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Research article

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The effect of leg ischemia/reperfusion injury on the liver in an experimental breast cancer model

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Ethics Committee Approval
This study was conducted at Hacettepe University Experimental Animal Application and Research Center (Ankara, Turkey) after obtaining the approval of the Experimental Animals Local Ethics Committee of the university (Approval No: 2015/46-07).
All animal experiments were performed according to the World Medical Association Code of Ethics (Helsinki Declaration).

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

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Abstract

Background/Aim: Ischemia/reperfusion (I/R) injury occurs during breast cancer surgery, especially those involving a modified radical mastectomy, lumpectomy, and axillary lymph node dissection. Tissue damage and stress due to I/R alter immune system functions, especially those of the myeloid cells. The immunologic impact of this I/R injury on myeloid-derived cancerous cells remains unknown. We sought to investigate the effect of I/R injury in the extremity close the breast tumor location on myeloid cell population in the liver and liver metastasis.

Methods: 4T1 breast tumors were created in the left inguinal breast region of the experimental animals. When the tumor reached 0.5 cm in diameter, ischemia was produced on the left down-extremity for 90 min and reperfusion was induced for short (3 days), middle (7 days), and long terms (14 days). At the end of the reperfusion period, proximal limbs and livers were harvested. The limb and liver samples were histopathologically examined with H&E staining. Immune cell percentages were determined in the liver by flow cytometry.

Results: There was an increase in muscle fiber degeneration and disorganization in the I/R induced proximal legs on days 3 and 7 of I/R in both tumor free and tumor bearing animals with a further impact in tumor bearing mice. Even though I/R injury did not affect tumor metastasis to the liver, it had an impact on liver myeloid cell percentages in both tumor free and tumor bearing animals. Additionally, tumor bearing mice demonstrated higher myeloid cell percentages in both the pre-I/R and post-I/R experimental groups. There was a remarkable change in the levels of granulocytic, and monocytic myeloid cells and macrophages due to the I/R injury.

Conclusion: With the formation of short-term I/R injury in a distant site, tumor development and/or seeding to metastasis sites after surgery could be prevented. This study contributes to the understanding of the inflammatory process after I/R injury occurring during interventions.

Keywords: Breast cancer, Ischemia, I/R injury, Myeloid cells, MDSCs, Liver

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Introduction

Breast cancer is the most common cancer among women and affects 2.1 million women every year, worldwide. It ranks the first cancer-related death among women, with about 15% [1]. In 2018, 22.345 women were diagnosed with breast cancer and 5.452 women lost their lives in Turkey [2]. Various methods were used in the treatment of breast cancer including surgery, radiation therapy, chemotherapy, hormone therapy, targeted drug therapy, and immunotherapy. Most women undergo breast cancer surgery, and many receive adjuvant therapies such as chemotherapy, hormone, or radiation therapy. However, during breast cancer surgery, especially those involving modified radical mastectomy, lumpectomy, axillary lymph node dissection, upper extremity and surrounding tissues are exposed to ischemia. When the surgery is completed, blood supply restarts, which leads to I/R damage.

The term "ischemia" was first expressed in early the 19th century as a restriction in blood supply to tissues by the occlusion of arterial flow. Restoration of blood flow to a previously ischemic tissue or organ is called reperfusion. The longer ischemic duration results in worse clinical complications such as functional failure of tissues and organs due to insufficient oxygen uptake [3]. Because of this, it has tremendous side effects, and increases morbidity and mortality rates. I/R injury is not restricted to the prior ischemic site, also accounts for remote organ damage, which brings out serious results like multiple organ failure [4-6]. Complications related to immune system causing tissue damage begins at reperfusion [7]. The main factors which cause distant organ damage by exiting the ischemic area and entering circulation are leukocytes, inflammatory mediators, and reactive oxygen species (ROS). The production of reactive nitrogen species is also effective in cellular and systemic response to ischemia. These reactive molecules induce cellular damage and structural changes and lead to apoptotic/necrotic cell death [8, 9].

In response to various stimuli, leukocytes migrating to the I/R region and pro-inflammatory molecules disrupt the cellular contact, thereby increasing vascular permeability [10], resulting in even more infiltration of leukocytes into the I/R region [11]. Among the leukocytes, the role of granulocytes in I/R injury is critical, and neutralizing the antibodies against CD11b and/or CD18 were utilized to prevent this damage [12]. Neutrophil granulocytes are the fastest migrating and extensively found cell group in the I/R region. They further induce tissue damage by the production of high levels of ROS, hydrolytic enzymes and pro-inflammatory cytokines and chemokines (IL-1β, IL-6, IL-12, IFN-γ, TNF-α, and MCP-1) [13, 14]. Although the contribution of monocytes and macrophages to the inflammatory process in the I/R region is limited and overshadowed by neutrophils, these cells are also known to migrate to damaged areas [15]. It is undeniable that myeloid cells play the central role in I/R damage, because they are both the foremost and fastest responding immune cell group.

The relationship between cancer development and inflammation has long been investigated [16]. Epidemiological evidence indicates the connection between inflammation and cancer development. For example, longer periods of inflammation induce dysplasia [17]. Furthermore, chronic inflammation-like processes are the characteristics of tumor micro-environment [18]. Secreted various cytokines, chemokines or growth factors (M-CSF, GM-CSF, PGE2, VEGF, IL-1β, IL-4, IL-6, IL-10 and IL-13) induce hematopoiesis and the production of myeloid cells [19]. However, these cells enter the circulation before their maturation step; therefore, they disrupt inflammatory response due to insufficient immune stimulation and/or immune suppression. Myeloid cells which regulate immune responses by diverse mechanisms are called myeloid-derived suppressor cells (MDSC) and divided into subtypes as PMN-MDSC: $CD14^{-}$ $CD11b^{+}CD15^{+}$ (or $CD66b^{+}$), E-MDSC: Lin^{-} $(CD3/14/15/19/56)/HLA\text{-}DR^{-}/CD33^{+}$, M-MDSC: $CD11b^{+}CD14^{+}HLA\text{-}DR^{low/-}$ $CD15^{-}$ in human; PMN-MDSC: $Gr\text{-}1^{+}CD11b^{+}Ly6C^{lo}Ly6G^{+}$ and M-MDSC: $Gr\text{-}1^{+}CD11b^{+}Ly6C^{hi}Ly6G^{-}$ in mice [20]. The presence and increase of MDSCs have also been shown in peripheral blood and tumor samples of cancer patients. The ratio of MDSCs in peripheral blood augments when cancer is in the progress. For example, the percentage of MDSCs is 1.96% in stage 1–2, 2.46% in stage 3 and 3.77% in stage 4 breast cancer [21].

MDSCs are gathered in the liver in addition to the spleen, bone marrow, blood, and the tumor. Liver hematopoiesis also contributes to the expansion of MDSCs in this organ [22]. It was indicated that intravenous injection of exogenous bone marrow-derived mononuclear cells into mice with colorectal cancer are able to migrate to the liver and induce tumor liver metastasis [23]. However, the contribution of hepatic I/R injury to cancer growth and metastasis in the liver has been explored in different studies [24]. Moreover, hepatic ischemia enhances the number of metastatic nodules in the liver in experimental rat and mice colon cancer models [25-27]. In other preclinical experimental studies, the impact of hepatic I/R injury on tumor growth and metastasis in different tumor models such as hepatocellular carcinoma and pancreatic cancer has been investigated [28, 29]. In hepatic ischemia, cytokines, growth factors, and adhesion molecules produced in the I/R region are the mediators of induction of tumor progression and liver metastasis [24]. However, none of these studies demonstrated the impact of I/R injury in a distant organ on tumor liver metastasis. Although immune suppression capacity, therefore, the tumor-promoting effect of MDSCs is enhanced under hypoxia, information about how these cells behave during I/R injury is limited [30]. In this study, we assumed that I/R damage to a distant organ affects the level of myeloid cells in the liver as a host repair response in the presence of a tumor and might affect tumor growth and liver metastasis. Therefore, we herein established an extremity I/R model in mice harboring breast tumors in the mammary fat pad and showed that I/R can induce the accumulation of MDSCs, especially G-MDSCs, in the liver and tumor growth without an effect on tumor metastasis to the liver.

Materials and methods

This study was conducted at Hacettepe University Experimental Animal Application and Research Center (Ankara, Turkey) after obtaining the approval of the Experimental Animals Local Ethics Committee of the university (Approval

No: 2015/46-07). All animal experiments were performed according to the World Medical Association Code of Ethics (Helsinki Declaration).

Animal model and cell lines

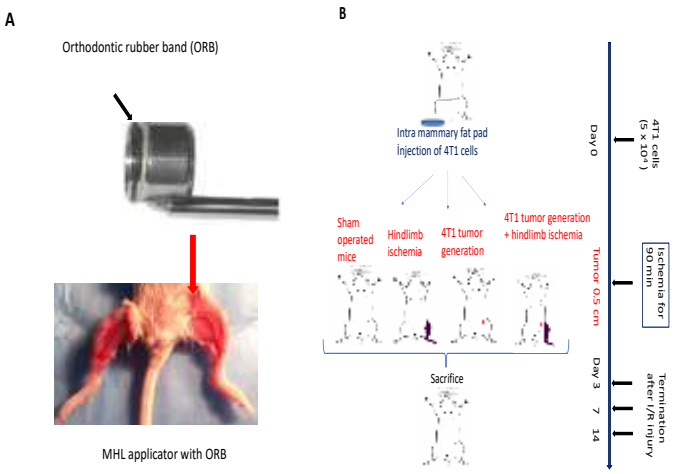
Female BALB/c mice, 6–8 weeks old, (Kobay A.S., Ankara, Turkey) were housed under standard conditions. Animals were divided into 8 groups: Extremity I/R early (Day 3 (n = 5)), middle (Day 7 (n = 6)), and late (Day 14 (n = 6)), breast cancer + I/R early (Day 3 (n = 5)), middle (Day 7 (n = 6)), and late (Day 14 (n = 6)), breast cancer only (n = 6), and no I/R control groups (n = 5).

Breast cancer cell line, 4T1, (American Type Culture Collection, LGC Promochem, Rockville, MD, USA) were cultured in RPMI 1640 medium (Biowest, Nuaillé, France) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% FBS in humidified atmosphere with 5% CO_2 at 37°C. 4T1 cells ($5x10^4$ cells/100 µl) were subcutaneously inoculated into the left-inguinal mammary fat pad of BALB/c mice.

Anesthesia and surgical procedure

For all groups, 5 mg/kg Xylazine (Alfazyne- %2) and 150 mg/kg ketamine hydrochloride (Ketalar- %5) were injected intraperitoneally for general anesthesia. Thirty minutes after the induction of anesthesia, the McGivney Ligasure was used to apply the ORB on the left limb [31] (Figure 1A). Supplemental anesthesia was administered to keep the mice under anesthesia throughout the duration of ischemia. Ischemia was induced on the hindlimbs for 90 min in the first 6 groups, and ORB was cut to induce reperfusion for 3, 7, and 14 days. Mice in 4th, 5th and 6th groups were first administered 4T1 cells into left mammary pad s.c. (Figure 1B). Tumors were monitored twice a week and when the tumor reached 0.5 cm in diameter, 90 min of ischemia was created. Mice in the 7th group were only inoculated with tumor cells while mice in 8th group served as controls. At the end of reperfusion, animals were euthanized (Figure 1B). Both limbs, livers and tumors were harvested. Body weights were followed biweekly. Limbs were collected just under the region where ORB was applied and the skin was removed.

Figure 1: Establishment the model of hind limb I/R injury. A) Representative position of ORB and MHL on mouse hind limb and operative view of hind limb ischemia injury. Orthodontic rubber band (ORB) is applied by McGiveny hemorrhoidal ligator (MHL). Red arrow indicates the position of ORB. B) Animals were inoculated with 4T1 cells s.c. (5 x 10^4 cells/100 µl) into left mammary fat pad and 90 min of ischemia was induced on the left hind limb when 4T1 tumors were 0.5 cm in diameter in (n =23 mice). Mice were sacrificed after 3-, 7- and 14-days following reperfusion and livers were harvested for histological and immunological assessment. n=6 mice were only injected with tumor cells. I/R injury was induced in n=18 mice w/o tumor challenge and n=5 mice left as controls.



Immunological Analyses

Cell suspensions from the livers and tumors were obtained with mechanical agitation in phosphate buffered saline (PBS) and passed through 40 µm pore-sized filters. The leukocytes in the cell suspension were further separated by Ficoll-1119 (Sigma, Steinheim, Germany) density gradient centrifugation. The cells were labeled with monoclonal antibodies against CD45 (clone 30/F11), CD11b (clone M1/70), Gr-1 (clone RB6-8C5), Ly6C (clone HK1.4), Ly6G (clone 1A8), and F4/80 (clone BM8) and isotype controls were used (Biolegend, San Diego, CA, USA). The percentage of positive cells was calculated by comparison with the appropriate isotype-matched antibody controls. Studies and analyses were conducted on a FACS Aria II flow cytometer (Becton Dickinson, San Jose, CA, USA) and FACS Diva software, respectively.

Histopathology and immunohistochemistry

Proximal limb tissues were harvested, fixed in 3.7% formaldehyde, embedded in paraffin, sliced, and stained with hematoxylin and eosin for histopathological evaluation. Histopathological scores for muscle fiber degeneration and disorganization were found in 5 tumor-free mice in the no I/R group, 8 in 3 days post-I/R, 5 in 7 days post-I/R, 6 in 14 days post-I/R and among tumor bearing mice, 6 in the no I/R group, 5 in 3 days post-I/R, 4 in 7 days post-I/R, and 6 in 14 days post-I/R groups. They were scored as severe = 3, mild = 2, and occasional = 1. In the liver, metastatic loci and myeloid cell groups were examined. For granulocytic myeloid cells, Ly6G marker was analyzed by immunohistochemical staining. Metastatic foci were determined within 4 µm sections cut across the liver. The geometric mean of the largest vertical and horizontal dimensions of these foci was calculated as a measure of the size. A metastasis score was calculated for each animal by considering the frequency and size of foci (metastasis score per liver = number of the foci x average size of the foci).

Relative-quantitative RT-PCR

Total RNA (5 µg) was isolated (Animal Tissue RNA Purification Kit, Norgen Biotek, Thorold, ON, Canada) and converted into cDNA (RevertAid First Strand cDNA Synthesis Kit, Thermo, Waltham, MA, USA). Real-time PCR (SsoAdvanced Universal SYBR Green supermix, Bio-Rad, Hercules, CA, USA) was performed with the forward and reverse primer oligonucleotides, respectively, specifically designed for IL-1β, 5'-TGCCACCTTTTGACAGTGATG-3' and 5'-AAGGTCCACGGGAAAGACAC-3'; calciumbinding protein A9 (S100A9), 5'-AGATGGCCAACAAAGCACCT-3' and 5'-TCTCTTTCTTCATAAAGGTTGCCA-3'; matrix metalloproteinase 9 (MMP-9), 5'-GCGGTCCTCACCATGAGTCC-3' and 5'-TAGCGGTACAAGTATGCCTCTGC-3'; vascular endothelial growth factor A (VEGFA), 5'-CAGATCATGCGGATCAAACCTC-3' and 5'-TTGTTCTGTCTTTCTTTGGTCTGC-3'; CCL2 5'-AGCTGTAGTTTTTGTCACCAAGC-3' and 5'-GTGCTTGAGGTGGTTGTGGA-3'; βactin, 5'-GGCACCACACCTTCTACAATG-3' and 5'-GGGGTGTTGAAGGTCTCAAAC-3'. For each gene of interest, threshold cycle (Ct) was determined and normalized according to the housekeeping β-actin Ct.

Statistical analysis

Statistical analyses were performed using Student's t-test and one-way ANOVA where appropriate (SPSS software, IBM, Turkey). For each test, *P*-values ≤0.05, ≤0.01, ≤0.001 and ≤0.0001 were considered statistically significant, more significant, significant, most significant, respectively ((*), (**), (***), and (****), respectively). Unless otherwise noted, the data are shown as mean ± SEM.

Results

The effect of I/R injury on muscle damage in the proximal limb tissues

Histopathological sections from the extremities were examined for muscle fiber degeneration and disorganization. Muscle fiber degeneration was 50% with score 3, 37.5% with score 2, and 12.5% with score 1 on day 3 post-I/R in the proximal limb of tumor free animals, while it was 80% with score 3 and 20% with score 2 in tumor-bearing mice (Figure 2A). Muscle fiber disorganization in the proximal leg of tumor-bearing animals was 40% with score 3, 40% with score 2 and 20% with score 1. In tumor-free mice, it was 50% with score 3, 12.5% with score 2 and 37.5% with score 1 on day 3 post-I/R (Figure 2B). Muscle fiber degeneration was 100% with score 3 in the proximal leg of tumor-bearing animals and 40% with score 3, 60% with score 2 on day 7 post-I/R in tumor-free mice (Figure 2A). In the proximal leg of tumor free animals, muscle fiber disorganization was 40% with score 3, and 60% with score 1, whereas in tumor-bearing mice, it was 100% with score 3 on day 7 post-I/R (Figure 2B). Furthermore, muscle fiber degeneration was 83.3% with score 2, and 16.6% with score 1 in tumor free mice, whereas in tumor-bearing mice, it was 100% with score 2 on day 14 post-I/R (Figure 2A). Muscle fiber disorganization in the proximal leg of tumor-bearing animals was 66.6% with a score 2 and 33.3% with score 1. In tumor-free mice, it was 33.3% with score 2 and 66.6% with score 1 on day 14 post-I/R (Figure 2B). The histopathological analysis of the proximal limb tissues demonstrated that the I/R procedure induces muscle fiber degeneration and disorganization in both tumor bearing and tumor-free animals with a higher impact on tumor-bearing mice and on day 7 post-I/R (Figure 2).

The effect of I/R injury on the liver

The breast tumor cells may spread locally or metastasize via the lymphatics and blood vessels. Frequently, breast cancer metastasizes to the liver and the lung through blood vessels [32]. Therefore, we first examined the effect of I/R injury on liver metastasis. Liver metastasis was not found in cancer-bearing mice. Moreover, the liver weights were not altered significantly among the tumor-free and tumor-bearing groups except on day 14 post-I/R (Figure 3A).

It is known that myeloid-derived cells have a tumor-promoting function in hematopoiesis-related organs, such as the liver, in cancer patients. Hence, we examined the liver tissue histologically. There was an average of 3 myeloid cell groups in the control group, 14 in the breast cancer group, 3 on day 3 post-I/R, 4 on day 7 post-I/R, and 2 on day 14 post-I/R groups. However, there was 11 on day 3 post-I/R, 30 on day 7 post-I/R and 85 on day 14 post-I/R in tumor-bearing animals.

Figure 2: Analysis of muscle damage in I/R injury induced proximal limb tissues. A) Muscle fiber degeneration in the proximal hind limb of tumor free animals without I/R injury (n = 5), on days 3 (n = 8), 7 (n = 5), and 14 (n = 6) post-I/R and 4T1 tumor bearing animals without I/R injury (n = 6), on days 3 (n = 5), 7 (n = 4), and 14 (n = 6). B) Muscle fiber disorganization in the proximal hind limb of tumor-free animals without I/R injury (n = 5), on days 3 (n = 8), 7 (n = 5), and 14 (n = 6) post-I/R and 4T1 tumor bearing animals without I/R injury (n = 6), on days 3 (n = 5), 7 (n = 4), and 14 (n = 6). Muscle fiber degeneration and disorganization scores were determined by hematoxylin and eosin staining. C) Representative H and E-stained histological images, 1 out of all samples in each group are shown.

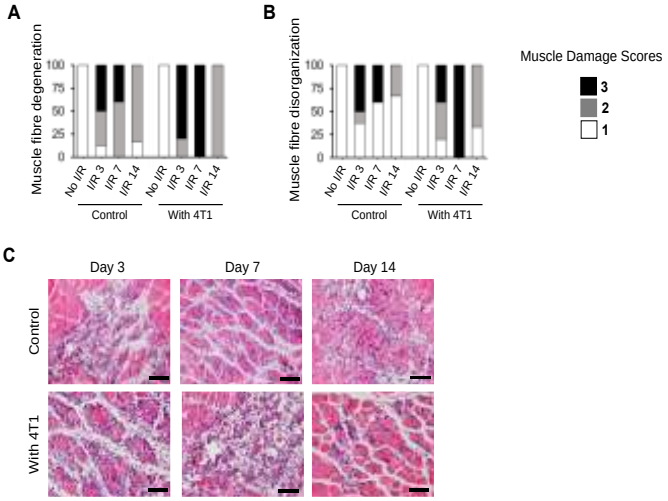
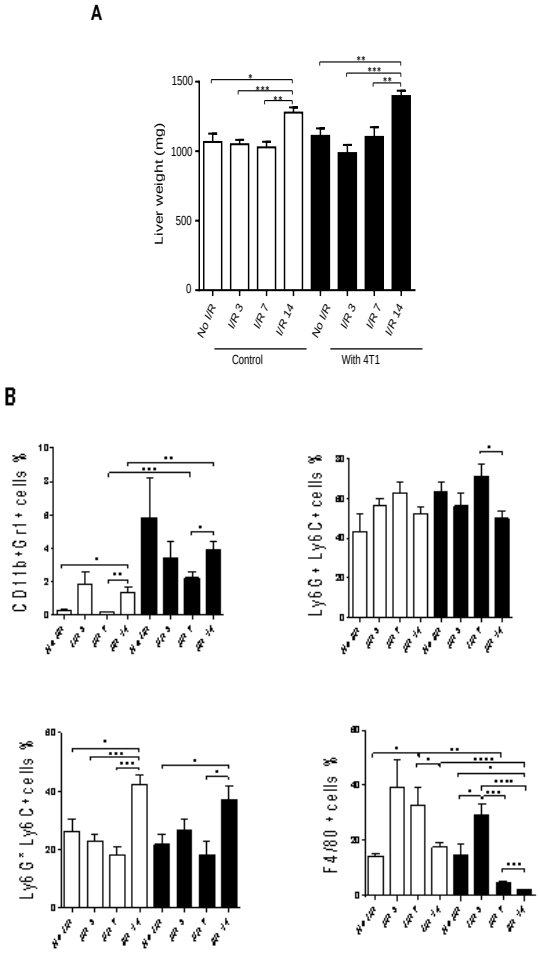


Figure 3: Hind limb I/R injury influence on the liver myeloid cell population percentages in tumor free and tumor-bearing mice. A) Liver weights were measured and average weight of livers in each group were graphed. The data are shown as mean ± SEM. B) Low-density cells (<1.077 g/mL) were isolated from fresh liver samples and CD45+ CD11b+ Gr-1+ total MDSCs, CD45+ CD11b+ Gr-1+ Ly6G+ Ly6C+ MDSCs, CD45+ CD11b+ Gr-1+ Ly6G-Ly6C+ MDSCs and CD45+ CD11b+ F4/80+ macrophages were assessed by flow cytometry. The data are shown as mean ± SEM. Significance was determined by one-way ANOVA (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001) and only significant values were indicated.



Due to the difference in the number of myeloid cell groups in liver sections, the percentage of myeloid cells was analyzed by flow cytometry. There was 0.22% (0.06%) of CD11b $^{+}$ Gr1 $^{+}$ total myeloid cells on average before the induction of I/R injury in the livers of tumor free animals (Figure

3B). This level was increased to the average of 1.83 (0.65) by day 3 after the I/R, reduced to 0.16 (0.02) by day 7 post - I/R, and enhanced to 1.29 (0.35) on day 14 post - I/R in tumor free mice. Total myeloid cell percentage on day 14 after the I/R was significantly more than the control I/R and day 7-post - I/R groups (P<0.05 and 0.02, respectively). However, there was 5.85% (2.2%) CD11b $^{+}$ Gr1 $^{+}$ total myeloid cells in tumor-bearing animals before the I/R and this percentage reduced to 3.4 (0.95) on day 3 after the I/R, 2.2 (0.34) on day 7 post-I/R (Figure 3B). However, CD11b $^{+}$ Gr1 $^{+}$ cell percentage was increased significantly to 3.92 (0.48) on day 14 compared to day 7 after the I/R (P<0.05). Furthermore, there was significantly more liver myeloid cell level on day 7 and 14 in tumor-bearing animals compared with tumor-bearing mice (P<0.001 and 0.02, respectively) (Figure 3B).

