caffeine have the highest incidences of both osteoporosis and hip fractures worldwide (3).

Encouraging women, particularly in the postmenopausal period, to follow and control their daily caffeine intake may protect them from hip fractures and possibly from other osteoporotic fractures. The amount of caffeine in commercially available drinks is provided by the suppliers either on the product label or on the internet. There are already useful mobile phone applications to follow caffeine intake.

The economic cost of hip fractures has been reported as an important issue in developed countries, even estimated to be higher than that of other diseases with high disabling potential, such as Parkinson's disease, rheumatoid arthritis, and stroke. The costs increase substantially when additional expenses of work loss and rehabilitation are added (4).

The work by Adıgüzel and Köroğlu (1) is appreciated for emphasizing the need for improving public awareness of the potential risks of high caffeine intake from a different view. In this context, follow-up of daily caffeine intake and taking reasonable amounts are warranted especially for post-menopausal women, with the hope of reducing the overwhelming cost of hip and other fractures on health expenditure worldwide.

Ethics

Peer-review: Internally peer-reviewed.

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

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Reply to: Comment on "Caffeine intake and bone mineral density in postmenopausal women"

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Keywords: Osteopenia, osteoporosis, caffeine, bone mineral densitometry, dual-energy X-ray absorptiometry, DXA

Dear Editor,

We would like to thank the author for the comments on our article entitled titled "Caffeine intake and bone mineral density in postmenopausal women" (1).

As the author has also addressed and we have emphasized in our article that high caffeine-containing energy drinks are increasingly consumed, and caffeine intake gets higher over the world (2). In contrast to prescription drugs, other medicines, and harmful chemicals, there is no safe limit for daily caffeine intake. However, over 200 mg single dose or 400 mg of daily caffeine may cause undesired effects as reported in the European Food Safety Authority Scientific Opinion document (3).

In our article, we did not aim to report the biological effects of caffeine via receptors on bony tissues or other body structures. On the other hand, adenosine receptors and vitamin D metabolism are the potential pathways to be affected (4). Considering the high percentage of adults that consume at least one caffeine-containing beverage daily, the total impact of caffeine on human health should be emphasized (5).

As the author stated, the undesired effects of caffeine can be minimized if its daily amount is carefully controlled. Nevertheless, factors like biased patient reports and differences in metabolism and absorption make the correct assessment of caffeine intake difficult.

Ethics

Peer-review: Internally peer-reviewed.

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

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