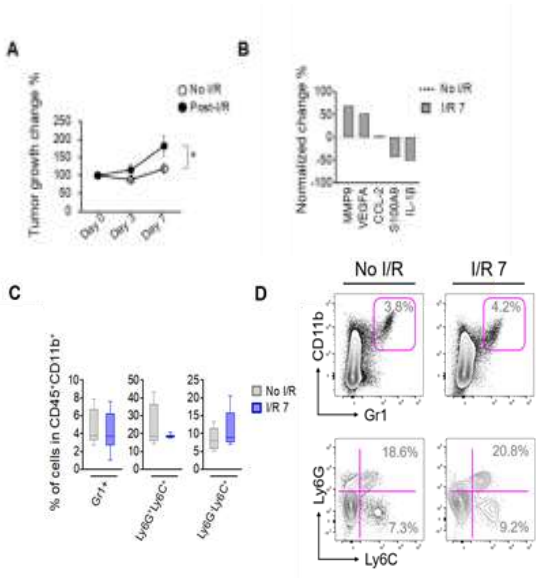
We further examined the sub-populations of myeloid cells and determined that the amount of $Ly6G^{+}C^{+}$ cell percentages enhanced to an average of 56.8 (2.75) on day 3, 62.9 (5.54) on day 7 compared to the control I/R group 43.2 (7.92) and reduced to 52.2 (3.4) on day 14 after the I/R (Figure 3B). In contrast, there was 63.4 (4.87)% of $Ly6G^{+}C^{+}$ cells before I/R in tumor-bearing animals and this percentage decreased to 56.3 (5.68)% on day 3, increased to 70.9 (6.01)% on day 7 and diminished to 49.8 (4.57)% on day 14 post - I/R. The difference in the percentage of $Ly6G^{+}C^{+}$ cells between day 7 and day 14-post - I/R was significant (P<0.05) (Figure 3B). There were augmented percentages of $Ly6G^{+}C^{+}$ cells in tumor bearing mice compared to tumor free animals before the I/R and on day 7 after the I/R while lower percentages on day 3 and 14 after the I/R. The amount of $Ly6G^{-}C^{+}$ cells were 26.1 (3.65)% on average before the induction of I/R injury in tumor free mice, which reduced to an average of 22.8 (1.83)% by day 3 and 18.1 (2.85)% by day 7 after the I/R. On the contrary, these levels were increased to 42.1 (3.3)% by day 14 post - I/R. $Ly6G^{-}C^{+}$ myeloid cell percentage on day 14 after the I/R was significantly more than the control no I/R, day 3 and 7 post-I/R groups (P<0.05, 0.001 and 0.001, respectively) (Figure 3B). The percentage of $Ly6G^{-}C^{+}$ cells were 21.6 (3.32)% on average before the I/R injury in tumor-inoculated mice, and increased to 26.7 (3.5) by day 3, decreased to 18.1 (4.41) by days 7 and increased to 34.3 (5.15) by day 14 after the I/R. $Ly6G^{-}C^{+}$ cell percentage on day 14 after the I/R was significantly more than the control no I/R, and day 7 post - I/R groups (P<0.05) (Figure 3B). There were reduced percentages of $Ly6G^{-}C^{+}$ cells in tumor-bearing mice compared to tumor free animals before the I/R and on day 14 after the I/R, while a higher percentage was observed on day 3 and the same percentage was seen on day 7 post- I/R.

Moreover, the percentage of $CD11b^{+}$ $F480^{+}$ macrophages was 14.3 (0.76)% in tumor free animals before the I/R and increased to 39.3 (8.43)% on day 3 post – I/R. Then, their levels decreased to 32.8 (6.24)% on day 7 and 17.6 (1.7)% on day 14 (Figure 3B). However, day 7 post-I/R level was still significantly higher than before I/R control and on day 14 after the I/R (P<0.05) (Figure 3B). In tumor-bearing mice, the macrophage level was 14.8 (3.58)% before the ischemia. This percentage significantly increased on day 3 post - I/R (29.1 (3.78) compared to before I/R control, day 7 and day 14 post-I/R groups (P<0.05, 0.001 and 0.0001, respectively) since the levels were decreased to 4.8 (0.47)% on day 7 and 1.99 (0.26)% on day 14. Furthermore, macrophage level on day 14 after I/R was significantly lower than before I/R control and day 7 groups (P<0.05 and 0.001, respectively) (Figure 3B). Macrophage percentages in tumor-inoculated mice were lower than tumor-free mice in all groups except for the before I/R control group with a significant difference on days 7 and 14 after the ischemia (P=0.02 and P<0.001, respectively) (Figure 3B).

Tumorigenesis after ischemia/reperfusion injury

Since MDSC are implicated in tumor progression [33], next, we evaluated the impact of the limb I/R injury on the tumor size and composition of the tumor microenvironment. A positive influence of the limb I/R on the tumor growth (a change in tumor size, 82 (2.7)% was observed on day 7, compared to a group of tumor-bearing mice without the I/R injury (change in tumor size, 19 (1.2) % (Figure 4A). Accordingly, the expression of angiogenesis- related MMP-9 and VEGFA genes was enhanced in the tumor tissue from the animals with I/R injury, whereas the expression of inflammatory factors S100A9 and IL-1β was decreased. The mRNA level of CCL2 was not changed (Figure 4B). In the tumor tissue, no difference was observed in the percentage of myeloid cells. Albeit insignificant, the amount of tumor-infiltrating Ly6G+Ly6C+ granulocytic cells on day 7 tended to decrease (tumor-bearing group without I/R, 24.3 (3.82)%; tumor-bearing group with I/R, 18.55 (0.46)% (Figure 4C and D). It might be speculated that the accumulation of G-MDSC into the I/R limb tissue reduced the influx of these cells into the tumor microenvironment. Hence, the expression pattern of the genes studied may indicate a relationship between tumor growth, angiogenesis and inflammatory regulation in the tumor tissue upon the I/R response.

Figure 4: Influence of the hind limb I/R on 4T1 breast tumors. A) The change observed in the tumor growth on days 3 and 7 is plotted. A group of tumor-bearing animals without I/R injury served as a control group (n=5). B) In the tumor tissues on the day 7 post-I/R, expression of the inflammation-associated genes was studied by semiquantitative real time PCR. The data were normalized, and percent change was calculated in comparison to the data obtained from the tumors from the control mice that did not undergo the I/R procedure (n=5). C) The percentage of Gr-1+, Ly6G+Ly6C+, Ly6GLy6C+ myeloid cells amongst tumor-infiltrating CD45+CD11b+ cells and D) representative counter plots from a mouse out of 6 used are shown. The data are shown as mean ± SEM. Significance was determined by one-way ANOVA (ns, not significant; *P<0.05).



Discussion

Although significant advances have been made in the diagnosis and treatment of breast cancer, a rational number of patients still develop resistances. I/R injury might develop regionally and/or in the extremities during great operations such as breast cancer surgery. It is known that the tissue damage and stress caused by this injury affect the functional character of the immune system, especially myeloid cells, and these cells display tumor-promoting function in the tumor microenvironment and in the organs related to hematopoiesis.

First of all, in this study, breast tumors were developed in the left inguinal breast region of experimental animals. Afterwards, short, middle and long term I/R injury was formed on left down-extremity. The short, middle, and long-term effect of the injury in tumor-bearing and tumor-free mice was evaluated histopathologically with sections taken from the extremities. It was observed that muscle fiber degeneration and disorganization increased gradually on the 3rd and 7th days while decreased on day 14 after ischemia. Our result is consistent with the literature findings since I/R injury is responsible for about 40% of the muscle damage [39]. Moreover, in the presence of breast tumor, the muscles were more damaged in the proximal limbs. This damage is a result of increased oxidative stress due to ROS production and facilitated probably by neutrophils since neutrophils accumulate more at the ischemic site in tumor bearing mice than in the controls [39]. This might be due to hematopoiesis induced by local inflammation within the tumor microenvironment. However, it is necessary to determine the cell types and functional characteristics of the cells in the extremities.

MDSCs play an important role in tumor development. Liver hematopoiesis contributes to the expansion of MDSCs in this organ [22] and these cells have tumor-promoting function in hematopoiesis-related organs of cancer patients. Therefore, liver weights were measured, and no significant difference was found between tumor free and tumor-bearing groups. However, liver weights on day 14 post-I/R were significantly higher than the control no I/R groups, short and mid-term groups. This might be a result of tumor or immune cell migration. Therefore, myeloid cell groups were analyzed histologically within the livers. It has been determined that locus numbers of myeloid cells were more in breast cancer bearing mice than in control animals, and more in ischemia-performed mice than the controls. After all, we sought to investigate the percentage of total and subpopulations of MDSCs within the liver by flow cytometry. Total myeloid cell percentages were enhanced on day 3 post-I/R, diminished to the level previous of the ischemia on day 7 post-I/R and again came up to higher percent on day 14 post-I/R in tumor free mice. The decrease in the myeloid cell populations within the liver on day 7 post-I/R suggests that cells might be migrating to the I/R region as a repair mechanism (40) as the level increased on day 14 post-I/R. On the other hand, there was already high levels of myeloid cells before the I/R and this percent decreased gradually on days 3 and 7 post-I/R and increased back on day 14 post-I/R in tumor harboring animals. Additionally, tumor-bearing mice demonstrated higher myeloid cell percentages at each experimental timepoint. The higher level of total myeloid cells prior to the I/R injury is most probably due to the presence of tumor. Later on, they progressively might have migrated to the I/R region and by day 14 post-I/R, the percentages came up to higher levels due to the tumor effect. Furthermore, the percentage of Ly6G+C+ cells increased gradually on day 3 and 7 and reduced on day 14 post-I/R in tumor-bearing mice. However, the level of these cells decreased on day 3, increased on day 7 and reduced below to the percentage before ischemia in tumor bearing animals. Ly6G-C+ cells diminished gradually on day 3 and 7 and enhanced on day 14 post-I/R in tumor free mice. Their level increased on day 3, decreased on day 7 and augmented significantly on day 14 after ischemia in tumor inoculated mice. The gradual increase in Ly6G+C+ and decrease in Ly6G-C+ on days 3 and 7 NA may be due to the I/R affect in tumor free mice. However, in the presence of I/R injury and a tumor, Ly6G+C+ and Ly6G-C+ levels significantly changed on day 14 post-I/R. However, CD11b+ F480+ macrophage percentages were diminished progressively on day 3, 7 and 14 post-I/R in tumor free mice. In contrast, macrophage levels increased on day 3 and gradually reduced on day 7 and 14 in tumor-bearing mice. Before I/R was created, macrophage levels were similar between tumor-free and tumor-bearing groups. However, the levels dramatically augmented on day 3 post-I/R while reduced gradually on day 7 and 14 post-I/R in both groups. The level of PMN, G-MDSC and macrophages in the peripheral blood and other hematopoietic organs should be determined to understand their trafficking. Furthermore, not only myeloid cell levels but also their functionality should be studied to conclude their role in the model.

Different groups have reported that tumor development and metastasis are induced due to the induction of cytokines, growth factors and adhesion molecules in I/R injury models [24, 41, 42]. However, reperfusion injury was performed in the organ where metastasis is studied in the previously published studies. In our model, we evaluated the effect of limb I/R injury on the metastasis of breast cancer cells to the liver and liver metastasis was not observed in any group. Generation of I/R injury in extremities prevents ischemic damage in distant organs [42, 43]. Providing protection of tissues against severe I/R injury that will be induced later by the generation of short-term periods of I/R injury is called ischemic preconditioning (IPC). Therefore, I/R injury in distant organs prevents the accumulation of myeloid cells in the liver and migration of tumor cells to the liver through myeloid cells. On the other hand, it has been demonstrated that intravenous injection of exogenous bone marrow derived mononuclear cells into mice with colorectal cancer were able to migrate to the liver and induced tumor liver metastasis [23]. In our experimental approach we could not detect this effect. This might be a timing issue in which the duration of the study was insufficient to study metastasis since there were extremely high numbers of myeloid cell groups in the liver of tumor bearing mice on day 14 in the post-I/R group.

Temporary clamping of blood vessels is used to prevent bleeding and may cause I/R injury during oncological surgery [34]. The pro-tumorigenic effects of I/R and its impact on surgical wounds were previously reported to be associated with the upregulation of angiogenic factors, generation of an inflammatory environment, activation of sympathetic nervous system, and induction of hypoxia [35, 36]. In contrast to a study that reported the increment of tumor-infiltrating MDSC upon

skin incision surgery [37], here, the I/R injury did not alter the myeloid cell compartment in the tumors. MDSCs also contribute to the formation of pre-metastatic niche [19]. Even though we did not monitor the impact of limb I/R on metastatic burden in the tumor-bearing animals, a previous study indicated the surgery induced wound-healing mechanisms as a factor that restricts T cell-mediated anti-tumor responses through elevation of circulating and tumor-infiltrating myeloid cells [38]. The influence of I/R-induced G-MDSC on metastasis and anti-tumor immunity remains to be better defined.

Conclusion

This study provides a basis for actualization of more comprehensive studies and formation of new hypotheses in the future. Furthermore, these findings contribute the understanding of inflammatory processes depending on I/R injury that developed during the clinical applications. Understanding the interactions of ischemia formed tissue and tumor microenvironment as two separate sterile inflammation sites, explaining the role of I/R injury in tumor growth and metastasis is a critical necessity for development of new treatment and surgical methods. With the formation of short-term I/R injury in a distant site, tumor development and/or seeding to metastasis sites after surgery could be prevented.

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References

1. https://www.who.int/activities/preventing-cancer.
2. https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/.
3. Bulkley GB. Free radical-mediated reperfusion injury: a selective review. Br J Cancer Suppl. 1987;8:66-73. Epub 1987/06/01. PubMed PMID: 3307876.
4. Barry MC, Kelly C, Burke P, Sheehan S, Redmond HP, Bouchier-Hayes D. Immunological and physiological responses to aortic surgery: effect of reperfusion on neutrophil and monocyte activation and pulmonary function. Br J Surg. 1997;84(4):513-9. Epub 1997/04/01. PubMed PMID: 9112905.
5. Esme H, Fidan H, Koken T, Solak O. Effect of lung ischemia--reperfusion on oxidative stress parameters of remote tissues. Eur J Cardiothorac Surg. 2006;29(3):294-8. Epub 2006/01/28. doi: 10.1016/j.ejcts.2005.12.008. PubMed PMID: 16439150.
6. Vaghasiya JD, Sheth NR, Bhalodia YS, Jivani NP. Exaggerated liver injury induced by renal ischemia reperfusion in diabetes: effect of exenatide. Saudi J Gastroenterol. 2010;16(3):174-80. Epub 2010/07/10. doi: 10.4103/1319-3767.65187. PubMed PMID: 20616412.
7. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012;298:229-317. Epub 2012/08/11. doi: 10.1016/B978-0-12-394309-5.00006-7. PubMed PMID: 22878108.
8. Altamirano F, Wang ZV, Hill JA. Cardioprotection in ischaemia-reperfusion injury: novel mechanisms and clinical translation. J Physiol. 2015;593(17):3773-88. Epub 2015/07/15. doi: 10.1113/JP270953. PubMed PMID: 26173176.
9. Ong SB, Gustafsson AB. New roles for mitochondria in cell death in the reperfused myocardium. Cardiovasc Res. 2012;94(2):190-6. Epub 2011/11/24. doi: 10.1093/cvr/cvr312. PubMed PMID: 22108916.
10. Karatzas T, Neri AA, Baibaki ME, Dontas IA. Rodent models of hepatic ischemia-reperfusion injury: time and percentage-related pathophysiological mechanisms. J Surg Res. 2014;191(2):399-412. Epub 2014/07/19. doi: 10.1016/j.jss.2014.06.024. PubMed PMID: 25033703.
11. Ferrari RS, Andrade CF. Oxidative Stress and Lung Ischemia-Reperfusion Injury. Oxid Med Cell Longev. 2015;2015:590987. Epub 2015/07/15. doi: 10.1155/2015/590987. PubMed PMID: 26161240.
12. Fan C, Zwacka RM, Engelhardt JF. Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med (Berl). 1999;77(8):577-92. Epub 1999/10/30. doi: 10.1007/s001099900029. PubMed PMID: 10543390.
13. Elias-Miro M, Jimenez-Castro MB, Rodes J, Peralta C. Current knowledge on oxidative stress in hepatic ischemia/reperfusion. Free Radic Res. 2013;47(8):555-68. Epub 2013/06/07. doi: 10.3109/10715762.2013.811721. PubMed PMID: 23738581.
14. Rodrigues SF, Granger DN. Role of blood cells in ischaemia-reperfusion induced endothelial barrier failure. Cardiovasc Res. 2010;87(2):291-9. Epub 2010/03/20. doi: 10.1093/cvr/cvq090. PubMed PMID: 20299333.
15. Ioannou A, Dalle Lucca J, Tsokos GC. Immunopathogenesis of ischemia/reperfusion-associated tissue damage. Clin Immunol. 2011;141(1):3-14. Epub 2011/08/16. doi: 10.1016/j.clim.2011.07.001. PubMed PMID: 21839685.
16. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539-45. Epub 2001/03/07. doi: 10.1016/S0140-6736(00)04046-0. PubMed PMID: 11229684.
17. Rakoff-Nahoum S. Why cancer and inflammation? Yale J Biol Med. 2006;79(3-4):123-30. Epub 2007/10/18. PubMed PMID: 17940622.
18. Akbulut GD, Özkazanç, D. , Esendağlı, G. Th1 cells in cancer-associated inflammation. Turkish Journal of Biology. 2017;41(1):20-30.
19. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9(3):162-74. doi: 10.1038/nri2506. PubMed PMID: 19197294.
20. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun. 2016;7:12150. Epub 2016/07/07. doi: 10.1038/ncomms12150. PubMed PMID: 27381735.
21. Markowitz J, Wesolowski R, Papenfuss T, Brooks TR, Carson WE, 3rd. Myeloid-derived suppressor cells in breast cancer. Breast Cancer Res Treat. 2013;140(1):13-21. Epub 2013/07/06. doi: 10.1007/s10549-013-2618-7. PubMed PMID: 23828498.
22. Ilkovitch D, Lopez DM. The liver is a site for tumor-induced myeloid-derived suppressor cell accumulation and immunosuppression. Cancer Res. 2009;69(13):5514-21. Epub 2009/06/25. doi: 10.1158/0008-5472.CAN-08-4625. PubMed PMID: 19549903.
23. Audollent R, Eveno C, Contreres JO, Hainaud P, Rampanou A, Dupuy E, et al. Bone marrow-derived endothelial and hematopoietic precursors cells enhance the metastasis of colon cancer in an orthotopic murine model. Int J Cancer. 2011;129(9):2304-5. Epub 2012/06/28. doi: 10.1002/ijc.25882. PubMed PMID: 22736413.
24. Orci LA, Lacotte S, Oldani G, Morel P, Mentha G, Toso C. The role of hepatic ischemia-reperfusion injury and liver parenchymal quality on cancer recurrence. Dig Dis Sci. 2014;59(9):2058-68. doi: 10.1007/s10620-014-3182-7. PubMed PMID: 24795038.
25. Kurata M, Okajima K, Kawamoto T, Uchiba M, Ohkohchi N. Antithrombin reduces reperfusion-induced hepatic metastasis of colon cancer cells. World J Gastroenterol. 2006;12(1):60-5. doi: 10.3748/wjg.v12.i1.60. PubMed PMID: 16440418.
26. Nicoud IB, Jones CM, Pierce JM, Earl TM, Matrisian LM, Chari RS, et al. Warm hepatic ischemia-reperfusion promotes growth of colorectal carcinoma micrometastases in mouse liver via matrix metalloproteinase-9 induction. Cancer Res. 2007;67(6):2720-8. doi: 10.1158/0008-5472.CAN-06-3923. PubMed PMID: 17363593.
27. Yazdani HO, Tohme S. Murine Model of Metastatic Liver Tumors in the Setting of Ischemia Reperfusion Injury. J Vis Exp. 2019(150). Epub 2019/09/17. doi: 10.3791/59748. PubMed PMID: 31524857.
28. Man K, Ng KT, Lo CM, Ho JW, Sun BS, Sun CK, et al. Ischemia-reperfusion of small liver remnant promotes liver tumor growth and metastases--activation of cell invasion and migration pathways. Liver Transpl. 2007;13(12):1669-77. doi: 10.1002/lt.21193. PubMed PMID: 18044786.
29. Yoshimoto K, Tajima H, Ohta T, Okamoto K, Sakai S, Kinoshita J, et al. Increased E-selectin in hepatic ischemia-reperfusion injury mediates liver metastasis of pancreatic cancer. Oncol Rep. 2012;28(3):791-6. Epub 2012/07/07. doi: 10.3892/or.2012.1896. PubMed PMID: 22766603.
30. Noman MZ, Hasmim M, Messai Y, Terry S, Kieda C, Janji B, et al. Hypoxia: a key player in antitumor immune response. A Review in the Theme: Cellular Responses to Hypoxia. Am J Physiol Cell Physiol. 2015;309(9):C569-79. Epub 2015/08/28. doi: 10.1152/ajpcell.00207.2015. PubMed PMID: 26310815.
31. Crawford RS, Hashmi FF, Jones JE, Albadawi H, McCormack M, Eberlin K, et al. A novel model of acute murine hindlimb ischemia. Am J Physiol Heart Circ Physiol. 2007;292(2):H830-7. doi: 10.1152/ajpheart.00581.2006. PubMed PMID: 17012358.
32. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9(4):274-84. Epub 2009/03/25. doi: 10.1038/nrc2622. PubMed PMID: 19308067.
33. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009;58(1):49-59. doi: 10.1007/s00262-008-0523-4. PubMed PMID: 18446337.
34. Van der Bilt JD, Livestro DP, Borren A, van Hillegersberg R, Borel Rinkes IH. European survey on the application of vascular clamping in liver surgery. Dig Surg. 2007;24(6):423-35. doi: 10.1159/000108325. PubMed PMID: 17855781.
35. Van der Bilt JD, Soeters ME, Duyverman AM, Nijkamp MW, Witteveen PO, van Diest PJ, et al. Perinecrotic hypoxia contributes to ischemia/reperfusion-accelerated outgrowth of colorectal micrometastases. Am J Pathol. 2007;170(4):1379-88. Epub 2007/03/30. doi: 10.2353/ajpath.2007.061028. PubMed PMID: 17392176.
36. Chen Z, Zhang P, Xu Y, Yan J, Liu Z, Lau WB, et al. Surgical stress and cancer progression: the twisted tango. Mol Cancer. 2019;18(1):132. doi: 10.1186/s12943-019-1058-3. PubMed PMID: 31477121.
37. Ma X, Wang M, Yin T, Zhao Y, Wei X. Myeloid-Derived Suppressor Cells Promote Metastasis in Breast Cancer After the Stress of Operative Removal of the Primary Cancer. Front Oncol. 2019;9:855. Epub 2019/09/26. doi: 10.3389/fonc.2019.00855. PubMed PMID: 31552179.
38. Krall JA, Reinhardt F, Mercury OA, Pattabiraman DR, Brooks MW, Dougan M, et al. The systemic response to surgery triggers the outgrowth of distant immune-controlled tumors in mouse models of dormancy. Sci Transl Med. 2018;10(436). Epub 2018/04/13. doi: 10.1126/scitranslmed.aan3464. PubMed PMID: 29643230.
39. Lejay A, Meyer A, Schlagowski AI, Charles AL, Singh F, Bouitbir J, et al. Mitochondria: mitochondrial participation in ischemia-reperfusion injury in skeletal muscle. Int J Biochem Cell Biol. 2014;50:101-5. doi: 10.1016/j.biocel.2014.02.013. PubMed PMID: 24582887.
40. Butterfield TA, Best TM, Merrick MA. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. J Athl Train. 2006;41(4):457-65. PubMed PMID: 17273473.
41. Nagai S, Yoshida A, Facciuto M, Moonka D, Abouljoud MS, Schwartz ME, et al. Ischemia time impacts recurrence of hepatocellular carcinoma after liver transplantation. Hepatology. 2015;61(3):895-904. Epub 2014/08/08. doi: 10.1002/hep.27358. PubMed PMID: 25099130.
42. Orci LA, Berney T, Majno PE, Lacotte S, Oldani G, Morel P, et al. Donor characteristics and risk of hepatocellular carcinoma recurrence after liver transplantation. Br J Surg. 2015;102(10):1250-7. Epub 2015/06/23. doi: 10.1002/bjs.9868. PubMed PMID: 26098966.
43. Lai IR, Chang KJ, Chen CF, Tsai HW. Transient limb ischemia induces remote preconditioning in liver among rats: the protective role of heme oxygenase-1. Transplantation. 2006;81(9):1311-7. Epub 2006/05/16. doi: 10.1097/01.tp.0000203555.14546.63. PubMed PMID: 16699460.

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Emergency peripartum hysterectomy: Five-year experience in a university hospital

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Ethics Committee Approval
Health Sciences University Bursa Yüksek İhtisas Training and Research Hospital ethics committee approved the study with the decision number 2011-KAEK-25 2021/04-25 on 14.04.2021. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Postpartum hemorrhage (PPH) is an important cause of maternal death and morbidity worldwide. Its incidence is still increasing in some countries. This study aimed to evaluate the incidence, indications, risk factors, complications, maternal and fetal outcomes, mortality rates and management strategies of emergency peripartum hysterectomy (EPH) in women with life-threatening postpartum hemorrhage.

Methods: This retrospective cohort study included 94 cases of EPH performed at tertiary obstetric center between January 2016-January 2021. The demographic data, current pregnancy and delivery mode, hysterectomy indications, operative complications, postoperative conditions, and maternal and fetal outcomes were analyzed. EPH was performed for hemorrhage which cannot be controlled with other conventional treatments within 24 hours of delivery.

Results: The incidence of EPH was 1.71 per 1000 deliveries. In our study, the indications of hysterectomy were placenta previa in 47 cases (50%), placental insertion anomalies in 23 cases (24.5%), uterine atony in 22 cases (23.4%), and uterine rupture in 2 cases (2.1%). Hysterectomy was performed after vaginal delivery in 7 patients (7.4%) and during cesarean section in 87 cases (92.6%). Total and subtotal hysterectomy were performed in 88 (93.6%) and 6 cases (6.4%), respectively. Major complications occurred in 40 patients (42.6%). In total, 97.9% of the patients required a transfusion of blood and blood products. The mean postoperative hospital stay was 8.1 (6.1) days. There were two (2.2 %) maternal deaths due to pulmonary embolism and coagulopathy.

Conclusion: EPH operation has high maternal morbidity and mortality but is lifesaving when performed at the appropriate time. The most common indication for EPH was placental abnormality (74.5%), followed by uterine atony (23.4%). Together with a significant increase in cesarean rates, significant increases in the frequency of placental abnormalities are remarkable. Limiting the number of cesarean deliveries will have a significant impact on reducing the risk of EPH.

Keywords: Emergency peripartum hysterectomy, Placenta previa, Abnormal placentation, Uterine atony, Obstetric hemorrhage

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Introduction

Emergency peripartum hysterectomy (EPH) is a life-saving operation, performed as a last-line treatment in massive obstetric hemorrhage that does not respond to any conservative interventions within 24 hours of delivery. Hysterectomy was used only in emergencies in the 1950s, and in later years, it was also used as a sterilization method and prophylaxis in malignant diseases. The first cesarean hysterectomy was performed by Storer in 1966, but the patient died in the early postoperative period. The first known successful surgery was performed by Edward Porro in 1876 to prevent maternal death from obstetric hemorrhage and peritonitis [1].

The reported worldwide incidence of EPH ranges from 0.20 to 5.09 per 1000 deliveries. These rates may differ regionally and nationwide [2, 3]. The risk factors for peripartum hysterectomy (PH) include high parity, advanced maternal age, prolonged delivery, abnormal placentation and a history of one or more cesarean sections [4, 5]. The indications for PH may have changed in recent years, as there has been an increase in EPH rates due to high cesarean rates all over the world [6].

This retrospective study aimed to examine the incidence, indications, risk factors, complications, and maternal and fetal prognosis of patients who underwent EPH and to present our experience in the management of these cases.

Materials and methods

We retrospectively analyzed 94 EPH operations performed between January 2016 and January 2021 at Bursa Yüksek İhtisas Training and Research Hospital, Obstetrics and Gynecology Department. Health Sciences University Bursa Yüksek İhtisas Training and Research Hospital ethics committee approved this study with the decision number 2011-KAEK-25 2021/04-25 on 14.04.2021.

Maternal characteristics such as age, gravidity, parity, body mass index, gestational age and a history of previous cesarean section were recorded. The mode of delivery, incidence, indication and type of hysterectomy, peri-operative or post-operative complications, additional surgical procedures, need for transfusions, post-operative hospitalization days, maternal and fetal outcomes and mortality rate of the patients were noted. A gestational age of less than 24 weeks at delivery was the only exclusion criterion.

EPH was performed in case of massive vaginal bleeding that could not be stopped with other medical (uterotonic agents such as oxytocin, methergine and misoprostol) or conservative surgical (curettage, uterine tamponade (Bacri balloon) etc.) treatments within 24 hours of delivery. The operations were performed by the experienced surgeons of our clinic. Both operative notes and pathology reports were used to determine the precise indication for the procedure. The number of erythrocyte suspension and fresh frozen plasma units delivered during hospitalization were recorded.

Statistical analysis

Statistical analyses were performed with the Windows-based SPSS 24.0 statistical analysis program (SPSS Inc., USA). To determine whether the data were normally distributed, the variables were examined via visual (histograms, probability plots) and analytical methods (Shapiro-Wilk's test and Kolmogorov-Smirnov tests). Variables were descriptively specified as mean (standard deviation), median (min-max), frequency (n) and percentage (%).

Results

This retrospective study reviewed 54.897 deliveries at Bursa Yüksek İhtisas Training and Research Hospital over a 5-year period (2016-2021). Of these, 31.164 (57%) were delivered vaginally and 23.733 (43%) by cesarean section. The overall incidence of EPH was 1.71 per 1.000 deliveries. In our series, seven hysterectomies were performed after vaginal delivery (0.22/1.000 vaginal deliveries) and the remaining 87 hysterectomies were performed after cesarean section (0.36/1,000 cesarean sections). Of these 87 patients, 6 had given only one and 81 had given two or more previous cesarean deliveries.

The demographic and obstetric characteristics of the patients are shown in Table 1. The mean age of the patients was 35.57 (5.3) years. The median gestational age was 35 weeks (24-41 weeks) with a mean birth weight of 2627 (784) grams. The clinical condition of the newborns was good in all cases.

Table 1: Demographic and obstetric characteristics of the patients

Characteristics (n=94)	Mean (standard deviation) Median (Minimum – maximum)
Age (years)	35.57 (5.3)
Gravidity	3.5 (1.0-14.0)
Parity	2.0 (0.0-10.0)
BMI (kg/m^2)	26 (4.2)
Gestational age (weeks, n %)	<35w n=33 (35%)
	35-37w n= 37 (39.4%)
	>37w: n=24 (25.6%)
Mode of delivery (n %)	Vaginal: 7 (7.4%)
	Previous C/S: 6 (6.4%)
	Repeat C/S: 81 (86.2%)
Birth weight (g)	2627 (784)
APGAR 1st min.	7.5 (0.0-9.0)
APGAR 5th min.	8.5 (0-10)
Fetal gender (n, %)	Male n=51 (54.3%)
	Female n=43 (45.7%)

g: grams, kg: kilograms, min: minute, n: frequency, %: percentage. Descriptive analyses were presented using mean (SD) and median (min-max) for normally and non-normally distributed data, respectively

Analysis of indications for emergency hysterectomy, operation types and conditions are detailed in Table 2. Most cases (58.5%) were operated under emergency conditions and general anesthesia. The most common indication for hysterectomy was placental abnormality (74.5%) followed by uterine atony (23.4%) and uterine rupture (2.1%). In this study, all patients (n=23) with placental insertion abnormality and 42 of 47 patients (89.4%) with placenta previa had a history of previous cesarean section.

Table 2: Analysis of indications for emergency hysterectomy, operation types and conditions

Characteristics (n=94)	
Operation condition (n,%)	Elective: n=39 (41.5%)
	Emergency: n=55 (58.5%)
Operation types (n.%)	Total hysterectomy: n=88 (93.6%)
	Subtotal hysterectomy: n=6 (6.4%)
Indications (n,%)	Placenta previa: n=47 (50%)
	Placenta insertion abnormality n=23 (24.5%)
	Uterine atony: n=22 (23.4%)
	Uterine rupture: n=2 (2.1%)
Hypogastric artery ligation (n,%)	n=26 (27.7%)
Blood and blood products transfusion (n,%)	n=92 (97.7%)
Red cell transfusion (units) (n,%)	<2U: n=10 (10.6%)
	2-6U: n=70 (74.5%)
	>7U: n=14 (14.9%)

n: frequency, %: percentage. Descriptive analyses were presented using n and % for categorically distributed variables.

Table 3 defines the complete blood count characteristics of the patients and the quantity of blood and blood products transfused. In total, 97.9% of the patients required blood and

blood product transfusions. The mean unit of erythrocytes transfused to the patients was 5.1 (4.1). Only two patients did not need erythrocyte transfusion. Also, fresh frozen plasma (FFP) replacement was performed in 78 (83%) patients and 19 (22.3%) patients were given thrombocyte suspension. While fibrinogen treatment was not required in 76 patients, we administered 2 grams to 9 patients, 1 gram to 6 patients, and 6 grams to 1 patient. We observed that thrombocytes were not generally given to the patients (n=88).

Table 3: Table of hemoglobin and hematocrit characteristics of the patients, quantity of blood and blood products transfused

Characteristics (n=94)	Mean (standard deviation) Median (Minimum – maximum)
Erythrocyte transfusion (units)	4.0 (0.0-9.0)
Fresh frozen plasma transfusion (units)	2.0 (0.0-10.0)
Platelet transfusion (units)	0.0 (0.0-8.0)
Fibrinogen transfusion (units)	0.0 (0.0-6.0)
Preop. hemoglobin (g/dL)	10.89 (1.5)
Preop. hematocrit (%)	32.99 (4.0)
Postop. hemoglobin (g/dL)	9.15 (1.7)
Postop. hematocrit (%)	28.13 (5.2)

n: frequency, %: percentage, SD: standard deviation, min: minimum, max: maximum preop: Preoperative, postop: postoperative. Descriptive analyses were presented using mean (SD) and median (min-max) for normally and non-normally distributed data, respectively.

Table 4 shows the intra- and postoperative conditions, postoperative intensive care unit needs, complications, and mortality rate. Twenty-one patients were followed in the intensive care unit for a certain period, 5 patients were followed for 2 days, and 7 patients were followed for 3 days. One of the patients was followed up in the intensive care unit for 36 days and one for 22 days. Forty patients (42.6%) suffered major complications. In 12 cases (12.7%), a relaparotomy was performed. Seventy-three patients needed intensive care follow-up (77.7%). There were two (2.2 %) maternal deaths due to pulmonary embolism and coagulopathy.

Table 4: Intra and postoperative characteristics, complications, and mortality rate

Characteristics (n=94)	Mean (standard deviation) Median (Minimum – maximum)
Intensive care unit (ICU) admission	n=21 (22.3%)
ICU stay (days)	0.0 (0.0-36.0)
Postoperative hospitalization days	6 (3-120)
Mortality	n=2 (2.2%)
Complication rate	n=40 (42.6%)
Complications	Urinary injury: n=20 (21.3%) Ureter injury: n=4 (4.3%) Bladder injury: n=16 (17%) Bladder+ ureter injury: n=2 (2.1%) Intestinal injury: n=1 (1.1%) Re-laparotomy: n=12 (12.7%) DIC: n=4 (4.3%) Hematoma: n=3 (3.2%)

n: frequency, %: percentage, SD: standard deviation, min: minimum, max: maximum Descriptive analyses were presented using mean (SD) and median (min-max) for normally and non-normally distributed data, respectively.

Discussion

EPH is a life-saving surgery performed as a last resort in the treatment of massive obstetric hemorrhage. The incidence of EPH is reported between 0.20 and 5.09 per 1.000 deliveries worldwide. The median incidence was 0.61 per 1000 deliveries [7]. These rates may differ regionally and nationwide [5, 8]. The incidence of EPH in our country varies according to the regions. Akar et al. [9] reported this rate as 0.26/1000 when the population of the Middle Anatolia region was included. Zeteroğlu et al. [3] found this rate to be 5.09/1000 in Eastern Anatolia. These differences can be explained by the environmental and socioeconomic characteristics of different populations [10]. Our incidence was 1.17 per 1000 deliveries, consistent with the literature. Our hospital, located in West Anatolia, is a referral, tertiary care center with approximately 10.000 deliveries performed per year. Most patients (62.8%) were referred from other hospitals. If the patients had undergone routine antenatal follow-up, preventive measures could have been taken to reduce the rate of EPH.

In the past years, uterine atony was reported as the most common indication for a hysterectomy. Recent studies have shown that abnormal placentation replaced uterine atony as the most common indication for EPH [11]. This should be due to higher cesarean delivery rates and the development of conservative treatments for uterine atony [12]. Between 1985-1989, Zorlu et al. [13] reported that the incidence of EPH for placenta abnormality increased from 25.6% to 41.7% and the incidence of EPH due to uterine atony decreased from 41.9% to 29.2%. In 1984, Clark et al. [14] reported that the most common reason of EPH was uterine atony (43.4%). A study from the same institution in 1993 stated that their primary indication was placenta accreta (45%) followed by uterine atony (20%). Similarly, the indication rates of placental abnormality were reported as 46%, 42.4% and 47% by Kayabaşoğlu et al. [15] , Karayalçın et al. [16] and, Kwee et al. [17], respectively. Our findings also agreed with all these results. In this study, the most common placental abnormality was placenta previa (50%), which may be due to the high number of patients with previous cesarean section (92.6%). Current data show that placenta previa is a significant risk factor that increases the rate of post-cesarean hysterectomy [4, 7, 18]. Previous cesarean section and placenta previa are the main risk factors for the development of placental insertion anomaly, especially if both are combined.

Cesarean section became the most preferred surgical procedure in obstetrics at the end of the 20th century. High cesarean rates ranging from 23% to 60% in the literature, and the presence of uterine scarring are the most important reasons for the increased incidence of placental abnormalities [19, 20]. Therefore, cesarean delivery is strongly associated with emergency peripartum hysterectomy. Recently, Cara et al. [7], and Lone et al. [21] identified previous cesarean delivery as a risk factor associated with placental abnormality. According to our findings, 92.6% of our patients had a history of at least one cesarean section (n: 86/94). This ratio seems to be higher than the previous studies from Turkey [22, 23]. Cesarean delivery itself is also a risk factor for EPH. It is known that this risk increases with each additional cesarean section [7]. In this study, 81 of 94 patients (86.2%) had undergone two or more previous cesarean sections. The incidences of PH in the vaginal and cesarean delivery groups were 0.22 and 0.36 in 1000 deliveries, respectively. The risk of EPH increased approximately 13 times in our cases with previous cesarean section. Selo-Ojeme et al. [24] and Kwee et al. [17] found a roughly 10-fold increase in EPH after cesarean section. Every effort should be made to reduce the cesarean delivery rate by performing this operation only under essential clinical indications.

Subtotal hysterectomy rate was reported as 36% by Kayabasoğlu et al. [15] and 75% by Zeteroğlu et al [3]. Our subtotal hysterectomy rate was lower than these studies. We preferred total hysterectomy because of placenta previa in most our cases. In the case of uterine atony, subtotal hysterectomy may be safer and faster. However, it is not suitable for bleeding from the lower uterine segment associated with placenta previa

and/or accreta. Engelson et al. [2] preferred total hysterectomy if the patient is hemodynamically stable, especially in the presence of uterine atony or a low-implanted placenta. Clark et al. [14] suggested a total hysterectomy instead of a subtotal hysterectomy in placental invasion pathologies. In the literature, total hysterectomy was recommended instead of subtotal hysterectomy to prevent bleeding from the cervical branch of the uterine artery. In our opinion, the decision of subtotal or total hysterectomy should be individualized according to the patient's condition. Total hysterectomy is a more appropriate procedure in emergency situations, but subtotal hysterectomy may be a better choice in some cases where the surgery needs to be completed in a shorter time.

EPH is associated with excessive blood loss and the need for transfusion. Zeteroglu et al. [3] and Kayabasoglu et al. [15] reported the incidence of blood transfusion as 100%. In our study, 97.6% (n:92/94) of cases required blood transfusion. The mean amount of erythrocyte suspensions transfused to the patients was 5.1 (4.1) units. This finding is similar with recent reports. To achieve optimal outcomes, cases with risk factors for PH should be identified and transferred to appropriate tertiary centers with blood transfusion units.

EPH is associated with high rates of maternal morbidity and mortality. In our study, postoperative maternal morbidity was 42.6%. Karayalcin et al. [16] and Kayabaşoğlu et al. [15] reported postoperative complication rates of 31.5% and 54%, respectively. Al-Jallad et al. [25] reported complications in 44% of 61 PH cases. Our results confirm previous observations. Urinary complications were the most common (21.3%) complication in this study group. Bladder injury was found at a rate of 17%. The rate of bladder injury was 13% in the study of Yucel et al. [10], and 18% in that of Habek et al. [26]. In our study, 75% (n:12/16) of patients with bladder injury had a history of previous cesarean section. This is associated with secondary adhesions in the vesico-uterine space due to previous cesarean section operations. Only one case had bowel injury due to intense intra-abdominal adhesions (1.1%). Re-laparotomy was performed in 12 cases (12.7%). Zeteroglu et al. [3], and Kwee et al. [17] reported the rates of relaparotomy as 13% and 25%, respectively. There were two maternal deaths (2.2%). One was due to consumptive coagulopathy, and she was referred from another hospital. The other one was because of a pulmonary embolism. In the literature, rates of maternal mortality range from 0 to 16.7% [7].

The most common cause of maternal death is peripartum uterine hemorrhage, which is responsible for 27.1% of all maternal deaths in the world [27, 28]. Since this condition is preventable, early diagnosis and effective treatment are essential. Risk factors for peripartum hysterectomy must be determined antenatally. It should be noted that the probability of EPH is very high in cases of placenta previa and/or placenta accreta with previous cesarean sections. The operation should be performed under appropriate clinical settings by experienced surgeons when these risk factors are identified.

A potential limitation of this study is the retrospective design, which likely introduces some degrees of bias. The other limitation was the series' relatively small sample size. In our opinion, prospective case-control studies with larger number of patients are needed to clarify this issue.

Conclusion

Placental invasion abnormalities were the most common indication for EPH. Uterine atony and uterine rupture were identified as other risk factors and the obstetrician should bear in mind that PH may be required. Emergency postpartum hysterectomy is an intervention that requires rapid evaluation to reduce maternal morbidity and mortality. Timely recognition of the need for emergency hysterectomy in high-risk patient groups and poor prognostic deliveries will help improve the maternal outcomes of pregnancy.

References

1. Sturdee DW, Rushton DI. Caesarean and post-partum hysterectomy 1968–1983. BJOG: An International Journal of Obstetrics & Gynaecology. 1986;93:270–4.
2. Engelsen IB, Albrechtsen S, Iversen OE. Peripartum hysterectomy-incidence and maternal morbidity. Acta obstetricia et gynecologica Scandinavica. 2001;80:409–12.
3. Zeteroglu S, Ustun Y, Engin-Ustun Y, Sahin G, Kamacı M. Peripartum hysterectomy in a teaching hospital in the eastern region of Turkey. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2005;120:57–62.
4. Van Den Akker T, Brobbel C, Dekkers OM, Bloemenkamp KWM. Prevalence, indications, risk indicators, and outcomes of emergency peripartum hysterectomy worldwide. Obstetrics & Gynecology. 2016;128:1281–94.
5. Campbell SM, Corcoran P, Manning E, Greene RA, Group IMMA. Peripartum hysterectomy incidence, risk factors and clinical characteristics in Ireland. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2016;207:56–61.
6. Kallianidis AF, Maraschini A, Danis J, Colmorn LB, Deneux-Tharaux C, Donati S, et al. Epidemiological analysis of peripartum hysterectomy across nine European countries. Acta obstetricia et gynecologica Scandinavica. 2020;99:1364–73.
7. Cara Z, Thompson EL, O'Rourke K, Nembhard WN. Cesarean section and the risk of emergency peripartum hysterectomy in high-income countries: a systematic review. Archives of gynecology and obstetrics. 2015;292:1201–15.
8. Machado LSM. Emergency peripartum hysterectomy: incidence, indications, risk factors and outcome. North American journal of medical sciences. 2011;3:358.
9. Akar ME, Yilmaz ES, Yuksel B, Yilmaz Z. Emergency peripartum hysterectomy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2004;113:178–81.
10. Yucel O, Ozdemir I, Yucel N, Somunkiran A. Emergency peripartum hysterectomy: a 9-year review. Archives of gynecology and obstetrics. 2006;274:84–7.
11. Flood KM, Said S, Geary M, Robson M, Fitzpatrick C, Malone FD. Changing trends in peripartum hysterectomy over the last 4 decades. American journal of obstetrics and gynecology. 2009;200:632-e1.
12. Knight M, Kurinczuk JJ, Spark P, Brocklehurst P. Cesarean delivery and peripartum hysterectomy. Obstetrics & Gynecology. 2008;111:97–105.
13. Zorlu CG, Turan C, Işik A, Danişman N, Mungan T, Gökmen O. Emergency hysterectomy in modern obstetric practice changing clinical perspective in time. Acta obstetricia et gynecologica Scandinavica. 1998;77:186–90.
14. Clark SL, Yeh SY, Phelan JP, Bruce S, Paul RH. Emergency hysterectomy for obstetric hemorrhage. Obstetrics and gynecology. 1984;64:376–80.
15. Kayabasoglu F, Guzin K, Aydogdu S, Sezginsoy S, Turkgeldi L, Gunduz G. Emergency peripartum hysterectomy in a tertiary Istanbul hospital. Archives of gynecology and obstetrics. 2008;278:251–6.
16. Karayalçın R, Özcan S, Özyer Ş, Mollamahmutoğlu L, Danışman N. Emergency peripartum hysterectomy. Archives of gynecology and obstetrics. 2011;283:723–7.
17. Kwee A, Bots ML, Visser GHA, Bruinse HW. Emergency peripartum hysterectomy: a prospective study in The Netherlands. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2006;124:187–92.
18. Oyelese Y, Smulian JC. Placenta previa, placenta accreta, and vasa previa. Obstetrics & Gynecology. 2006;107:927–41.
19. Hamilton BE, Martin JA, Ventura SJ. Births: preliminary data for Natl Vital Stat Rep. 2007. 2006.
20. NHS Maternity Statistics - England, 2004-2005. NHS Maternity Statistics. 2005. https://files.digital.nhs.uk/publicationimport/pub01xxx/pub01674/nhs-mater-eng-2004-2005-rep.pdf.
21. Lone F, Sultan AH, Thakar R, Beggs A. Risk factors and management patterns for emergency obstetric hysterectomy over 2 decades. International Journal of Gynecology & Obstetrics. 2010;109:12–5.
22. Baskett TF. Emergency obstetric hysterectomy. Journal of Obstetrics and Gynaecology. 2003;23:353–5.
23. Bakshi S, Meyer BA. Indications for and outcomes of emergency peripartum hysterectomy. A five-year review. The Journal of reproductive medicine. 2000;45:733–7.
24. Selo-Ojeme DO, Bhattacharjee P, Izuwa-Njoku NF, Kadir RA. Emergency peripartum hysterectomy in a tertiary London hospital. Archives of Gynecology and Obstetrics. 2005;271:154–9.
25. El-Jallad MF, Zayed F, Al-Rimawi HS. Emergency peripartum hysterectomy in Northern Jordan: indications and obstetric outcome (an 8-year review). Archives of gynecology and obstetrics. 2004;270:271–3.
26. Habek D, Bečareviç R. Emergency peripartum hysterectomy in a tertiary obstetric center: 8-year evaluation. Fetal diagnosis and therapy. 2007;22:139–42.
27. Huque S, Roberts I, Fawole B, Chaudhri R, Arulkumaran S, Shakur-Still H. Risk factors for peripartum hysterectomy among women with postpartum haemorrhage: analysis of data from the WOMAN trial. BMC pregnancy and childbirth. 2018;18:1–8.
28. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller A-B, Daniels J, et al. Global causes of maternal death: a WHO systematic analysis. The Lancet global health. 2014;2:e323–33.

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Research article

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The effect of preemptive ketamine on postoperative analgesia in lower extremity surgery

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Ethics Committee Approval
Ankara Training and Research Hospital Local Ethics Committee, 2005/01/033
The study was carried out at University of Health Sciences, Ankara Health Application and Research Center.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Preventing sensitization by an analgesic administered before a painful stimulus is the basis for preemptive analgesia. Preemptive use of pain medication prior to the application of painful stimuli results in better-controlled pain. This study aimed to compare the effects of preoperatively administered low-dose ketamine and lornoxicam on postoperative analgesia, their side effects and patient satisfaction in orthopedic operations on the lower extremities.

Methods: Seventy-eight patients aged 18-70 years who were admitted for lower extremity surgery under general anesthesia were enrolled in this prospective, randomized, and double-blind study. The patients were randomly allocated to one of the three following groups: Four milliliters of physiological saline were administered to the patients in Group P, 0.15 mg kg^{-1} ketamine was given to the patients in Group K and 8 mg lornoxicam was administered to those in Group L, all in 4 ml of volume, intravenously, 15 minutes before anesthesia induction. Postoperative pain was evaluated at rest and during movement at 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 12^{th}, 20^{th} and 24^{th} postoperative hours with the Visual Analogue Scale (VAS) and the Verbal Pain Scale (VPS). Total fentanyl consumption, additional meperidine usage, side effects and patient satisfaction were recorded at these times.

Results: The mean area under the VAS and VPS at rest and movement-time curves were lower in Group K compared to the other groups. Group P and Group L were comparable in terms of the area under the VAS at rest and movement-time curves, but the mean area under the VPS at rest and movement-time curves were higher in Group P compared to Group L. Total fentanyl consumption was lower in Group K than the other two groups (P=0.001).

Conclusion: Low-dose ketamine administered preoperatively to patients for lower extremity surgery decreased postoperative pain scores more than lornoxicam or placebo.

Keywords: Ketamine, Preemptive analgesia, Lornoxicam, Lower extremity orthopedic surgery

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Introduction

Postoperative pain, estimated as moderate by 20-40% and severe by 50-70% of the patients, starts with surgical trauma and gradually resolves with tissue healing. Opioid analgesics, used frequently for postoperative pain treatment, are potent and effective drugs; however, respiratory and cardiovascular side effects limit their use and make them inadequate for pain management [1]. Multimodal analgesia, defined as analgesic drugs and methods with different mechanisms of action used together, increases the quality and efficacy of pain treatment while possibly decreasing side effects [2], hospital stay and cost [3]. It is reported to be effective for pain control after lower extremity surgeries [4]. Opioids have been combined with various nonopioid analgesics for this purpose [2].

When an analgesic is administered before a painful stimulus, sensitization may be diminished in the first place. Preventing sensitization is the basis for preemptive analgesia. It has been documented that the preemptive use of pain medication prior to noxious stimuli results in better-controlled pain versus waiting to treat pain until the noxious stimuli has already occurred [5]. The use of preoperative nonsteroidal anti-inflammatory drugs (NSAIDs) for pain treatment in orthopedic surgical interventions decreases the requirement for postoperative opioids or other analgesics and their relevant side effects. Lornoxicam, a member of the oxicam group of NSAIDs, possesses potent anti-inflammatory and analgesic activities. Lornoxicam is widely recommended for the symptomatic treatment of pain and inflammation in patients with osteoarthritis and rheumatoid arthritis, as well as preoperative and postoperative pain associated with gynecologic, orthopedic, abdominal, and dental surgeries [6].

The NMDA receptor antagonist ketamine has a direct analgesic effect and plays a key role in preventing the hyper-excitability of the spinal cord neurons and central sensitization to peripheral nociceptor stimulation [7]. Preventing central sensitization is of great importance in preemptive analgesia. Pain activation areas in the brain show decreased activity when ketamine is used, correlating with a decrease in pain sensing and processing [8].

This study aimed to compare the effects of preoperatively administered low-dose ketamine and lornoxicam on postoperative analgesia quality, side effects and patient satisfaction in lower extremity orthopedic surgeries.

Materials and methods

After the approval of the local ethics committee (Ankara Training and Research Hospital Ethics Committee-2005/01/0033) was obtained, a total of 78 patients aged 18-70 years, with ASA I-II, admitted for lower extremity surgery under general anesthesia were enrolled in this prospective, randomized and double-blind study. Exclusion criteria included a history of chronic pain, chronic intake of analgesics or opioids, psychiatric disease, history of a drug or alcohol use, peptic ulcers, pregnancy, body mass index≥35 kg/m^2 and known allergy to NSAIDs and/or opioid analgesics.

The patients were informed about the patient-controlled analgesia device, the Visual Analog Scale (VAS) and the Verbal Pain Score (VPS) at the preoperative visit, the day before the surgery, by an investigator who was not informed of the study drugs used. For VAS, the patients were shown a ruler, 10 cm long, and asked to mark it. VPS scores were 0 for no pain, 1 for mild pain, 2 for moderate pain and 3 for severe pain.

The patients were taken into the operation room without premedication and 0.9% NaCl infusion was started after intravenous access was obtained with a 20 Gauge catheter. Electrocardiogram, peripheral oxygen hemoglobin saturation (SpO_2) and blood pressure (noninvasive) monitorization were performed (DRAGER PM 8060 Vitara). The patients were randomly allocated to one of the three groups using a computer-generated random number sequence. The study drugs were prepared by an anesthesiologist who did not participate in the study. All group of drugs were at the same volume, and not labeled. Another anesthetist administered 4 ml physiological saline to the patients in Group P, 0.15 mg kg^{-1} ketamine (KETALAR® Pfizer Pharmaceuticals Ltd., Istanbul-Turkey) to the patients in Group K and 8 mg lornoxicam (XEFO® Abdi İbrahim Pharmaceuticals, Istanbul-Turkey) to the patients in Group L in a total volume of 4 ml intravenously 15 minutes before anesthesia induction.

In all groups, anesthesia was induced with thiopental 5-7 $mg.kg^{-1}$, vecuronium 0.1 $mg.kg^{-1}$ and maintained with sevoflurane 2% in $N_2O50\%$-$O_250\%$. 0.01 mg/kg vecuronium was used as required for maintaining the muscle relaxation. Remifentanil 1 µg/kg was administered intravenously within 30 seconds when the heart rate or mean blood pressure during surgery reached 20% higher than the pre-induction value. The hemodynamic interventions used for the patients, consisting of remifentanil administration and the total amount administered, were noted.

Anesthesia maintenance was stopped once the surgical procedure was over (last skin suture) and the remaining neuromuscular junction block was antagonized with 2.5 mg neostigmine and 0.5 mg atropine. All patients were extubated when they fulfilled the recovery criteria. The anesthesia duration (the period between the start of anesthesia induction and the end of anesthesia maintenance), the surgery duration (the period between the first surgical incision and the last skin suture) and the recovery period (the period between the end of anesthesia maintenance and opening of the eyes on verbal stimuli) were noted. The PCA (patient control analgesia) (fentanyl) device was adjusted to 5 mcg/ml with a 25-mcg loading dose, 15-mcg bolus dose and a 15-minute lock duration.

Postoperative pain was evaluated at rest and during movement at the 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 12^{th}, 20^{th} and 24^{th} postoperative hours with VAS and VPS by an independent investigator who was unaware of the study drugs administered. Total fentanyl consumption, additional meperidine usage, side effects and patient satisfaction were recorded at these times.

Patients who were not within the evaluation range were not awakened. The results of these patients from this period were obtained by the retrospective evaluation of the patient at the next evaluation if the patient was awake. Meperidine 100 mg was administered intramuscularly if the patient reported a VAS value of 4 or more. The total meperidine amount administered was noted.

The patients were queried 24 hours after the surgery on nausea, vomiting, dry mouth, dyspepsia, lightheadedness and itching (yes/no) within the last 24 hours. They were evaluated regarding hypotension, respiratory depression, bronchospasm or allergic reaction (yes/no) following the surgery.

Statistical analysis

A power analysis was performed before the initiation of the study to determine the minimum number of patients for each group. Twenty-six patients per group were needed to ensure a type-1 error of 0.05 and a type-2 error of 0.20. All statistical analyses were performed with the statistical package program, SPSS version 21.0 (Statistical Program for Social Sciences, Chicago, IL, USA) with an IBM-compatible personal computer. Demographic data such as age, weight and operation time were analyzed with the one-way analysis of variance (ANOVA) with the Bonferroni test as a post-hoc analysis. Analysis of variance for repeated measures with the t-test was used to assess the pain. Global patient satisfaction scores, postoperative total meperidine and fentanyl consumption data were also analyzed with one-way analysis of variance (ANOVA) with the Bonferroni test as a posthoc analysis and the data were presented as mean and standard deviation. The incidence of side effects (such as nausea, vomiting, itching, sedation, hypotension, etc.) was evaluated with the Chi-square test and the Fisher's exact test. The area under the pain variables versus time curves were calculated and further analyzed with one-way ANOVA with Bonferroni correction. Data were presented as mean and standard deviation, percentages, and the number of patients. *P*-values of less than 0.05 were considered significant.

Results

The data of 75 patients were analyzed. One patient from Group K and two patients from Group L were excluded from the study because of not adjusting to the PCA device. There were no differences between the groups regarding demographic data such as age, sex, height and weight, and the anesthesia and surgery duration (Table 1), or the amount of preoperative remifentanil administered.

Table 1: Patient characteristics

	Group P (n=26)	Group K (n=25)	Group L (n=24)	*P*-value
Age (years)	52(14)	46(15)	52(14)	0.112
Gender (F/M) (n)	17/13	12/18	14/15	0.342
Height (cm)	164(7)	169(9)	165(8)	0.823
Weight (kg)	75(9)	74(9)	73(10)	0.843
Anesthesia time (min)	89(22)	81(12)	87(23)	0.169
Surgery time (min)	83(21)	78(13)	81(21)	0.217
Total remifentanil use (μg)	74 (0-92)	80 (0-94)	70 (0-86)	0.357
Recovery time (min)	4.3(1.1)	4.5(1)	3.9(1.2)	0.425

Data mean (SD), median (interquartile distribution) and number of patients. Group P: Control, Group K: Ketamine, Group L: Lornoxicam

The results of the area under the pain scores (VAS and VPS at rest and movement)-time curves were calculated and presented in Table 2. The mean area under the VAS and VPS at rest and movement-time curves were lower in Group K compared to other groups (P=0.001). On the other hand, Group P and Group L were comparable in terms of area under the VAS at rest and movement-time curves, but the mean area under the VPS at rest and movement-time curves were higher in Group P compared to Group L (P=0.012 and P=0.026).

The total amount of fentanyl consumed was 1127(73) μg in Group P, 705(26) μg in Group K and 1152(64) μg in Group L. The total fentanyl consumption was lower in Group K than the other two groups (P=0.001).

The number of patients who did not receive meperidine as an additional analgesic was higher in Group K (13) than in Group P (2) or Group L (3) (P=0.001).

The patients experienced nausea, vomiting and hypotension as side effects. The number of patients with nausea and vomiting was statistically significantly lower in Group K, who received ketamine, than in Groups P or L (Table 3).

Table 2: Area under the curve values for VAS and VPS at rest and movement-time and global satisfaction-time curves

	Group P (n=26)	Group K (n=25)	Group L (n=24)	*P*-value
VAS at rest x time (cm^2)	30.6(6.7)	15.0(3.7)*	28.1(5.3)	0.001*
VAS at movement x time (cm^2)	42(9.6)	17.9(3.6)*	37.9(6.7)	0.001*
VPS at rest x time (cm^2)	17(3.3)**	10.1(1.1)*	15.2(2.2)	0.001* 0.026**
VPS at movement x time (cm^2)	22(4)***	12.7(1.97)*	19.6(2.8)	0.001* 0.012***
Global satisfaction x time (cm^2)	79(14)	83(12)	80(15)	0.524

Data are mean (standard deviation), Group P: Control, Group K: Ketamine, Group L: Lornoxicam, * P=0.001 Group K vs Group L and Group P, ** P=0.026 Group P vs Group L, ***P=0.012 Group P vs Group L

Table 3: Side effects

	Group P (+/-) (n=26)	Group K (+/-) (n=25)	Group L (+/-) (n=24)	*P*-value
Nausea	13/13	2/23*	7/17	0.001
Vomiting	1/25	0/25**	6/18	0.005
Hypotension	2/24	0/25	2/22	0.610

Group P: Control, Group K: Ketamine, Group L: Lornoxicam, *P=0.001 Group K vs. Group P and Group L, **P=0.005 Group K vs Group P and Group L

The ketamine group performed better than the other groups regarding preemptive analgesia.

Discussion

We evaluated the effects of preoperative low-dose ketamine and lornoxicam on postoperative pain parameters and analgesic use in patients who had undergone surgery for lower extremity fracture in this randomized, double-blind, and placebo-controlled study. The most important result of the study was the decreased pain severity, increased patient satisfaction and decreased 24-hour fentanyl consumption in the postoperative period with low-dose ketamine administered preoperatively.

Postoperative pain causes various problems and complications for the patient and the physician and is caused by the changes created in the medulla spinalis, posterior horn neurons by the surgical incision. The painful stimuli that may develop during the surgical intervention or the perioperative period are known to increase postoperative pain by causing changes in the nervous system. Analgesia administration before a surgical trauma has been shown to decrease the posttraumatic sensitivity and secondary hyperalgesia in the spinal cord [9]. We therefore administered our study drugs 15 minutes before the surgical incision.

Peripheral sensitization is defined as a decrease in the threshold value of afferent terminal receptors while central sensitization is an increase in spinal neuron excitability due to activity [10, 11]. Stimulation of C fibers through surgical stimuli triggers central sensitization and this sensitization continues during the postoperative period. Considering that orthopedic surgery consists of quite painful interventions and that it may cause adequate central sensitization and peripheral hypersensitivity, orthopedic lower extremity fracture surgical cases are selected in our study to compare the effects of these two drugs.

Many previous studies have evaluated the role of various NSAIDs on moderate to severe postoperative pain treatment [2]. However, there are very few painful conditions where NSAIDs are adequate for postoperative pain treatment as a single agent. NSAIDs provide a more effective analgesia profile in a multimodal analgesia approach in addition to other agents or techniques, as an alternative to conventional postoperative pain treatment [2]. Lornoxicam is reported to be safe and effective when used as a part of multimodal postoperative pain management in adults [12]. A study comparing the postoperative analgesic efficacy of preoperative intramuscular 8 mg lornoxicam and 100 mg ketoprofen in abdominal hysterectomy found both drugs to be effective when compared to the control group; however, lornoxicam was more effective than ketoprofen in the early postoperative period [13]. Although we found a decrease in postoperative pain in patients who received lornoxicam in our study, there was no statistically significant difference between the placebo group and Group L that received lornoxicam. The efficacy of lornoxicam was less than that of ketamine in decreasing postoperative pain. The results of our study differ from the study of Karaman et al. [13]. Nevertheless, another study of the same author which compared patients to whom 8 mg lornoxicam was administered prior to a laparoscopic cholecystectomy with the patients who received 1 g paracetamol and a control group reported that lornoxicam and paracetamol had similar analgesic efficacy, but the use of analgesics was higher in the lornoxicam group [14].

Ketamine is a non-competitive antagonist of NMDA receptors and plays a key role in preventing central sensitization via the nicotinic and muscarinic receptors [15]. High-dose intravenous ketamine provides analgesia by affecting the spinal cord and brain. However, it also makes recovery after anesthesia difficult, and the patients are confused for a long time [16]. We therefore administered low doses of ketamine in our study. Various results were obtained in different studies with low-dose ketamine administration for postoperative pain treatment. Low-dose ketamine administered for anterior cruciate ligament repair surgery provides postoperative analgesia without any side effects while increasing the duration until the first analgesic requirement [17].

In this study, we evaluated the effect of low-dose ketamine and the NSAID lornoxicam administered preoperatively on pain parameters and total fentanyl use in the postoperative period. Preoperative ketamine (0.15 mg kg^{-1}) decreased postoperative pain scores more than lornoxicam (8 mg) or placebo and decreased fentanyl consumption in the postoperative period. The areas under pain vs. time curves that evaluate the postoperative period globally were lower in the group receiving ketamine. These data show that using preoperative low-dose ketamine decreased postoperative pain levels.

Roytblat et al. [18] have similarly found that the pre-incisional administration of 0.15 mg kg^{-1} IV ketamine led to a 40% decrease in the postoperative opioid requirement. Fu et al. [19] administered pre-incisional 0.5 mg kg^{-1} ketamine as an intravenous bolus, 10 mcg kg^{-1} ketamine infusion during surgery to one group and 0.5 mg kg^{-1} ketamine iv bolus to another group post-incisionally in patients undergoing abdominal surgery. Although there was no difference regarding postoperative pain scores between the two groups, the group receiving pre-incisional ketamine showed a significant decrease in the 1st and 2nd-day opioid requirement.

In a recent meta-analysis by Wang et al, [20] in which twenty-one randomized controlled trials about the analgesic effect of perioperative ketamine use for hip and knee arthroplasties are analyzed, perioperative ketamine was reported as a safe and an effective analgesic. Of these twenty-one studies, in one study [21], intravenous ketamine was used only pre-incisionally. In the remaining studies, ketamine was either used via the neuraxial route or infused throughout the operation after the initial bolus dose. A single low-dose ketamine seems an easier method of administration. In that study, Özbakış et al. [21] reported that the administration of small doses of intravenous ketamine and midazolam improved postoperative pain, patient satisfaction, and second day VAS scores, decreased total postoperative meperidine consumption, and delayed the time of first analgesic administration compared with the control group. However, the combination did not improve these results significantly over the intravenous ketamine-only group. Our results support the main findings of this study in lower extremity operations.

Patient satisfaction following the procedure is currently considered one of the most important postoperative monitoring parameters. Inadequate pain treatment also prolongs patient recovery, increases hospital expenses, and decreases patient satisfaction [22, 23]. A study evaluating patient satisfaction found the most important factor regarding satisfaction to be "worst pain score" [24].

We did have not any unsatisfied patients in our study. The highest satisfaction scores were in the ketamine group.

We evaluated the efficacy of preoperatively administered lornoxicam and ketamine in decreasing postoperative pain and found low-dose ketamine to be more efficient than lornoxicam in decreasing pain. The rest and motion VAS and VPS values, as well as fentanyl consumption, were much lower in the ketamine group than in the control and lornoxicam groups. Additional analgesic requirement was also significantly lower in the group which received ketamine. Although various factors play a role in the etiology of postoperative nausea and vomiting, opioids are the most important factor in its increase. We believe that the reason for the significantly higher incidence of nausea and vomiting in the group receiving placebo and lornoxicam compared to the group receiving ketamine was the higher fentanyl consumption in these two groups.

Conclusion

Low-dose ketamine administered preoperatively to patients for lower extremity surgery decreased postoperative pain scores more than lornoxicam (8 mg) or placebo in the postoperative period and enabled the patient to have a more comfortable postoperative period.

References

1. Oden R. Acute postoperative pain: incidence, severity and the etiology of inadequate treatment. Anaesthesiol Clin Nam. 1989;7:1-15.
2. Kehlet H, Dahl JB. The value of "Multimodal" or "Balanced Analgesia" in postoperative pain treatment. Anesth Analg. 1993;77(5):1048-56.
3. Goodman SB. Multimodal analgesia for orthopedic procedures. Anesth Analg. 2007;105:19-20.

4. Hannon CP, Keating TC, Lange JK, Ricciardi BJ, Waddell BS, Della Valle JC. Anesthesia and analgesia practices in total joint arthroplasty: a survey of the American association of hip and knee surgeons membership. J Arthroplasty. 2019;34:2872–7.
5. Wegorowski P, Stanistawek A, Domzat-Drzewicka R, Sysiak J, Rzaca M, Milanowska J, et al. The effect of preemptive analgesia on the level of postoperative pain in women undergoing surgery for breast neoplasm. Contemporary Oncology. 2016;20(2):158-64.
6. Mowafi HA, Elmakarim EA, Ismail S, Al Mahdy M, El-Saflan AE, Elsaid AS. Intravenous lornoxicam is more effective than paracetamol as a supplemental analgesic after lower abdominal surgery: a randomized controlled study. Worl J Surg. 2012;36:2039-44.
7. Oliveira C, Issy A, Sakata R, Carcia J, Martins G. Preemptive effect of IV S(+)-ketamine for hysterectomy. Acute Pain. 2005;7:139-43.
8. Niesters M, Khalili-Mahani N, Martini C, Aarts L van Gerven J, van Buchem M, et al. Effect of subanesthetic ketamine on intrinsic functional brain connectivity: a placebo-controlled functional magnetic resonance imaging study in healthy male volunteers. Anesthesiology. 2012;117(4):868-77.
9. Wall PD. The prevention of postoperative pain. Pain. 1988;33:289.
10. Woolf CJ. Somatic pain-pathogenesis and prevention. Br J Anaesth. 1995;75(2):169-76.
11. Treede RO, Meyer RA, Raja SN. Peripheral and central mechanism of cutaneous hyperalgesia. Progress in Neurobiology. 1992;38:397.
12. Hillstrom C, Jahobsson J. Lornoxicam: pharmacology and usefulness to treat acute postoperative and musculoskeletal pain a narrative review. Expert Opin.Pharmacother. 2013;14:12.
13. Karaman S, Gunusen I, Uyar M, Firat V. The effect of pre-operative lornoxicam and ketoprofen application on the morphine consumption of post-operative patient-controlled analgesia. J Int Med Res. 2006;34(2):168-75.
14. Karaman T, Kuzucuoglu T, Arslan G, Karaman S, Hatun M. Comparison of the analgesic effects of preemptive lornoxicam and paracetamol after laparoscopic cholecystectomy. Int J Anesthetic Anesthesiol. 2016;3:47.
15. Brau ME, Sander F, Vogel W, Hempelmann G. Blocking mechanisms of ketamine and its enantiomers in enzymatically demyelinated peripheral nerve as revealed by single-channel experiments. Anesthesiology. 1997;86:394-404.
16. Aida S, Yamakura T, Baba H, Taga K, Fukuda S, Shimoji K. Preemptive analgesia by intravenous low-dose ketamine and epidural morphine in gastrectomy. Anesthesiology. 2000;92:1624-30.
17. Menigaux C, Fletcher D, Dupont X, Guignard B, Guirimand F, Chauvin M: The benefits of intraoperative small-dose ketamine on postoperative pain after cruciate ligament repair. Anesth Analg. 2000;90:129-35.
18. Roytblat L, Korotkoruchko A, Katz J, Glazer M, Greemberg L, Fisher A. Postoperative pain: the effect of low-dose ketamine in addition to general anesthesia. Anesth Analg. 1993;77(6):1161-5.
19. Fu ES, Miguel R, Scharf JE. Preemptive ketamine decreases postoperative narcotic requirements in patients undergoing abdominal surgery. Anesth Analg. 1997;84:1086-90.
20. Wang P, Yang Z, Shan S, Cao Z, Wang Z. Analgesic effect of perioperative ketamine for total hip arthroplasties and total knee arthroplasties: a PRISMA-compliant meta-analysis. Medicine. 2020;99:42(e228099).
21. Ozbakis Ç, Akkurt B, Inanoglu K, Kalaci A, Asfuroğlu Z, Tümkaya F. Effects of intravenous small dose ketamine and midazolam on postoperative pain following knee arthroscopy. Pain Pract. 2009;9:289–95.
22. Miaskowski C. Patient-controlled modalities for acute postoperative pain management. J Perianesthesia Nursing. 2005;20:255-67.
23. Summers S. Evidence-based practice part 3 acute pain management of the perianesthesia patient. J Perianesthesia Nursing. 2001;16:112-20.
24. Savaia A, Min JJ, Leber C, Erbacher K, Abrams F, Fink R. Postoperative pain management in elderly patients: correlation between adherence to treatment guidelines and patient satisfaction. J Am Geriatr Soc. 2005;53:274-82.

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The compliance of our practice of hepatitis B virus screening with the current guidelines in patients undergoing chemotherapy for hematological malignancies

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Ethics Committee Approval
This study was approved by 'Non-Invasive Research Ethics Committee of Cukurova University Medical Faculty' on November 6, 2020 (document number 105/66).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: The reactivation of Hepatitis B virus (HBV) among cancer patients is a critical issue which is preventable by precise detection of risky cases prior to the administration of chemotherapy drugs. This study aimed to investigate whether the evaluated serological tests for HBV screening before chemotherapy in adults with newly diagnosed hematological malignancies follow the guidelines.
Methods: In this retrospective cohort study, all patients with hematological malignancies who visited our hematology clinic between January 01, 2018-January 01, 2020, were examined and adult patients referred to the outpatient clinic for combined chemotherapy were included. All clinical data and laboratory results were obtained from the electronic hospital information system. Serological tests performed for HBV screening and their results were noted. The compliance of our clinical practice with the current guidelines was analyzed assuming that there are three mandatory serological tests for screening, HBsAg, anti-HBs and anti-HBc, recommended in the guidelines.
Results: A total of 91 newly diagnosed cases were included for analysis. HBV screening completely lacked in 10% of the patients and it did not follow the current guidelines in 30%. The most neglected serological test was anti-HBc. Regarding different hematological malignancies, the results were best in lymphoma patients (76% compliance with guidelines) and worst in MM (only 40% compliance with guidelines). The serological test results of eighty-two cases were also examined and the seropositivity rates for HBsAg and anti-HBc were 2%, and 41%, respectively.
Conclusion: We observed that the risk of reactivation was not adequately evaluated by serological screenings for HBV in adult patients receiving chemotherapy for hematological malignancy. To protect patients from this mostly preventable complication, it is necessary to increase the awareness on the subject and encourage more compliance with the related guidelines.

Keywords: Hepatitis B virus, Hematological malignancies, Reactivation

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Introduction

Hepatitis B virus (HBV) is an important health problem. According to the World Health Organization (WHO) data, our country is located where it is considered moderately endemic for HBV. One out of every three adults in Turkey is exposed to HBV [1,2]. Although the HBV vaccine is in the routine immunization program for children since 1998 and the vaccination of adults in the risk group is encouraged, epidemiological studies still reveal high rates of seropositivity in adults. One of the most important studies on HBV epidemiology in Turkey was conducted by the 'Turkish Association for the Study of the Liver' in 23 provinces that screened a total of 5460 individuals between 2009 and 2010. According to this study, 4% hepatitis B surface antigen (HBsAg) positivity and 30.6% total hepatitis B core antigen-antibody (anti-HBc) positivity were detected in individuals over 18 years of age [2].

On the other hand, the risk of treatment-related viral reactivation in cancer patients during chemotherapy is a well-known complication [3]. Serologically, HBsAg positivity is an indicator of chronic carriage, while anti-HBc positivity indicates previous exposure to HBV. Viral reactivation should be considered not only for HBsAg-positive cases, but also for HBsAg-negative/anti-HBc-positive patients [4]. Therefore, current guidelines recommend that HBsAg, anti-bodies against HBsAg (anti-HBs) and anti-HBc should be evaluated together in serological screenings before chemotherapy [5-8].

During the follow-up of cancer patients, the severity of the primary disease and the urgency of chemotherapy may cause missing or incomplete HBV screening practices. In our study, we aimed to investigate whether the evaluated serological tests for HBV screening before chemotherapy in newly diagnosed hematological malignancies were in accordance with the guidelines.

Materials and methods

In this retrospective cohort study, all adult patients with hematological malignancies who were referred to our hematology clinic between January 01, 2018-January 01, 2020, were examined. The patients aged 18 years and over who were referred to the outpatient unit to receive combined chemotherapy with a diagnosis of non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), chronic lymphocytic leukemia (CLL) or multiple myeloma (MM) were included in the study. The cases treated solely with steroids, those receiving chemotherapy due to recurrence or refractory disease, and patients with missing information on treatment or follow-up were excluded from the study. Patients whose treatment was continuing in our clinic but whose first treatment was started in another center were excluded as well.

All clinical data and laboratory results were obtained from the electronic information system used in our center for patient follow-up. Laboratory tests, which were conducted up to 3 months before the first day of chemotherapy were examined. Serological tests performed for HBV screening and their results were noted. To avoid bias during data collection, laboratory results were gathered from the system by an independent health worker who was blinded to the study details. Then, the compliance of our clinical practice with current guidelines was analyzed, assuming that there are three mandatory serological tests for screening: HBsAg, anti-HBs and anti-HBc, since these tests were recommended in both the national and international literature [5-8].

Ethical issues

This study was approved by the Non-Invasive Research Ethics Committee of Cukurova University Medical Faculty on November 6, 2020 (document number 105/66) and performed per the Helsinki Declaration guidelines.

Statistical analysis

Statistical analyses were performed by a biostatistician using SPSS Statistics version 17.0 (IBM). The normality of data was analyzed using the Kolmogorov-Smirnov test. For categorical values, p-values were calculated using the Chi-square test. All comparative tests were 2-tailed, and a *P*-value of less than 0.05 was considered statistically significant.

Results

In our study, 113 patients who were referred to our hematology clinic between January 01, 2018-January 01, 2020 and received combined chemotherapy in the outpatient unit were examined. Then, 91 newly diagnosed cases meeting the inclusion criteria were included for further analysis. The diagnoses of the cases were lymphoma in 56% (n = 51), CLL in 22% (n=20) and MM in 22% (n = 20). Among lymphoma patients, 80% (n = 41) were NHL and 20% (n = 10) were HL. The average age of study patients was 61 (20-81) years and 36% (n = 33) were female, while 64% (n = 58) were male.

Our HBV screening practice before chemotherapy is summarized in Table 1. It was observed that 90% (n=46) of lymphoma patients were screened with HBsAg, while 78% (n=40) were screened with anti-HBc. Similarly, 95% of CLL patients were screened for HBsAg (n=19), and 85% (n=17), for anti-HBc. Patients with MM were screened for HBsAg at a rate of 85% (n=17), while only 45% (n=9) were screened for anti-HBc. Compared to lymphoma and CLL, it seems that the ordering of appropriate serological tests was most often neglected in MM patients ($P<0.05$).

Table 1: Serological test ratios for HBV screening before chemotherapy

	Total (n=91)	Lymphoma (n=51)	CLL (n=20)	MM (n=20)	*P*-value*
Serological test					
HBs Ag, % (n)	90 (82)	90 (46)	95 (19)	85 (17)	0.570
anti-HBs, % (n)	88 (80)	88 (45)	95 (19)	80 (16)	0.345
anti-HBc, % (n)	72 (66)	78 (40)	85 (17)	45 (9)	0.027

*Chi-square test, HBV: Hepatitis B virus, CLL: Chronic Lymphocytic Leukemia, MM: Multiple Myeloma, HBsAg: Hepatitis B Surface Antigen, anti-HBs: Anti-Hepatitis B Surface Antigen Antibody, anti-HBc: Anti-Hepatitis B Core Antigen Antibody

Regarding all cases, HBV screening completely lacked in 10% (n=9) and it was not in accordance with the current guidelines in 30% (n=27). Concerning different hematological malignancies, the results of analysis about the compliance of our screening with the guidelines are summarized in Figure 1. It was best in lymphoma, since 76% was in accordance. The worst was in MM, because in 60% of the patients, the guidelines were not followed.

The evaluation of patients who received rituximab as a part of a combined chemotherapy protocol in a separate group revealed that 56 patients (n=40 NHL and n=16 CLL) were screened. The serological tests in 23% (n=13) were not in

accordance with the guidelines, and in 9% (n=5), screening was not performed at all.

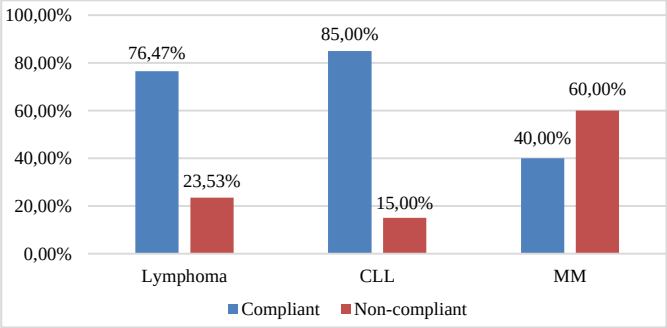
The results of serological tests in 82 cases who were screened for HBV were also examined. The rate of seropositivity for HBsAg was 2%, whereas it was 41% for anti-HBc. The rate of patients found to have anti-HBs positivity was 41% (Table 2).

Table 2: Serological test results in patients screened for HBV, n=82

	Total % (n*)	Lymphoma % (n*)	CLL % (n*)	MM % (n*)	*P*-value **
Rate of HBsAg seropositivity	2 (2/82)	4 (2/46)	0 (0/19)	0 (0/17)	0.448
Rate of anti-HBs seropositivity	41 (33/80)	47 (21/45)	42 (8/19)	25 (4/16)	0.318
Rate of anti-HBc seropositivity	41 (27/66)	30 (12/40)	65 (11/17)	44 (4/9)	0.050

* Number of seropositive patients divided by the number of screened patients, **Chi-square test, HBV: Hepatitis B virus, CLL: Chronic Lymphocytic Leukemia, MM: Multiple Myeloma, HBsAg: Hepatitis B Surface Antigen, anti-HBs: Anti-Hepatitis B Surface Antigen Antibody, anti-HBc: Anti-Hepatitis B Core Antigen Antibody

Figure 1: Compliance of our HBV screening practice with current guidelines (CLL: chronic lymphocytic leukemia; MM: multiple myeloma)



Discussion

HBV reactivation is preventable, provided that the risky cases are detected before the onset of chemotherapy and anti-viral agents are given prophylactically. On the other hand, if patients are not managed appropriately, past HBV exposure may result in asymptomatic hepatitis or sometimes life-threatening severe fulminant liver failure and death during chemotherapy [9].

In our study, routine screening for HBV was not performed before chemotherapy in 10% of the outpatients with hematological malignancies, and the serological evaluations were not in accordance with the current guidelines in about one third of the screened patients.

The most neglected serological test in our screening practice was anti-HBc. However, the exposure to the virus is recognized by the seropositivity of anti-HBc, while persistent HBsAg positivity indicates the presence of chronic infection. Following exposure, when the virus is eliminated from the blood, HBsAg becomes negative, but since viral DNA remains in the nuclei of infected hepatocytes, anti-HBc continues to be positive. Therefore, in individuals with a history of HBV exposure, even if HBsAg is negative, viral reactivation may occur in the case of immunosuppression [10].

When the cases were evaluated according to disease subgroups, our serological screening was found to be insufficient in about one fourth of the patients who received chemotherapy for lymphoma. When the literature on viral reactivation in patients with hematological malignancies undergoing chemotherapy is examined, it is seen that lymphoma patients are mainly included in the studies on the subject. In these patients, the reactivation rate can reach up to 50% and the risk is much higher for patients whose treatment protocol includes rituximab [3, 11].

Although the number of studies examining hematological malignancies other than lymphoma is less in the literature, there are also studies involving patients with MM [12]. In a retrospective study recently published from Turkey, a reactivation risk of 8% was reported for MM cases treated with new agents [13]. In our study, 60% of MM patients were insufficiently screened for Hepatitis B.

Regarding CLL, the ratio of insufficiently screened patients was 15%. Although the literature on CLL patients is scarce, these patients are also regarded to have increased risk for HBV reactivation, at least because of rituximab being the backbone of chemotherapy for CLL. The association of anti-CD20 monoclonal antibody ‘rituximab’ with viral reactivation is a well-known complication that has been the subject of many studies [14-16]. Rituximab usually exists in the treatment protocols for non-Hodgkin lymphoma and CLL. When our lymphoma and CLL patients who have rituximab in their treatment protocol were evaluated together, the ratio of patients whose serological screening was not performed per the guidelines was 23%, which is high.

In another study from Turkey, the rate of correct screening with serological tests in a different center was much lower than that recommended in the literature [17]. In the same study, the rate of patients who were positive for only HBsAg was 4%, while the rate of patients who were positive for anti-HBc was 16%. Similarly, our seropositivity rate for HBsAg was 4%, whereas it was 41% for anti-HBc in screened patients.

In a recent study involving 37 centers from our country, the awareness and clinical practice of physicians, 21% of which were hematologists, about HBV reactivation was investigated. While 88% of the total 430 physicians included in this study stated that they performed screening before immunosuppressive treatment in all patients, the rate of use of HBsAg as a screening test was 97%, while the rate of screening with anti-HBc was only 63% [18]. Our results are in parallel with these findings, as we mentioned that anti-HBc was the most neglected serological test.

The literature published from other countries reveals that the awareness deficit on the subject is a problem in many countries, including developed ones [19-21]. However, the fact that our country is in a region that is considered moderately endemic for HBV increases the importance of the issue.

The most important limitations of our study are its retrospective and single-center design. However, it is obvious that awareness on the subject should be increased, and our research may contribute to the future, larger scale studies.

Conclusion

We found that serological screening for HBV in adult patients with hematological malignancies receiving chemotherapy were not adequately ordered to assess the risk of reactivation during the treatment process and encouraging greater compliance with relevant guidelines is necessary to protect patients from this, often preventable, complication.

References

1. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017. Report No: CC BY-NC-SA 3.0 IGO.

2. Tozun N, Ozdogan O, Cakaloglu Y, Idilman R, Karasu Z, Akarca U, et al. Seroprevalence of hepatitis B and C virus infections and risk factors in Turkey: a fieldwork TURHEP study. Clin Microbiol Infect. 2015;21:1020-6. doi: 10.1016/j.cmi.2015.06.028.
3. Lubel JS, Angus PW. Hepatitis B reactivation in patients receiving cytotoxic chemotherapy: diagnosis and management. J Gastroenterol Hepatol. 2010;25:864-71. doi: 10.1111/j.1440-1746.2010.06243.x.
4. Pattullo V. Prevention of Hepatitis B reactivation in the setting of immunosuppression. Clin Mol Hepatol. 2016;22:219-37. doi: 10.3350/cmh.2016.0024.
5. Aygen B, Demir AM, Gümüş M, Karabay O, Kaymakoğlu S, Köksal AŞ, et al. Immunosuppressive therapy and the risk of hepatitis B reactivation: Consensus report. Turk J Gastroenterol . 2018;29:259-69. doi: 10.5152/tjg.2018.18263.
6. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67:370-98. doi: 10.1016/j.jhep.2017.03.021.
7. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10:1-98. doi: 10.1007/s12072-015-9675-4.
8. Weinbaum CM, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, et al. Centers for Disease Control and Prevention (CDC). Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. MMWR Recomm Rep. 2008;57:1-20.
9. Hoofnagle JH. Reactivation of hepatitis B. Hepatology. 2009;49:156-65. doi: 10.1002/hep.22945.
10. Pattullo V. Hepatitis B reactivation in the setting of chemotherapy and immunosuppression - prevention is better than cure. World J Hepatol. 2015;7:954-67. doi: 10.4254/wjh.v7.i7.954.
11. Hsu C, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, et al. Taiwan Cooperative Oncology Group. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. Hepatology. 2014;59:2092-100. doi: 10.1002/hep.26718.
12. Gentile G, Andreoni M, Antonelli G, Sarmati L. Screening, monitoring, prevention, prophylaxis and therapy for hepatitis B virus reactivation in patients with haematologic malignancies and patients who underwent haematologic stem cell transplantation: a systematic review. Clin Microbiol Infect. 2017;23:916-23. doi: 10.1016/j.cmi.2017.06.024.
13. Ataca Atilla P, Yalçıner M, Atilla E, İdilman R, Beksaç M. Hepatitis B Reactivation Rate and Fate Among Multiple Myeloma Patients Receiving Regimens Containing Lenalidomide and/or Bortezomib. Turk J Haematol. 2019;36:266-73. doi: 10.4274/tjh.galenos.2019.2019.0103.
14. Evens AM, Jovanovic BD, Su YC, Raisch DW, Ganger D, Belknap SM, et al. Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: meta-analysis and examination of FDA safety reports. Ann Oncol. 2011;22:1170-80. doi: 10.1093/annonc/mdq583.
15. Tsutsumi Y, Yamamoto Y, Ito S, Ohigashi H, Shiratori S, Naruse H, et al. Hepatitis B virus reactivation with a rituximab-containing regimen. World J Hepatol. 2015;7:2344-51. doi: 10.4254/wjh.v7.i21.2344.
16. Drößler L, Lehmann C, Töpelt K, Nierhoff D, Vehreschild JJ, Rybniker J, et al. HBsAg-negative/anti-HBc-positive patients treated with rituximab: prophylaxis or monitoring to prevent hepatitis B reactivation? Infection. 2019;47:293-300. doi: 10.1007/s15010-019-01271-z.
17. Sarı NG, Yıldırım Aİ, Doğu H, Yörük G. Hematolojik Maligniteli Hastalarda Hepatit B Virüs Serolojileri, Antiviral Profilaksi ve Reaktivasyon Hepatit Oranlarının Değerlendirilmesi. Turkiye Klinikleri J Med Sci. 2020;40(1):74-8. doi: 10.5336/medsci.2019-71432.
18. Korkmaz P, Demirtürk N, Aydın G, Ceken S, Aygen B, Toka O, et al. Awareness of hepatitis B virus reactivation among physicians administering immunosuppressive treatment and related clinical practices. Klimik Derg. 2019;32:146-53. doi: 10.5152/kd.2019.33.
19. Kawsar HI, Shahnewaz J, Gopalakrishna KV, Spiro TP, Daw HA. Hepatitis B reactivation in cancer patients: role of prechemotherapy screening and antiviral prophylaxis. Clin Adv Hematol Oncol. 2012;10:370-8.
20. Marignani M, Marzano A, Begini P, Vitolo U, Luminari S, Levis A, et al. Perception of hepatitis B virus infection reactivation-related issues among specialists managing hematologic malignancies: result of an Italian survey. Leuk Lymphoma. 2014;55:2564-71. doi: 10.3109/10428194.2013.879712.
21. Yuen MF. Need to improve awareness and management of hepatitis B reactivation in patients receiving immunosuppressive therapy. Hepatol Int. 2016;10:102-5. doi: 10.1007/s12072-015-9694-1.

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Comparison of two drospirenone-containing oral contraceptives for their effect on the ovary, menstrual cycle, acne, and side-effect profile: 20 µg ethinylestradiol/3 mg drospirenone (24/4) versus 30 µg ethinylestradiol/3 mg drospirenone (21/7)

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Ethics Committee Approval
Ethics committee approval for the study was obtained from the Ethics Committee of Health Sciences University Etlik Zübeyde Hanım Gynecology Training and Research Hospital (Decision no:127).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

Financial Disclosure
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Previous Presentation
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Abstract

Background/Aim: Serious side effects, depending on the length of the hormone-free interval and the estrogen dose, cause the discontinuation of combined oral contraceptives (COCs). Therefore, it is important to identify COCs with minimal side effects which provide effective contraception. This study aimed to compare the effects of two different drospirenone-containing oral contraceptives (COCs) on ovarian suppression, cycle control, premenstrual symptoms, pain, acne, and the incidence of side-effects.
Methods: This prospective randomized controlled study was conducted with eighty women aged between 17-40 years. Patients were randomized to either 3mg drospirenone/30mcg ethinylestradiol (21/7 tablets) (Group 1) or 3mg drospirenone/20mcg ethinylestradiol (24/4 tablets) (Group 2) COCs. On Day-3 of the pre-treatment cycle, menstrual cycle patterns, serum hormone and lipid levels, menstrual complaints were recorded, followed by an evaluation of Day-21 progesterone levels, sonographic evaluation of endometrial thickness and the ovaries. Same assessment was repeated after pill use and the findings of the two cycles were compared.
Results: Both COC formulations suppressed serum hormone levels, decreased endometrial thickness and reduced incidence of dysmenorrhea-dyspareunia, and acne while serum HDL-cholesterol level was increased. Progesterone, FSH and endometrial thickness were lower, and serum cholesterol level was higher in Group 2 (P=0.007, P=0.044, P<0.001, P=0.035; respectively). Breast tenderness was significantly less in Group 2 (P=0.02). The incidence of follicular development, menstrual irregularity, and a headache was higher in Group 1, but the difference was not significant except for headaches (P=0.027).
Conclusion: 24/4 tablets might be a better alternative to 21/7 tablets with the advantage of tolerability as well as providing effective contraception.

Keywords: Combined oral contraceptive, Ovarian suppression, Premenstrual disorders, Side-effects

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Introduction

Combined oral contraceptives (COCs) are widely used all over the world and almost 16 to 30% of women of reproductive age have used COCs at one stage of their lives [1, 2]. The primary mechanism of action of COC use is to suppress dominant follicle development, and thus inhibit ovulation by suppressing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and preventing fertilization besides preventing implantation by thickening the cervical mucus and making the endometrium thinner [3].

The progestin component suppresses ovulation while the estrogen component is added for cycle control [4]. Estrogen also has an additive effect on suppression of the follicular development while preventing spotting and decreasing menstrual blood loss [5, 6]. With the improvement of contraceptive technology, new forms, formulations, and regimens have been developed to decrease the incidence of side-effects and increase users' compliances. Estrogen-related complaints such as fluid retention, nausea, mood changes, and breast tenderness are common reasons for COC discontinuation [7]. Estrogen in COCs has been reduced from 150 µg Mestranol to 20-30 µg estradiol (EE) with dose adjustment studies. Lower estrogen dosages that still have a suppressive effect on follicular growth and maintain cycle control are preferred to reduce the risk of venous thrombosis besides estrogen-related side effects. Different progestins with anti-androgenic, anti-glucocorticoid properties that stabilize the endometrium efficiently are developed and added to COC formulations. Hormonal fluctuations that cause irregular bleeding patterns and poor cycle control during COC use negatively impact patient compliance. To provide better suppression besides decreasing hormonal fluctuations, new regimens with the shorter hormone-free period or extended-formulations have been developed [8].

We aimed to compare the follicular suppression, cycle control, incidence of premenstrual symptoms, and side-effects encountered in a 21/7 tablet COC, which contains 30 µg ethynyl estradiol (EE) + 3 mg of drospirenone, with a 24/4 tablet COC regimen with 20µg EE + 3 mg of drospirenone.

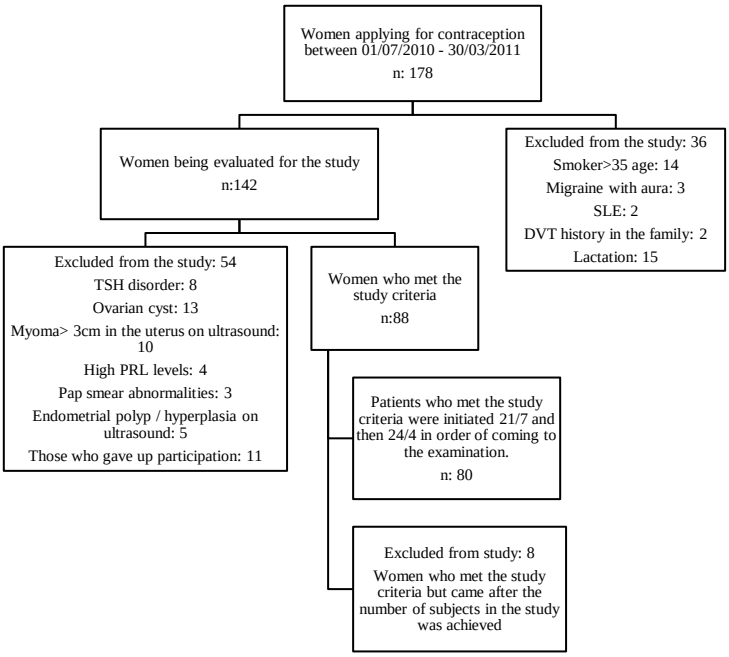
Materials and methods

The study was conducted at the Family Planning Clinic of a tertiary center. Ethics committee approval for the study was obtained from the Ethics Committee of Health Sciences University Etlik Zübeyde Hanım Gynecology Training and Research Hospital (Decision no:127). The study was conducted according to the Helsinki Declaration principles. The study group consisted of women who visited Etlik Zübeyde Hanım Gynecology Training and Research Hospital for contraception between July 1, 2010, and March 30, 2011 and requested combined oral contraceptives after comprehensive counseling for family planning methods. The medical eligibility for COC use for the recruitment of the women was based on the recommendations of the WHO eligibility criteria [9].

Inclusion criteria included 1) Being a non-smoker aged 17-40 years, 2) Not being pregnant or lactating, 3) Not having any organic gynecologic pathologies, 4) Having a Body Mass Index (BMI)< 30kg/m^2, 5) Not using hormonal contraception during the last six months.

The exclusion criteria were as follows: Smokers over the age of 35, query of pregnancy or a malignancy, having migraines, endocrine disorders (diabetes mellitus, thyroid dysfunction vs.), unexplained vaginal bleeding, using drugs that could interact with COCs (barbiturate, rifampin, hydantoin), having a history of deep venous thrombosis (DVT) or pulmonary embolus, women with SLE, hepatic, or renal disease, and an abnormal pap smear within the last year. All volunteering women received comprehensive counseling for COC use and signed informed consent forms. The patients were evaluated on the second month of the COC use. The flowchart of the study is shown in Figure 1.

Figure 1: Flow-chart of the study



Two groups were formed: Group 1: 40 patients who met these criteria received COC containing 3mg drospirenone/30mcg ethinyl estradiol (21/7, Yasmin, Bayer Germany) and Group 2: 40 patients were given 3mg drospirenone/20mcg ethinyl estradiol (24/4, Yazz, Bayer, Germany) COC. To avoid bias in the study, oral contraceptives were started randomly on the 2nd or 3rd day of the menstrual cycle. Randomization was done by assigning tablets sequentially: the 21/7 tablet for the first patient and the 24/4 tablet for the subsequent patient until the groups were completed.

During the cycle before using the pill

Pre-pill Day 2-3: Age, parity, educational status, smoking, lactation, previous pelvic surgery, chronic disease, PAP smear results, menstrual pattern, premenstrual symptoms, dysmenorrhea-dyspareunia complaints (the severity of dysmenorrhea and dyspareunia was evaluated with the visual analog scale (VAS)), acne and other skin manifestations of the women in both groups were questioned and recorded. After physical examination and recording of the blood pressure, height and weight measurements, organic pathologies that could cause abnormal bleeding were excluded by gynecological examination and transvaginal ultrasonography (TVUSG). Evaluation of the ovaries and endometrial thickness were noted. In the morning, fasting blood samples were collected and FSH, LH, estradiol

(E2), prolactin (PLR), thyroid stimulating hormone (TSH) levels, fasting blood sugar, blood lipid (triglyceride (TG), low-density lipid (LDL), very low-density lipid (VLDL), high-density lipid (HDL), total cholesterol) levels, liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST)) and kidney (blood urea nitrogen (BUN), creatinine) function tests were requested and the results were recorded.

Pre-pill Day-21: Blood samples were collected for serum progesterone (P) level, and ovaries and endometrial thickness were re-evaluated by TVUSG.

In the cycle following one month of pill use

Post-pill Day 2-3: Analysis of serum hormone and lipid levels, transvaginal ultrasonographic evaluation of the ovaries and the endometrium were conducted.

Post-pill Day-21: Serum P level assessment, TVUSG for evaluation of follicular development and measurement of endometrial thickness were conducted. Menstrual patterns, presence of premenstrual symptoms and skin findings, signs of deep vein thrombosis and side effects due to pill use (breast tenderness, nausea, headache) were questioned, and the severity of dysmenorrhea and dyspareunia was re-evaluated with the VAS score.

BMI and blood pressure were recorded at each visit. Premenstrual symptoms included breast tenderness, anxiety, increased appetite, and sleep disturbance. Symptoms that occurred for at least two cycles, began within five days before menstruation, disappeared with menstruation and caused distress in daily social or work-related activities were considered premenstrual symptoms [10].

In terms of menstrual pattern, cycles lasting 4-7 days, with 21-35-day intervals and bleeding up to 35-80 ml were considered regular cycles.

A Logiq P5 ultrasonography device was used for TVUSG. Blood hormone samples were analyzed with the E170 device using kits from Roche, and blood lipid samples were analyzed with dry chemistry kits with the Vitros Fusion 5.1 device.

Power analysis

Sample size was determined with the G Power 3.1 program. In the study of Krol et al. [11], considering that the rate of side effects in women using oral contraceptives between 18-35 years of age was 9.0%, 0.70 effect size (d = 0.70) was calculated according to the double-tailed hypothesis method. The confidence interval was 90% and the margin of error was 5%. Thirty-six women were needed in the control group, and 36 women were required in the study group. Considering possible data loss, the study was completed with 80 women, 40 women in each group.

Statistical analysis

Data were analyzed using the SPSS 21.0 program. Mann-Whitney U test and Wilcoxon signed-rank test were used to compare variables not conforming to normal distribution. In cases with normal distribution, Independent Sample T-Test and Paired Sample T-Test were used. Chi-square test or Fisher's exact probability test were used to determine whether frequency distributions of categorical variables were homogeneously distributed among the groups. Results with $P<0.05$ were considered statistically significant.

Results

Both groups were homogeneous in terms of demographic characteristics (Table 1).

When compared with the pre-pill values, in both groups, post-pill serum FSH, LH, E2 and P levels were significantly suppressed, and endometrial thickness was decreased ($P<0.05$). However, serum FSH and P levels and endometrial thickness were lower with 24/4 tablets than 21/7 tablets. With 21/7 tablets, seven patients had follicular development (follicle diameter 11-16mm), while in the 24/4 group only two had follicles (diameter 12 and 14 mm). However, this was not statistically significant ($P>0.05$) (Table 2).

The pre-pill menstrual pattern in Group 1 was as follows: All women had regular cycles, 3 had spotting, and one woman had heavy menstrual bleeding (HMB). After pill use, the patient with HMB improved. However, five patients developed spotting episodes, and menstrual irregularity was observed in three patients. These differences in bleeding patterns before and after pill use were not significant ($P>0.05$) (Table 2).

Table 1: Comparison of the groups in terms of demographic characteristics (age, BMI, parity, educational status, chronic disease, previous surgery), smoking status, systolic and diastolic blood pressures before pill use, serum TSH and PRL levels

		21/7 Tablet n (40)	24/4 Tablet n (40)	*P*-value
Age (year)		26.55 (4.04)	27.38 (3.76)	0.348
BMI (kg/m2)		25.45 (3.12)	25.30 (2.84)	0.531
Education	Primary school	5 (12.5%)	2 (5.0%)	0.587
	Middle school	6 (15.0%)	5 (12.5%)	
	High School	22 (55.0%)	23 (57.5%)	
	University	7 (17.5%)	10 (25.0%)	
Parity	Nulliparous	3 (7.5%)	5 (12.5%)	0.712
	Multiparous	37 (92.5%)	35 (87.5%)	
Previous operation		9 (22.5%)	7 (17.5%)	0.576
Chronic disease		4 (10.0%)	6 (15.0%)	0.499
Smoking		12 (30.0%)	7 (17.5%)	0.189
TSH (mIU / L)		2.22 (1.72)	1.88 (1.05)	0.806
PRL (mlU/ml)		11.02 (4.85)	9.93 (3.50)	0.285

P-values were calculated with the independent T test (age), Mann Whitney U Test (BMI, TSH, PRL), and Chi-Square Test, PRL: Prolactin, TSH: Thyroid stimulating hormone

Table 2: Comparison of the effects of 21/7 and 24/4 tablets on ovarian suppression and cycle control

	Before the 21/7 tablet n (40)	After the 21/7 tablet	*P*-value	Before the 24/4 tablet n (40)	After the 24/4 tablet	*P*-value	Difference with the 21/7 tablet n (40)	Difference with the 24/4 tablet n (40)	*P*-value
FSH (U/L) (D3)	5.07 (2.21)	1.62 (1.00)	<0.001	5.82 (1.99)	1.67 (1.80)	<0.001	3.45 (2.18)	4.15 (2.46)	0.044
LH (U/L) (D3)	5.87 (3.14)	1.60 (1.15)	<0.001	6.55 (3.20)	1.87 (1.38)	<0.001	4.27 (3.50)	4.67 (3.56)	0.581
E2 (pg/ml) (D3)	89.10 (146.94)	17.35 (20.52)	0.004	65.00 (33.48)	14.92 (10.27)	<0.001	71.75 (150.14)	50.07 (37.36)	0.248
P (ng/ml) (D21)	4.65 (4.01)	0.32 (0.21)	<0.001	6.49 (4.11)	0.25 (0.19)	<0.001	4.32 (3.94)	6.23 (4.14)	0.007
ET (mm) (D21)	11.65 (3.72)	4.02 (0.99)	<0.001	16.60 (2.56)	3.25 (1.12)	<0.001	7.62 (3.69)	13.35 (2.86)	<0.001
Follicle development (D21)	-	7 (17.5%)	-	-	2 (5.0%)	-	7 (17.5%)	2 (5.0%)	0.077
Menstrual cycle	n (%)	n (%)	*P*	n (%)	n (%)	*P*	n (%)	n (%)	*P*
Regular	40 (100.0)	37 (92.5)	0.241	40(100.0)	39(97.5)	1.00	37 (92.5)	39 (97.5)	0.615
Spotting*	3 (7.5)	8 (20.0)	0.105	0 (0.0)	3 (7.5)	0.241	5** (12.5)	3 (7.5)	0.712
Menorrhagia*	1 (2.5)	0 (0.0)	1.00	1 (2.5)	0 (0.0)	1.00	0 (0.0)	0 (0.0)	-
Amenorrhea	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
Irregularity	0 (0.0)	3 (7.5)	0.241	0 (0.0)	1 (2.5)	1.00	3 (7.5)	1 (2.5)	0.615

P-values were calculated with Paired Sample T Test and Wilcoxon Test in dependent groups and Chi-Square Test. ET: Endometrial thickness, *: with regular cycles, **: new spotting complaint, D3-D21: 3rd and 21st day of the menstrual cycle.

All women in Group 2 had regular cycles before COC use and only one woman had HMB. After pill use, the patient with HMB improved. However, menstrual irregularity developed in one woman and spotting episodes, in three. There was no significant difference in terms of the menstrual pattern changes in Group 2 ($P>0.05$) (Table 2).

Menstrual irregularity and spotting bleeding were more common after 21/7 tablet use compared to 24/4 tablet use, but the difference was not significant ($P>0.05$) (Table 2).

There was a significant decrease in cholesterol and VLDL and a significant increase in HDL after 21/7 tablet use. The decrease in cholesterol levels was more significant in Group 2 while this group also had a significant decrease in HDL levels ($P<0.05$) (Table 3).

In Group 1, the incidence of breast tenderness, nausea, and headache were significantly higher and there was a significant increase in systolic blood pressure. In Group 2, breast tenderness and nausea were significantly high when compared to the pre-pill period. The increase in the incidence of breast tenderness was higher in Group1 when compared to Group 2. No patients had deep vein thrombosis or any other vascular complications (Table 3).

When the effects on premenstrual symptoms were compared, both oral contraceptives positively affected appetite and sleep disturbances, but this did not reach statistical significance ($P>0.05$) (Table 4).

Table 3: Comparison of the side effects of the tablets after use

	Before the 21/7 tablet n (40)	After the 21/7 tablet	*P*-value	Before the 24/4 tablet n (40)	After the 24/4 tablet	*P*-value	Difference with the 21/7 tablet n (40)	Difference with the 24/4 tablet n (40)	*P*-value
FBG (mg/dl) (D3)	94.70 (22.54)	90.72 (16.58)	0.362	91.37 (15.34)	95.90 (16.27)	0.181	3,97 (27.23)	-4.52 (23.60)	0.140
TG (mg/dl) (D3)	131.32 (60.64)	124.00 (52.04)	0.094	108.82 (60.45)	106.15 (44.74)	0.608	7.32 (26.98)	2.67 (32.76)	0.182
Cholesterol (mg/dl) (D3)	166.47 (27.35)	151.55 (26.12)	0.001	160.25 (24.53)	154.65 (22.80)	0.097	14.92 (27.19)	5.60 (20.80)	0.035
HDL (mg/dl) (D3)	53.92 (12.72)	64.52 (11.30)	<0.001	60.05 (12.41)	72.95 (11.67)	<0.001	10.60 (13.04)	12.90 (11.82)	0.927
LDL (mg/dl) (D3)	102.52 (29.25)	103.17 (29.04)	0.850	106.15 (28.87)	101.92 (25.75)	0.246	-0.65 (21.63)	4.22 (22.67)	0.148
VLDL (mg/dl) (D3)	28.40 (13.04)	23.00 (8.38)	0.001	22.32 (14.94)	19.27 (11.64)	0.138	5.40 (9.36)	3.05 (12.74)	0.264
Breast tenderness	0 (0.0%)	15 (37.5%)	<0.001	0 (0.0%)	6 (15.0%)	0.026	15 (37.5%)	6 (15.0%)	0.022
Nausea	0 (0.0%)	12 (30.0%)	<0.001	0 (0.0%)	6 (15.0%)	0.026	12 (30.0%)	6 (15.0%)	0.108
Headache	0 (0.0%)	5 (12.5%)	0.027	0 (0.0%)	2 (5.0%)	0.494	5 (12.5%)	2 (5.0%)	0.432
DVT	0 (0.0%)	0 (0.0%)	-	0 (0.0%)	0 (0.0%)	-	0 (0.0%)	0 (0.0%)	-
SBP (mmHg)	110.50 (8.14)	114.00 (10.57)	0.046	112.00 (9.92)	114.00 (0.57)	0.401	3.50 (10.75)	2.00 (14.88)	0.597
DBP (mmHg)	71.00 (7.08)	73.50 (8.33)	0.115	72.50 (6.69)	73.75 (8.06)	0.442	2.50 (9.80)	1.25 (10.17)	0.512

P-values were calculated with the independent T test and Mann Whitney U Test. FBG: Fasting blood glucose, TG: Triglyceride, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, D3: 3rd day of the menstrual cycle, DVT: Deep vein thrombosis.

Table 4: Comparison of the effects of tablets on premenstrual symptoms, dysmenorrhea-dyspareunia complaints and acne.

		Before the 21/7 tablet n (40)	After the 21/7 tablet	*P*-value	Before the 24/4 tablet n (40)	After the 24/4 tablet	*P*-value	Difference with the 21/7 tablet n (40)	Difference with the 24/4 tablet n (40)	*P*-value
Effects of tablets on premenstrual symptoms								Comparison of patients whose symptoms have improved		
Breast tenderness		5 (12.5%)	4 (10.0%)	0.5	3 (7.5%)	3 (7.5%)	1.00	1 (2.5%)	0 (0.0%)	1.00
Anxiety		4 (10.0%)	4 (10.0%)	1.00	3 (7.5%)	2 (5.0%)	0.5	0 (0.0%)	1 (2.5%)	1.00
Increased appetite		9 (22.5%)	5 (12.5%)	0.130	10 (25.0%)	4 (10.0%)	0.077	4 (10.0%)	6 (15.0%)	0.499
Sleeping disorder		5 (12,5%)	2 (5.0%)	0.216	6 (15%)	3 (7.5%)	0.214	3 (7.5%)	3 (7.5%)	1.00
Comparison of the effects of tablets on dysmenorrhea and dyspareunia in women with VAS scores								Comparison of differences in VAS scores		
Dysmenorrhea	Present	11 (27.5%)	11 (27.5%)		7 (17.5%)	7 (17.5%)		11 (27.5%)	7 (17.5%)	
	VAS	7.30 (1.05)	3.36 (1.02)	<0.001	7.00 (1.41)	3.29 (0.95)	0.002	3.82 (0.98)	3.71 (1.25)	0.846
Dyspareunia	Present	4 (10.0%)	4 (10.0%)		6 (15.0%)	6 (15.0%)		4 (10.0%)	6 (15.0%)	
	VAS	6.75 (1.25)	1.75 (0.50)	<0.001	6.33 (1.63)	3.00 (0.89)	0.003	5.00 (0.81)	3.33 (1.63)	0.099
Effects of tablets on acne and improvement of oily skin in women								Comparison of differences in skin complaints		
Acne and oily skin		4 (10.0%)	3 (7.5%)	0.5	3 (7.5%)	0 (0.0%)	0.120	1 (2.5%)	3 (7.5%)	0.615

P-values were calculated with the Mann Whitney U Test, independent T test and Chi-Square Test. VAS: Visual analog scale scores

In both groups, among women with dysmenorrhea and dyspareunia, pain intensity decreased significantly after using COCs and the results in both groups were similar ($P>0.05$) (Table 4).

Acne and oily skin improved in more women in Group 2 (n:3(7.5%)) when compared to Group 1 (n:1(2.5%)). However, the differences within or between the groups were not significant ($P>0.05$ for both) (Table 4).

Discussion

Although COCs provide effective contraception, compliance and continuity of the method depend on the side-effects' incidence and severity. New formulations aim to minimize the estrogen related side-effects by decreasing the estrogen dosages and using progestin that have favorable metabolic and systemic effects. While decreasing the estrogen dosages, it is important to maintain the suppressive effect of the formulation on ovulation. Hormone free interval (HFI) is another area under investigation with low dose COCs containing <50 mcg ethinyl estradiol (EE) as follicular activity is detected during the HFI [8]. Different regimens with shortened HFI are formulated to suppress ovarian function while using lower doses of EE effectively. In the presented study, we compared two formulations with 20 mcg and 30 mcg EE while the HFI was shorter with the 20-mcg-EE-containing formulation. In the presented study, both formulations suppressed ovulation effectively as proven by the serum levels of Day-3 FSH, Day-21 P values, and TVUSG findings related to follicular growth and endometrial thickness. However, suppression of the serum FSH and P levels and endometrial thickness were lower with 24/4 tablets than the 21/7 tablets, although this was not statistically significant. In the presented study, with the 21/7 tablet, follicles with a diameter of 10 mm or larger are observed and these follicles may easily develop and ovulate if there is a hormone free period [3].

Nausea, headaches, bloating, breast tenderness, decreased libido are the common side-effects related to COC use. While breast tenderness and nausea were observed with both tablets, headaches were also significantly more common with 21/7 tablets containing 30 mcg EE. During the HFI, side-effects related to estrogen withdrawal, a.k.a., "hormone-withdrawal-associated symptoms", might arise [12-14]. Similar to the natural cycle, prostaglandins are responsible for these side effects, which are caused by myometrial contractions and shedding of the endometrium and so dysmenorrhea might arise besides headache, nausea and bloating [15]. In our study, side effects were seen less with the 24/4 tablet, which had a shorter HFI when compared to the 21/7 tablet.

Another critical role of estrogen in COCs is providing cycle control and reducing spotting, which is a hormonal breakthrough bleeding. Studies show that spotting and intermittent bleeding are less with shorter HFI formulations when compared to the formulations with the same EE dose but a longer HFI [16]. In a meta-analysis of low-dose estrogen-containing tablets, there was less intermittent bleeding with tablets with a short hormone-free interval [17]. With extended use contraceptives that have a shorter interval, side effects are almost eliminated. Spotting episodes, menstrual irregularities were higher with 21/7 tablets. Hormonal contraceptives are widely used for the treatment of primary dysmenorrhea as they reduce prostaglandin secretion during menstruation and thus improve dysmenorrhea [18]. Drospirenone bearing COCs are effective in the treatment of premenstrual symptoms [19]. When we evaluated the effects on dysmenorrhea and dyspareunia, both

drospirenone containing tablets reduced dysmenorrhea and dyspareunia, but neither was superior. Both tablets had positive effects on premenstrual symptoms, dysmenorrhea, dyspareunia, oily skin, and acne, but only their effects on dysmenorrhea and dyspareunia were significant.

Metabolic side effects of COCs are also important. Drospirenone increases HDL and TG, increases LDL with long-term use. While estrogens increase TG and HDL, they support LDL catabolism [20]. In a study comparing 24/4 tablets containing 20 mcg EE/3mg drospirenone with 21/7 tablets containing 20 mcg EE/150 mcg desogestrel, both tablets increased HDL while decreasing LDL [21]. In our study, both tablets significantly increased HDL but were not superior to each other. The 21/7 tablet also increased systolic blood pressure and reduced cholesterol and LDL. Out of these variables, only the decrease in cholesterol with 21/7 was significant compared to 24/4.

Limitation and strength of the study

As the presented study evaluated the tablets' short-term effects, different results might be encountered after more prolonged use. However, since drospirenone levels become stable after the 8th day of the menstrual cycle with stabilized serum EE levels achieved after the second half of the cycle, making the assessment in the second month of COC use provided sufficient time for evaluation [22].

The strength of our work was the comparison of tablets with a fixed progestin dose (3mg drospirenone) but different EE doses (20mcg-30mcg) and different hormone-free intervals. Studies in the literature have mostly compared the tablets for cycle control and bleeding patterns or only in one aspect [23-25]. The presented study is a comprehensive, multifaceted study of two formulations with two different estradiol doses, including the incidence of side-effects, premenstrual symptoms, dyspareunia and dysmenorrhea pain scores, and effects on acne, together with cycle control.

Conclusion

In summary, among the oral contraceptives that showed efficacy, the 24/4 tablet containing low dose estrogen and having a shorter hormone-free interval can be considered an advantageous option compared to the 21/7 tablet due to relatively fewer side effects (especially breast tenderness), less stray follicle development and less menstrual irregularity in the short term.

References

1. Brynhildsen J. Combined hormonal contraceptives: prescribing patterns, compliance, and benefits versus risks. Ther Adv Drug Saf. 2014;5(5):201-13. doi: 10.1177/2042098614548857.
2. Skouby SO. Contraceptive use and behavior in the 21st century: a comprehensive study across five European countries. Eur J Contracept Reprod Health Care. 2004;9(2):57-68. doi: 10.1080/13625180410001715681.
3. Baerwald AR, Pierson RA. Ovarian follicular development during the use of oral contraception: a review. J Obstet Gynaecol Can. 2004;26(1):19-24. doi: 10.1016/s1701-2163(16)30692-2.
4. Endrikat J, Gerlinger C, Richard S, Rosenbaum P, Düsterberg B. Ovulation inhibition doses of progestins: a systematic review of the available literature and of marketed preparations worldwide. Contraception. 2011;84(6):549-57. doi: 10.1016/j.contraception.2011.04.009.
5. Milsom I, Korver T. Ovulation incidence with oral contraceptives: a literature review. J Fam Plann Reprod Health Care. 2008;34(4):237-46. doi: 10.1783/147118908786000451.
6. Burkman R, Bell C, Serfaty D. The evolution of combined oral contraception: improving the risk-to-benefit ratio. Contraception. 2011;84(1):19-34. doi: 10.1016/j.contraception.2010.11.004.
7. Rosenberg MJ, Waugh MS. Oral contraceptive discontinuation: a prospective evaluation of frequency and reasons. Am J Obstet Gynecol. 1998;179(3):577-82. doi: 10.1016/s0002-9378(98)70047-x.
8. London A, Jensen JT. Rationale for eliminating the hormone-free interval in modern oral contraceptives. Int J Gynaecol Obstet. 2016;134(1):8-12. doi: 10.1016/j.ijgo.2015.10.028.
9. WHO Guidelines Approved by the Guidelines Review Committee. Medical Eligibility Criteria for Contraceptive Use. Geneva: World Health Organization Copyright Â© World Health Organization 2015; https://www.who.int/publications/i/item/9789241549158
10. Mishell Jr DR. Premenstrual disorders: epidemiology and disease burden. Am J Manag Care. 2005;11(16 Suppl):S473-9.
11. Kroll R, Ackerman R, Feldman R, Howard B, Weiss H, Hsieh J, et al. Efficacy and safety of a 21/7-active combined oral contraceptive with continuous low-dose ethinyl estradiol. Contraception. 2016;93(3):249-56. doi: 10.1016/j.contraception.2015.10.007.
12. Nappi RE, Kaunitz AM, Bitzer J. Extended regimen combined oral contraception: A review of evolving concepts and acceptance by women and clinicians. Eur J Contracept Reprod Health Care. 2016;21(2):106-15. doi: 10.3109/13625187.2015.1107894.
13. Graziottin A. The shorter, the better: A review of the evidence for a shorter contraceptive hormone-free interval. Eur J Contracept Reprod Health Care. 2016;21(2):93-105. doi: 10.3109/13625187.2015.1077380
14. Bitzer J, Banal-Silao MJ, Ahrendt HJ, Restrepo J, Hardtke M, Wissinger-Graefenhahn U, et al. Hormone withdrawal-associated symptoms with ethinylestradiol 20 mug/drospirenone 3 mg (24/4 regimen) versus ethinylestradiol 20 mug/desogestrel 150 mug (21/7 regimen). IntJ Women's Health. 2015;7:501-509. doi: 10.2147/IJWH.S77942.
15. Baerwald AR, Olatunbosun OA, Pierson RA. Ovarian follicular development is initiated during the hormone-free interval of oral contraceptive use. Contraception. 2004;70(5):371-7. doi: 10.1016/j.contraception.2004.05.006.
16. Machado RB, Pompei LM, Andrade R, Nahas E, Guazzelli C, Wender MC, et al. Bleeding Pattern and Management of Unexpected Bleeding/Spotting with an Extended Regimen of a Combination of Ethinylestradiol 20 mcg and Drospirenone 3 mg. Int J Womens Health. 2020;12:235-42. doi: 10.2147/IJWH.S238294.
17. Endrikat J, Gerlinger C, Plettig K, Wessel J, Schmidt W, Grubb G, et al. A meta-analysis on the correlation between ovarian activity and the incidence of intermenstrual bleeding during low-dose oral contraceptive use. Gynecol Endocrinol. 2003;17(2):107-14.
18. Ferries-Rowe E, Corey E, Archer JS. Primary Dysmenorrhea: Diagnosis and Therapy. Obstet Gynecol. 2020;136(5):1047-58. doi: 10.1097/AOG.0000000000004096.
19. Appleton SM. Premenstrual Syndrome: Evidence-based Evaluation and Treatment. Clin Obstet Gynecol. 2018;61(1):52-61. doi: 10.1097/GRF.0000000000000339.
20. Silva-Bermudez LS, Toloza FJK, Perez-Matos MC. Effects of oral contraceptives on metabolic parameters in adult premenopausal women: a meta-analysis. Endocr Connect. 2020;9(10):978-98. doi: 10.1530/EC-20-0423.
21. Klipping C, Marr J. Effects of two combined oral contraceptives containing ethinyl estradiol 20 microg combined with either drospirenone or desogestrel on lipids, hemostatic parameters and carbohydrate metabolism. Contraception. 2005;71(6):409-16. doi: 10.1016/j.contraception.2004.12.005.
22. Fenton C, Wellington K, Moen MD, Robinson DM. Drospirenone/ethinylestradiol 3mg/20microg (24/4 day regimen): a review of its use in contraception, premenstrual dysphoric disorder and moderate acne vulgaris. Drugs. 2007;67(12):1749-65. doi: 10.2165/00003495-200767120-00007.
23. Larivée N, Suissa S, Coulombe J, Tagalakis V, Filion KB. Drospirenone-Containing Oral Contraceptive Pills and the Risk of Venous Thromboembolism: An Assessment of Risk in First-Time Users and Restarters. Drug Saf. 2017;40(7):583-96. doi: 10.1007/s40264-017-0525-2.
24. Jaisamrarn U, Santibenchakul S. A comparison of combined oral contraceptives containing chlormadinone acetate versus drospirenone for the treatment of acne and dysmenorrhea: a randomized trial. Contracept Reprod Med. 2018;3:5. doi: 10.1186/s40834-018-0058-9.
25. Uysal G, Akkaya H, Cagli F, Tutus S, Tayyar AT. A comparison of two different oral contraceptives in patients with severe primary dysmenorrhoea. J Obstet Gynaecol. 2018;38(6):828-32. doi: 10.1080/01443615.2017.1410533.

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The investigation of antifungal susceptibilities of *Kluyveromyces marxianus* and *Clavispora lusitaniae* strains isolated from various clinical specimens

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Ethics Committee Approval
Ethics approval and patient consent were not considered necessary since the isolates originated from the clinical samples obtained during routine laboratory activities.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: *C. albicans* remains the most common pathogen responsible for invasive candidiasis. On the other hand, increased rates of candidiasis have been reported worldwide, caused by other *Candida species* (such as *K. marxianus* and *C. lusitaniae*). Considering these uncommon yeasts may be crucial pathogens in the future, it is preferable to describe the in-vitro activities of antifungal agents as potential options for their treatments. This study aimed to evaluate the in-vitro activity of nine different antifungal agents that are routinely used to contribute to the treatment of the infections caused by *K. marxianus* and *C. lusitaniae*.

Methods: The study included 21 *K. marxianus* and eight *C.lusitaniae* strains isolated from various clinical specimens of patients with suspected invasive fungal infection. Conventional identification was confirmed using the molecular methodology of DNA sequencing analysis. Antifungal susceptibilities of the isolates were tested using the Sensititer Yeast One Test Panel Y06 kit, a colorimetric microdilution test.

Results: For *K. marxianus*, amphotericin B had the highest geometric mean MIC (1 µg/mL) and voriconazole had the lowest geometric mean MIC (0.010 µg/mL). For *C. lusitaniae*, flucytosine had the highest geometric mean MIC (8 µg/mL) and voriconazole had the lowest geometric mean MIC (0.011 µg/mL).

Conclusion: Considering that these two species, rare causes of invasive candidiasis nowadays, may become important pathogens in the future, it is reasonable to investigate the in-vitro activities of antifungal agents that can be used in their treatment.

Keywords: Antifungal susceptibility, Clavisopra lusitaniae, Kluyveromyces marxianus, Sequencing

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Introduction

Although five *Candida* species (*C.albicans*, *C. glabrata*, *C.parapsilosis sensu stricto*, *C.tropicalis*, and *C.krusei*) account for ≥95% of all candidemia or other forms of invasive candidiasis, other less common species (*Kluyveromyces marxianus*, *Clavispora lusitaniae*) may cause problems, particularly in cancer and leukemia patients [1-3]. Although *K. marxianus* is uncommonly documented in the literature, recent reports suggest that it may be an emerging pathogen, especially in patients with hematologic malignancies [4]. The ecology of the *K. marxianus* is not exactly understood, although it appears to grow in different habitats, including dairy products like kefir, fermented milk, cheese and yoghurt [5]. It was isolated from kefir in 1909, named *Saccharomyces fragilis* at first, then *C. pseudotropicalis,* and was reclassified as *K.marxianus* [5]. *C. lusitaniae* was first isolated from the gastrointestinal system of warm-blooded animals in 1959. It was first reported as an opportunistic human pathogen in a patient with acute myelogenous leukemia in 1979 [6]. Recent studies have indicated that the incidence of serious infections, especially blood stream infections caused by *K. marxianus* and *C. lusitaniae* are increasing. The clinical management of systemic infections caused by these organisms is challenging because of high MICs of amphotericin B for *K. marxianus* and the intrinsic amphotericin B resistance in some isolates of *C. lusitaniae* [5, 7]. Considering these uncommon yeasts may be crucial pathogens in the future, it is preferable to describe the in-vitro activities of both new and conventional antifungal agents as potential options for their treatments [2].

This study aimed to evaluate the in-vitro activity of nine different, routinely used antifungal agents to contribute to the treatment of infections caused by *K. marxianus* and *C. lusitaniae*.

Materials and methods

Identification

This study included 21 *K. marxianus* and eight *C.lusitaniae* strains isolated from various clinical specimens of patients with suspected invasive fungal infection, which were sent to Mycology Laboratory of Erciyes University Hospital, Faculty of Medicine. All yeast isolates were taken from different patients. Cultures were performed on Sabouraud dextrose agar (SDA) (Oxoid; United Kingdom), chloramphenicol SDA containing or not containing cycloheximide, and chromogenic media (CHROMagar *Candida*, Becton Dickinson, USA) in accordance with the standard identification procedure for fungal isolates. The Germ Tube Test (GTT) was performed on colonies grown on agar plates after 24 hours of incubation. Yeast isolates negative for GTT were cultured on corn meal-Tween 80 agar medium to identify their morphological characteristics and species. Twenty-one isolates identified as *K. marxianus* and eight isolates identified as *C.lusitaniae* based on the color they formed in chromogenic medium, colony morphology, and microscopic appearance in Corn Meal-Tween 80 Agar were confirmed with API 20 C AUX (Biomerieux, France). This initial identification was affirmed using the molecular methodology of DNA sequencing analysis. The sequences were compared with those available in the GenBank database using the BLASTN tool for species identification of each sequence.

Antifungal susceptibility test

Isolates were tested for their antifungal susceptibility with Sensititre Yeast One Test Panel Y06 kit (Trek Diagnostic Systems Inc., USA), a colorimetric microdilution test. A yeast suspension of 1.5-8 x 10^3 cells/mL was prepared in the Sensititre Yeast One broth, which was then added to the wells containing a certain amount of antifungal agent and incubated at 35°C for 24-48 hours. The value of the first well, where no red color was observed in the positive growth well, was considered the minimum inhibitory concentration (MIC).

Statistical analysis

Microsoft Excel 2010 software was used to determine the MIC ranges, MIC_{50} and MIC_{90} values of the isolates.

Results

K. marxianus species was isolated from the bronchoalveolar lavage (BAL) fluid (n=12), urine (n=4), peritoneal fluid (n=3) and blood (n=2) cultures and *C.lusitaniae* species was isolated from the urine (n=3), BAL fluid (n=1), peritoneal fluid (n=3) and blood (n=1) cultures of the patients who were hospitalized at the Erciyes University Hospital, Faculty of Medicine, in Kayseri. The ranges of minimum inhibitory concentrations (MICs), geometric mean MICs, MIC_{50} and MIC_{90} values (expressed in μg/ml) of the 21 *K. marxianus* and six *C. lusitaniae* isolates were detailed in Table 1. For *K. marxianus*, amphotericin B had the highest geometric mean MIC (1 μg/mL) and voriconazole had the lowest (0.010 μg/mL). For *C. lusitaniae*, flucytosine had the highest geometric mean MIC (8 μg/mL) and voriconazole had the lowest (0.011 μg/mL).

Table 1: The ranges of minimum inhibitory concentrations (MICs), geometric mean MICs and MIC_{50} and MIC_{90} values

Antifungal agent		Incubation time (24 hour)				Incubation time (48 hour)			
	Candida species	MIC range	GM	MIC_{50}	MIC_{90}	MIC range	GM	MIC_{50}	MIC_{90}
Amphotericin B									
(n: 21)	*C. kefyr*	0.5-2	1	1	1	1-2	1.935	2	2
(n: 6)	*C. lusitaniae*	0.12-0.5	0.246	0.25	0.5	0.25-1	0.629	0.5	1
Fluconazole									
(n: 21)	*C. kefyr*	0.12-32	0.238	0.25	0.25	0.12-64	0.423	0.25	0.5
(n: 6)	*C. lusitaniae*	0.12-4	0.442	0.25	0.5	0.12-4	0.702	0.5	1
Voriconazole									
(n: 21)	*C. kefyr*	0.008-0.5	0.010	0.008	0.008	0.008-2	0.014	0.008	0.015
(n: 6)	*C. lusitaniae*	0.008-0.06	0.011	0.008	0.008	0.008-0.06	0.015	0.015	0.015
Posaconazole									
(n: 21)	*C. kefyr*	0.015-2	0.039	0.03	0.06	0.03-2	0.064	0.06	0.06
(n: 6)	*C. lusitaniae*	0.008-0.25	0.021	0.015	0.015	0.015-0.25	0.042	0.03	0.06
Itraconazole									
(n: 21)	*C. kefyr*	0.03-1	0.062	0.06	0.06	0.03-4	0.070	0.06	0.06
(n: 6)	*C. lusitaniae*	0.03-0.25	0.060	0.06	0.06	0.012-0.25	0.135	0.12	0.12
Caspofungin									
(n: 21)	*C. kefyr*	0.03-0.06	0.046	0.06	0.06	0.03-0.12	0.052	0.06	0.06
(n: 6)	*C. lusitaniae*	0.015-0.12	0.06	0.06	0.12	0.03-0.5	0.246	0.5	0.5
Anidulafungin									
(n: 21)	*C. kefyr*	0.03-0.25	0.116	0.12	0.12	0.03-0.25	0.133	0.12	0.25
(n: 6)	*C. lusitaniae*	0.12-0.12	0.12	0.12	0.12	0.12-0.5	0.172	0.12	0.25
Micafungin									
(n: 21)	*C. kefyr*	0.06-0.12	0.062	0.06	0.06	0.06-0.25	0.105	0.12	0.12
(n: 6)	*C. lusitaniae*	0.03-0.25	0.067	0.06	0.06	0.12-0.25	0.153	0.12	0.25
Flucytosine									
(n: 21)	*C. kefyr*	0.06-4	0.180	0.12	2	0.06-4	0.287	0.12	4
(n: 6)	*C. lusitaniae*	0.5-64	8	16	64	1-64	17.95	64	64

Discussion

C. albicans remains the most common pathogen responsible for invasive candidiasis. On the other hand, increased rates of candidiasis are reported worldwide, caused by other *Candida species* (such as *K. marxianus* and *C. lusitaniae*) [8, 9]. *K. marxianus* is an increasingly important yeast. Studies report that a significant proportion of patients with hematologic malignancies, especially acute myelogenous leukemia patients,

are colonized with *C. kefyr* and there is a significant risk for subsequent bloodstream infection [5]. *C. lusitaniae* (anamorph: *Candida lusitaniae*) is an opportunistic yeast that can be isolated from plants, animals, industrial wastes and humans [10]. In the recent years, it has become increasingly recognized as an emerging nosocomial pathogen with a high mortality rate [11].

So far, it has been noted that amphotericin B has good sensitivity to most non-albicans *Candida* species, but there may be country-specific differences [2,12]. In a surveillance study on fungemia in adults in Germany, it was shown that the MIC of AMB was increased in 9% of all *K. marxianus* isolates [13], On the other hand, a study conducted in Spain reported that all *K. marxianus* strains were susceptible to AMB in-vitro (geometric mean [GM], 0.21 mg / L and MIC range, 0.03-1 mg / L) [8]. A study conducted in our country stated that amphotericin B had had MICs ≤1 µg / mL for all *Candida* species isolated from bloodstream infections except *K. marxianus* [14]. Due to the high MICs of amphotericin B for some *K. marxianus* strains, it can be predicted that the use of empirical therapeutics alongside antifungal prophylaxis may induce the selection of *K. marxianus* in the gastrointestinal flora, particularly in hemato-oncological patients [15]. Similar to the study of Gomez-Lopez A et al. [10], all azole compounds showed a great deal of activity against *K. marxianus* isolates with GM values of 0.238, 0.062, 0.015, 0.010 and 0.039 for FLC, ITC, VRC and POS, respectively, in our study. All *K. marxianus* strains revealed a similar susceptibility pattern against echinocandins, MIC_{50}, MIC_{90}, and the range of the measured MICs were ≤0.25 mg/L for caspofungin, anidulafungin and micofungin.

Due to the possibility that some *C. lusitaniae* species will not respond to chemotherapy, common candidiasis caused by this strain can have serious consequences [12]. *C. lusitaniae* infections respond poorly to amphotericin B despite low MIC values in in-vitro tests [16]. There are no CLSI/EUCAST antifungal susceptibility breakpoints for *C. lusitaniae*. However, several studies have established epidemiological breakpoints for *C. lusitaniae* to distinguish between wild type and non-wild type strains [17]. Investigating the caspofungin susceptibility of 105 *C.lusitaniae* isolates obtained from 91 institutions under the global surveillance program between 2001 and 2004, Pfaller et al. [18] reported that all isolates were inhibited at a concentration of 4 µg/mL. Caspofungin, anidulafungin, or micafungin did not have MIC values >0.25 µg/mL for any our isolates. In a case report, Desnos-Ollivier M et al. [9] reported that increased echinocandin MICs appeared in *C. lusitaniae* isolates 2 weeks after the initiation of caspofungin treatment and these isolates exhibited missense mutation S645F in the HS1 region. In our study, the MIC range for flucytosine was between 05-64 µg/mL and flucytosine had the highest geometric mean MIC. In their antifungal susceptibility study with 80 *C. lusitaniae* strains isolated from various clinical specimens, Favela et al. [19] reported that the MIC range for flucytosine was between 0.004-> 32 mg/L. These extremely high MIC values for flucytosine are important because flucytosine is often used in combination with amphotericin B or azoles to treat patients infected with this species.

Limitations

The small number of clinical samples and the antifungal susceptibility testing with a commercial colorimetric microdilution method, which is not the gold standard, are the limitations of the study.

Conclusions

Considering that these two species, rare causes of invasive candidiasis nowadays, may emerge as important pathogens in the future, it is reasonable to investigate the in-vitro activities of antifungal agents that can be used in their treatment. Further studies are required by testing large panels of geographically diverse clinical isolates.

References

1. Saleh Q, Kovàcs R, Kardos G, Gesztelyi R, Kardos T, Bozo A, et al. Decreased killing activity of micafungin against Candida guilliermondii, Candida lusitaniae and Candida kefyr in the presence of human serum. Microb Drug Resist. 2017;23:764-70. doi: 10.1089/mdr.2016.0241.
2. Gomez-Lopez A, Pan D, Cuesta I, Alastruey-Izquierdo A, Rodriguez-Tudela JL, Cuenca-Estrella M. Molecular identification and susceptibility profile in vitro of the emerging pathogen Candida kefyr. Diagn Microbiol Infect Dis. 2010;66:116-9. doi: 10.1016/j.diagmicrobio.2009.06.007.
3. Brandt ME, Lockhart SR. Recent taxonomic developments with Candida and other opportunistic yeasts. Curr Fungal Infect Rep. 2012;6:170-7. doi: 10.1007/s12281-012-0094-x.
4. Corpus K, Hegeman-Dingle R, Baijoka I. Candida kefyr, an uncommon but emerging fungal pathogen:report of two cases. Pharmacotherapy. 2004;24:1084-8. doi: 10.1592/phco.24.11.1084.36140.
5. Dufresne SF, Marr KA, Sydnor E,Staab JF, Karp JE, Lu K, et all. Epidemiology of Candida kefyr in patients with hematologic malignancies. J Clin Microbiol. 2014;52:1830-7. doi: 10.1128/JCM.00131-14.
6. Viudes A, Peman J, Canton E, Salavert M, Ubeda P, Lopez-Ribot JL, et al. Two cases of fungemia due to Candida lusitaniae and a literature review. Eur J Clin Microbiol. Infect Dis 2002;21:294-9. doi: 10.1007/s10096-002-0713-5.
7. McClenny NB, Fei H, Baron EJ, Gales AC, Houston A, Hollis RJ, et al. Change in colony morphology of Candida lusitaniae in association with development of amphotericin B resistance. Antimicrob Agents Chemother. 2002;46:1325-8. doi: 10.1128/aac.46.5.1325-1328.2002
8. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis. 2009;48:1695-703. doi: 10.1086/599039
9. Colak M, Asgin N. Retrospective assesment of fungal pathogens isolated from various clinical samples in a tertiary care hospital in Turkey: A cross-sectional study. J Surg Med. 2021;5:362-6.
10. Desnos-Ollivier M, Moquet O, Chouaki T, Guerin AM, Dromer F. Development of echinocandin resistance in Clavispora lusitaniae during caspofungin treatment. J Clin Microbiol. 2011;49: 2304-6. doi: 10.1128/JCM.00325-11
11. Zhang H, Ran Y, Li D, Liu Y, Xiang Y, Zhang R, Dai Y. Clavispora lusitaniae and Chaetomium atrobrunneum as rare agents of cutaneous infection. Mycopathologica. 2010;169: 373-80. doi: 10.1007/s11046-009-9266-9
12. Weichert S, Reinshagen K, Zahn K, Geginat G, Dietz A, Kilian AK, et al. Candidiasis caused by Candida keyfr in a neonate: case report. BMC Infect Dis. 2012;12:61. doi: 10.1186/1471-2334-12-61
13. Borg-von Zepelin M, Kunz L, Rüchel R, Reichard U, Weig M, Gross U: Epidemiology and antifungal susceptibilities of Candida spp. to six antifungal agents: results from a surveillance study on fungaemia in Germany from July 2004 to August 2005. J Antimicrob Chemother. 2007;60: 424-8 doi: 10.1093/jac/dkm145
14. Turk-Dagi H, Findik D, Senkles Ç, Arslan U. Identification and antifungal susceptibility of Candida species isolated from bloodstream infections in Konya, Turkey. Ann Clin Microbiol Antimicrob. 2016;15:36. doi: 10.1186/s12941-016-0153-1
15. Sendid B, Lacroix C, Bougnoux ME. Is Candida kefyr an emerging Pathojen in Patients with oncohematological diseases? Clin Infect Dis. 2006;43:666-7. doi: 10.1086/506573
16. Rahmati E, Correa AJ, She RC. A budding case infectious endocarditis: Candida lusitaniae. ID cases. 2019;19:e00679. doi: 10.1016/j.idcr.2019.e00679
17. Khan Z, Ahmad S, Al-Sweih N, Khan Seema, Joseph L. Candida lusitaniae in Kuwait: Prevalence, antifungal susceptibility and role in neonatal fungemia. PLoS ONE. 14(3):e0213532. doi: 10.1371/journal.pone.0213532.
18. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. In vitro susceptibilities of Candida spp. to caspofungin: four years of global surveillance. J Clin Microbiol. 2006; 44:760–3. doi: 10.1128/JCM.44.3.760-763.2006
19. Favel A, Michel-Nguyen A, Datry A, Challier S, Leclerc F, Chastin C, Fallague K, Regli P. Susceptibility of clinical isolates of Candida lusitaniae to five systemic antifungal agents. Antimicrob Chemother. 2004;53:526-9. doi: 10.1093/jac/dkh106.

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Critically ill Covid-19 patients with acute kidney injury: A single-center cohort study

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Ethics Committee Approval
The study was approved by the Baskent University Institutional Review Board (project no: KA 20/448).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Acute kidney injury (AKI) is a common serious complication associated with morbidity and mortality in critically ill COVID-19 patients. Although there is very limited data on the incidence of AKI in this cohort, conflicting results were recently reported. The incidence of AKI in critically ill COVID-19 patients ranged between 0.5-50% in the early studies. This study aimed to evaluate the incidence and determine the demographic parameters, clinical courses, and outcomes of AKI in critically ill COVID-19 patients admitted to the intensive care unit (ICU).

Methods: After ethics committee approval was obtained, critically ill COVID-19 patients admitted to our ICU between June 1- December 30, 2020, were analyzed in this retrospective cohort study. Patients over the age of 18 years who were admitted to the intensive care unit with the diagnosis of COVID-19 or whose real-time polymerase chain reactions (RT-PCR) test were positive were included in the study. Incidence and stages of AKI among the included critically ill COVID-19 patients were evaluated. The patients were divided into two groups according to the presence of AKI to define the risk factors and clinical outcomes. AKI was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines based on serum creatinine and urine output.

Results: We analyzed seventy-four critically ill confirmed COVID-19 patients. The mean age was 70.7 (14.8) years and 63.5% were male. Thirty-four patients (45.9%) had AKI, 12 patients in stage I (16.2%), 13 patients in stage II (17.6%), and 9 patients in stage III (12.1%). Renal replacement therapy (RRT) was initiated in 28.4% of patients with AKI; 16.2% received intermittent hemodialysis and 12.2%, continuous renal replacement therapy. APACHE II score and GCS at ICU admission were similar in patients with or without AKI ($P>0.05$), but the SOFA score was significantly higher in patients with AKI ($P=0.03$). ARDS and shock were significantly higher in patients with AKI than without ($P=0.01$ and $P=0.039$, respectively). Compared to the patients without AKI, those with AKI required higher amounts of oxygen therapy (high-flow oxygen therapy, non-invasive mechanical ventilation) and invasive mechanical ventilation ($P=0.01$ and $P<0.001$). The ICU mortality was 61.8% for the AKI group compared to 20% among those without ($P<0.001$).

Conclusions: Our study showed that AKI and renal replacement therapy are common in critically ill COVID-19 patients. SOFA score, ARDS, and shock rates were significantly higher among patients who developed AKI. The presence of AKI was associated with higher amounts of oxygen therapy and increased invasive mechanical ventilation. The severity of illness at ICU admission and ICU mortality were higher among those with AKI. Since AKI is seen in almost one in two patients and its development is associated with higher mortality, urine output, and creatinine values should be closely monitored in critically ill COVID-19 patients. It is recommended not to delay RRT therapy as soon as stage 2 AKI develops to preserve kidney function. In addition, optimal hemodynamic monitoring with appropriate fluid management and vasopressor drugs is required to ensure adequate renal perfusion.

Keywords: COVID-19, Coronavirus, Acute kidney injury, Critically ill, Intensive care unit

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Introduction

Coronavirus disease-19 (COVID-19), a multisystemic disease characterized by hyper-inflammation and hypercoagulation, caused by SARS-CoV-2, was first reported in Wuhan, China on December 31, 2019, and declared a pandemic by the World Health Organization on March 11, 2020 [1, 2]. SARS-CoV-2 uses angiotensin-converting enzyme II (ACE 2) receptors to enter the cell [3], which are found in various organs, including the cell membrane of the alveolar epithelial cells, small intestine enterocytes, arterial and venous endothelial cells, and arterial smooth muscle cells [4, 5]. Therefore, SARS-COV-2 can cause damage to the lungs as well as many organs in line with this mechanism, the kidney being another one.

Acute kidney injury (AKI) is a life-threatening disease associated with increased costs, poor outcomes, and mortality [6]. The risk of developing AKI is quite high in critically ill patients, and according to the latest data, the incidence of AKI is as high as 57% in patients followed in the intensive care unit (ICU) [7]. Studies show that this rate is comparable in COVID-19 patients, among which the incidence of AKI reportedly increases to 50% [8-10].

This study aimed to evaluate the incidence and determine the demographic parameters, clinical courses, and outcomes of AKI in critically ill COVID-19 patients admitted to the ICU.

Materials and methods

This study was approved by the Institutional Review Board of Baskent University (project no: KA 20/448). After ethics committee approval, critically ill COVID-19 patients admitted to our ICU between June 1- December 30, 2020, were analyzed retrospectively.

The patients who were not hospitalized with a diagnosis of COVID-19 or whose real-time polymerase chain reactions (RT-PCR) assay was negative, whose data could not be accessed, and those under the age of 18 years were excluded from the study.

Incidence and stages of AKI among the included critically ill COVID-19 patients were evaluated. Patients were divided into two groups according to the presence of AKI to define the risk factors and clinical outcomes. AKI was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines based on serum creatinine and urine output [11].

The following data were obtained from electronic medical and nursing records: Patient age, sex, complaints, exposure and travel history, comorbidities, Acute Physiology and Chronic Health Evaluation System (APACHE II) score, vital signs at ICU admission, microbiological sample type, PCR results, arterial blood gas analysis, need for intubation and mechanical ventilation (MV) (noninvasive or invasive), ventilation parameters (tidal volume, positive end-expiratory pressure [PEEP], fraction of inspired oxygen [FIO_2]), arterial partial pressure of oxygen [PaO_2], PaO_2/FIO_2 ratio, prone position, renal replacement therapy [RRT], laboratory values, treatment (vasopressors, antiviral and antibacterial agents, corticosteroids), length of hospital and ICU stay and ICU and in-hospital mortality.

SARS-CoV-2 was detected by real-time polymerase chain reaction (RT-PCR) assay. Laboratory examinations included complete blood count, D-dimer, coagulation profile, serum biochemical tests (renal and liver function tests, creatinine kinase, lactate dehydrogenase, and electrolytes), myocardial enzymes, ferritin, C-reactive protein (CRP), and procalcitonin (PCT). All patients underwent posterior-anterior chest radiography (PA-CR) and chest computed tomography (CT). The intensivist decided on the frequency of the examinations.

Confirmed cases were defined and the criteria for admission to the ICU were evaluated according to the guidelines of the Ministry of Health [9, 12, 13].

Fever was defined as a tympanic measurement of 37.8°C and higher. Sepsis and septic shock were defined according to the 2020 Surviving Sepsis Campaign: Guidelines on the Management of Critically Ill Adults with COVID-19 [14]. Secondary infection was considered when a positive culture of a new pathogen was detected in at least one of respiratory tract specimens, blood, urine, wound, drain sample after ICU admission [15]. Pneumonia was diagnosed based on the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA) criteria [16]. Acute respiratory distress syndrome (ARDS) was diagnosed according to the Berlin Definition [17, 18]. Disseminated Intravascular Coagulation (DIC) was defined as a cumulative score of five or more with regards to prolonged prothrombin time (PT), reduced platelets and fibrinogen, and elevated fibrin- related markers [12, 19, 20].

Statistical analysis

Data were summarized as mean (SD) and median (Min.-Max.) for continuous variables, and frequency (percentiles) for categorical variables. The student's t-test was used for independent group comparisons. The Chi-square test was used for proportions, and its counterpart, the Fisher's Exact test, was utilized when the data were sparse. A *P*-value of less than 0.05 was considered significant and SPSS 25.0 for Windows was used for all statistical analyses.

Results

During the designated period, 128 patients were admitted to the ICU with a preliminary diagnosis of COVID-19. COVID-19 RT-PCR was positive in 74, which were analyzed in this study (Figure 1). The mean age was 70.7 (14.8) years and 63.5% were male. Most patients were admitted from the other wards (51.4%, n=38) within our hospital and the emergency service (44.6%, n:33). Sixty-seven patients (90.5%) had medical etiologies and 7 patients (9.5%) had surgical causes. There were 6 renal (8.1%) transplant recipients. Dyspnea (75.7%) was the most common symptom and hypertension (73.0%) was the most common comorbidity**.** There were thirteen patients with malignancy (mostly lung cancer, 8.1%). Eight patients (10.8%) had a history of exposure, and six patients (8.1%) had a travel history. Twenty-eight patients (37.8%) were taking Angiotensin Receptor Blockers (ARBs) or Angiotensin-Converting Enzyme inhibitors (ACEi) **(**Table 1). The mean APACHE II score was 15.9 (7.2) at ICU admission (Table 2).

Figure 1: Flow chart of COVID-19 patients admitted to the intensive care unit (ICU) (AKI: acute kidney injury)

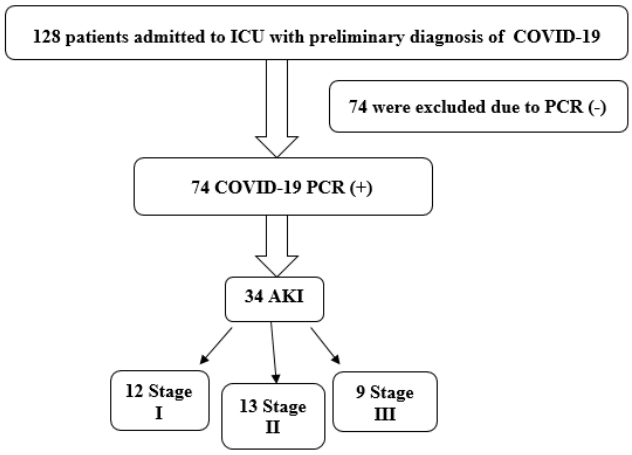

Table 1: Demographic and clinical characteristics of Covid-19 patients

Variables	Total: 74 (n/%)
Age, mean (SD)	70.7 (14.8)
Range, years	(28-95)
Sex	
Male	47 (63.5)
Female	27 (36.5)
Etiology	
Medical causes	67 (90.5)
Surgical causes	7 (9.5)
Admission from	
Emergency	33 (44.6)
Ward in hospital	38 (51.4)
Emergency from outer center	3 (4.1)
Transplant recipient	
Renal	6 (8.1)
Liver	0 (0)
History of exposure	8 (10.8)
History of travel	6 (8.1)
Smoking history	23 (31.1)
Use of ACEi/ARBs	28 (37.8)
Comorbidities	
Hypertension	54 (73.0)
Diabetes Mellitus	29 (39.2)
Cardiovascular disease	48 (48.6)
Obstructive pulmonary diseases	16 (21.6)
Malignancy	13 (17.6)
Cerebrovascular disease	9 (12.2)
Chronic kidney disease	11 (14.9)
Immunosuppression	23 (31.1)
Symptoms	
Fever	41 (55.4)
Fatigue	38 (51.4)
Dry cough	32 (43.2)
Dyspnea	56 (75.7)
Nausea	8 (10.8)
Vomit	5 (6.8)
Diarrhea	4 (5.4)
Myalgia	17 (23.0)
Sore throat	5 (6.8)
Altered mental status	20 (27.0)
Chest CT findings	
Unilateral ground glass	3 (4.1)
Bilateral ground glass	31 (41.9)
Unilateral consolidation	7 (9.5)
Bilateral consolidation	24 (32.4)

SD: Standard deviation, ACEi: Angiotensin-converting enzyme inhibitor, ARBs: Angiotensin Receptor Blockers, CT: Computed tomography

Table 2: Severity scores and vital signs on ICU admission of Covid-19 Patients

	Total (n:74) Mean (SD)
APACHE II score	15.9 (7.2)
SOFA score	6.0 (3.4)
GCS score	12.7 (4.1)
Temperature (^oC)	36.9 (1)
Heart rate, beats per min	100.0 (24.1)
Respiratory rate, breaths per min	26.0 (5.6)
Mean arterial pressure, mmHg	87.4 (20.4)
Oxygen Saturation (%)	86.6 (9.5)
Lactate (mmol/L) on admission	2.2 (1.8)

SD: Standard deviation, APACHE II: Acute Physiology and Chronic Health Evaluation System; SOFA: Sequential Organ Failure Assessment, GCS: Glasgow Coma Score

PaO_2/FIO_2 ratio was 100-200 among 35 patients (47.3%). Twelve patients (16.2%) received only low flow (nasal/ mask) oxygen; two had only high flow nasal oxygen (2.7%). Thirty-six patients (48.6%) required endotracheal intubation and 7 (9.5%) received invasive mechanical ventilation (IMV) only. Non-invasive mechanical ventilation (NIMV) was used in 36 patients (48.6%) (Table 3). Sixty-six patients (89.2%) had ARDS, 7 mild, 21 moderate, and 38 of which were severe. Thirty-three patients (44.6 %) were followed in the prone position. Recruitment maneuvers (RM) were performed on 14 (18.9%) patients. Bilateral pulmonary infiltrates were present in the lung X-ray in 45 (60.8%) patients. Bilateral ground-glass opacity (41.9%) and bilateral consolidation (32.4%) were the most common signs at thorax computer tomography (CT). All patients were given favipiravir as antiviral treatment. Five patients (6.8%) were given tocilizumab for macrophage activation syndrome (MAS). Low molecular weight heparin (LMWH) was used in 64 patients (86.5%) at a dose of 40 mg twice a day, and in 8 patients (10.8%), at 40 mg/day for thrombosis prophylaxis. Vitamin C and steroid therapy were given to all patients. Immune plasma therapy was administered to 22 patients (29.7). Forty patients (54.1%) did not receive any antibiotic treatment. If necessary, empirical antibiotic therapy was revised according to results of microbiological culture during the ICU stay. Secondary bacterial infections were detected among 34 patients (45.9%) (Table 4).

Table 3: Respiratory support therapies of patients with and without AKI

	Total (n:74) n (%)	With AKI (n:34) n (%)	Without AKI (n:40) n (%)	*P*-value
P/F on admission				0.004
>400	4 (5.4)	0	4 (10)	
300-400	4 (5.4)	0	4(10)	
200-300	9 (12.2)	2 (5.9)	7 817.5)	
100-200	35 (47.3)	16 (47.1)	19 (47.5)	
<100	22 (29.7)	16 (47.1)	6 (15)	
Types of respiratory support				0.001
Nasal/ Mask oxygen	12 (16.2)	2 (5.9)	10 (25)	
Nasal oxygen + NIMV	2 (2.7)	1 (2.9)	1 (2.5)	
IMV	7 (9.5)	5 (14.7)	2 (5)	
Nasal oxygen+ IMV	4 (5.4)	1 (2.9)	3 (7.5)	
HFOT+IMV	13 (17.6)	11 (32.4)	2 (5)	
HFOT+NIMV	16 (21.6)	4 (11.8)	12 (30)	
NIMV+IMV	11 (14.9)	9 (26.5)	2 85)	
Nasal oxygen+ HFOT	7 (9.5)	1 (2.9)	6 (15)	

AKI: acute kidney injury, P/F: PaO_2 FiO_2 ratio, NIMV: Noninvasive mechanical ventilation, IMV: Invasive Mechanical Ventilation, HFOT: High Flow Oxygen Therapy, $P<0.05$ was considered statistically significant.

Table 4: Treatments and complications of Covid-19 Patients

Types of treatments	Total (n:74) n (%)
Hydroxychloroquine	2 (2.7)
Oseltamivir	2 (2.7)
Favipiravir	74 (100.0)
Tocilizumab	5 (6.8)
Convalescent plasma therapy	22 (29.7)
Thromboprophylaxis	80 (86)
Mechanical	2 (2.7)
LMWH 40 gm /day	8 (10.8)
LMWH 2x 0.5 gm/kg	64 (86.5)
C vitamin	74 (100.0)
Steroid	54 (58.1)
Methylprednisolone	34 (48.6)
Hydrocortisone	4 (4.1)
Dexamethasone	51 (69.0)
Vasopressor therapy	34 (45.9)
RM	14 (18.9)
Prone position	33(44.6)
Tracheotomy	1 (1.4)
Shock	35 (47.3)
Septic	23 (31.1)
Secondary bacterial infections	34 (45.9)
Influenza type B	3 (4.1)
RSV	2 (2.7)
Pneumonia	70 (94.6)
ARDS	66 (89.2)
Mild	7 (9.5)
Moderate	21 (28.4)
Severe	38 (51.4)
DIC	13 (14.1)
≥5 points	2 (2.7)
<5 points	14 (18.9)

LMWH: Low Molecular Weight Heparin, RM: recruitment maneuvers, RSV: Respiratory syncytial virus, ARDS: Acute Respiratory Distress Syndrome, AKI: Acute Kidney Injury, DIC: Disseminated Intravascular Coagulation, $P<0.05$ was considered significant.

Thirty-four patients (45.9%) had AKI, 12 patients were in stage I (16.2%), 13 patients, in stage II (17.6%), and 9 patients, in stage III (12.2%). Renal replacement therapy (RRT) was initiated in 28.4% of patients with AKI among which 16.2% received intermittent hemodialysis and 12.2%, continuous renal replacement therapy (CRRT) (Table 5). Citrate anticoagulation was administered to 7 (77.8%) patients who underwent CRRT, and heparin anticoagulation was administered to two (22.2%). oXiris® hemofilter, high permeability polyacrylonitrile (AN69)-based membrane, was used in five (55.6%) patients who underwent CRRT.

Seventy (94.6%) patients had pneumonia. Thirty-two patients (43.2%) had septic shock and received vasopressor therapy: Thirteen patients (17.6%) received only norepinephrine, 13 patients (17.6%), norepinephrine and dobutamine, and 3 patients (4.1%), norepinephrine, dobutamine, and adrenalin (Table 4). None of the patients required extracorporeal membrane oxygenation (ECMO).

APACHE II score and GCS during ICU admission were similar in patients with or without AKI (P>0.05), but the SOFA score was significantly higher in patients with AKI (P=0.03). ARDS and shock were significantly higher in patients with AKI than in those without (P=0.01 and P=0.039). Compared to patients without AKI, patients with AKI required higher amounts of oxygen therapy (high-flow oxygen therapy, non-invasive mechanical ventilation) and invasive mechanical ventilation (P=0.01 and P<0.01) (Table 6). The ICU mortality was 61.8% among the AKI group compared to 20% among those without AKI (P<0.01) (Table 7).

Table 5: AKI stages and treatment modalities

	Total (n:74) (n/%)
AKI	34 (45.9)
Stage 1	12 (16.2)
Stage 2	13 (17.6)
Stage 3	9 (12.2)
RRT	21 (28.4)
IHD	12 (16.2)
CRRT	9 (12.2)
Type of anticoagulation	
Citrate	7 (77.8)
Heparin	2 (22.2)
Type of hemofilter	
Oxiris	5 (55.6)
Others	4 (44.4)

AKI: Acute kidney injury, RRT: renal replacement therapy, IHD: intermittent hemodialysis, CRRT: continuous renal replacement therapy

Table 6: Comparisons of patients with AKI and without AKI

	Total (n:74) mean (SD)	With AKI (n:34) mean (SD)	Without AKI (n:40) mean (SD)	*P*-value
Age, years, mean (SD)	70.7 (14.8)	75.7 (10.8)	66.6 (16.7)	0.036
APACHE II score	15.9 (7.2)	17.2 (7.7)	14.7 (6.5)	0.294
SOFA score	6.0 (3.4)	6.8 (3.8)	5.2 (2.9)	0.036
GCS score	12.7 (4.1)	11.8 (4.6)	13.6 (3.4)	0.099
Oxygen Saturation (%)	86.6 (9.5)	82.7 (10.1)	89.9 (7.6)	0.001
NLR on ICU admission	17.2 (13.8)	19.9 (14.4)	14.8 (13)	0.029
	n (%)	n (%)	n (%)	
ARDS	66 (89.2)	34 (100)	32 (80)	0.014
Shock	35 (47.3)	21 (61.8)	14 (35)	0.039
HFOT+NIMV	13 (17.6)	11 (32.4)	2 (5)	0.001
IMV	36 (48.6)	26 (76.5)	10 (25)	<0.001
NIMV+IMV	11 (14.9)	9 (26.5)	2(5)	0.001

SD: Standard deviation, AKI: acute kidney injury, APACHE II: Acute Physiology and Chronic Health Evaluation System; SOFA: Sequential Organ Failure Assessment, GCS: Glasgow Coma Score, NLR: neutrophil-to-lymphocyte ratio, ARDS: acute respiratory distress syndrome, HFOT: High Flow Oxygen Therapy, NIMV: Noninvasive mechanical ventilation, IMV: Invasive Mechanical Ventilation, P<0.05 was considered statistically significant

Table 7: Length of stay and outcomes of patients

	Total (n:74) mean (SD)	With AKI (n:34) mean (SD)	Without AKI (n:40) mean (SD)	*P*-value
LOS before ICU	3.9 (5.9)	5.9 (7.1)	2.2 (4.0)	0.008
LOS at ICU	7.7 (6.7)	9 (7.2)	6.6 (6.1)	0.061
LOS after ICU	2.3 (3.8)	1.6 (4)	2.9 (3.5)	0.007
LOS at Hospital	13.9 (10)	16.8 (10.5)	11.4 (8.9)	0.005
Outcome of ICU				<0.001
Exitus	29 (39.2)	21 (61.8)	8 (20)	
Discharge	45 (60.8)	13 (38.2)	32 (80)	
Outcome of hospital				<0.001
Deceased	30 (40.5)	22 (64.7)	8 (20)	
Discharge	44 (59.5)	12 (35.3)	32 (80)	

SD: Standard deviation, AKI: acute kidney injury, LOS: length of stay, ICU: Intensive care unit, P<0.05 was considered statistically significant.

Discussion

The incidence of AKI was 45.9 % in this retrospective study evaluating 74 critically ill confirmed COVID 19 patients. RRT was initiated in 61.8% of patients with AKI, of which 35.3% received IHD and 26.5%, CRRT. SOFA score, ARDS, and shock were significantly higher in patients with AKI. The presence of AKI was associated with higher amounts of oxygen therapy required and increased rates of invasive mechanical ventilation. AKI was associated with prolonged ICU stay and increased ICU mortality.

It is known that one of the most common problems encountered in patients followed in the ICU is AKI, with rates reaching 57% [7]. Studies show that this rate is almost comparable among COVID-19 patients, which increases up to 50% in critically ill COVID-19 patients [8-10]. We found an AKI incidence of 45.9% among our patients, in line with the published articles. However, there are also studies reporting an incidence of AKI as low as 0.5% to 20% in patients with COVID-19 [21-23]. The reason for this major difference in incidences among studies may be due to the differences in the patient populations (demographic characteristics, co-existing diseases), the number of patients included in the study, the severity of the disease, and the management differences at follow-up. However, publications are reporting that the reason for this difference may be due to the higher expression of angiotensin-converting enzyme 2 (ACE 2) in podocytes and proximal tubules in western populations compared to eastern individuals [9, 24].

In the FINNAKI study in which intensive care patients were investigated, the incidence of RRT was 9.4% [25]. In other studies, the incidence of RRT in patients with AKI ranged between 8-13.5% [26, 27]. Among COVID-19 patients, the need for RRT increased up to 61.5-96.6%. [9, 28]. In our study, the incidence of RRT was 28.4%. The lower RRT requirement in our study compared to that of Zamoner et al. [9] is attributed to the high rate of AKI stage III patients (58.9%) in the other study. In addition to absolute indications, the reason for the higher RRT rate in COVID 19 patients compared to other critically ill patients may be due to high cytokine storm, high fever, and positive fluid balance in this patient group.

After the development of the SOFA score in 1994 to evaluate organ failure in sepsis, it began to be used in patients without sepsis as well [29]. Many publications are showing the relationship between this scoring, which evaluates six organ systems (respiratory, cardiovascular, central nervous system, kidney, coagulation, and liver), with morbidity and mortality [30, 31]. However, publications are reporting different views on

SOFA scoring in COVID-19 patients. While some state that high SOFA scores are an independent risk factor for mortality, others report that the SOFA score is insufficient and weak in predicting mortality in COVID-19 patients [32, 33]. In our study, the SOFA score was significantly higher in patients with AKI. Like Gupta et al., we think that high SOFA scores are effective in indicating increased morbidity.

With a high mortality rate of 40%, the ARDS rate increases to 67% in COVID-19 patients and is one of the leading causes of death [34-36]. The incidence of AKI in critically ill patients with ARDS is as high as 35-50%, and the responsible mechanisms may be ARDS and related ventilator strategies [37]. The five mechanisms affecting the development of AKI in patients followed up with ARDS are hemodynamic effects, gas exchange impairment (hypoxemia/hypercapnia), acid-base dysregulation, hyper-inflammation, and neurohormonal effects [38]. Similar to these mechanisms, COVID-19 patients who develop ARDS have been reported to develop AKI [37]. AKI independently worsens ARDS by leading to increased production and decreased clearance of inflammatory cytokines, and downregulation of lung aquaporin and ion channels [39, 40]. Akin to these publications, the ARDS rate was significantly higher for our patients with AKI.

The incidence of shock can reach 35% among critically ill COVID 19 patients [35, 41]. The use of vasopressor agents causes renal blood flow dysregulation, including ischemia-reperfusion injury, metabolic reprogramming, and inflammation, resulting in AKI [42]. We found that shock was significantly higher in our patients with AKI.

Zamoner et al. [9] stated that mechanical ventilation is associated with the development of AKI in patients hospitalized with COVID-19. In a study evaluating 211 COVID-19 mechanically ventilated patients with ARDS, Chaibi et al. [43] reported a 49.8% incidence of AKI. Similar to these studies, we found that AKI was associated with increased oxygen therapy and invasive mechanical ventilation requirements.

There are many publications regarding the high in-hospital mortality in COVID-19 patients with AKI [9, 10, 43]. Cheng et al. [10] reported a rate of 79.4%. Similar to these, we found that AKI was associated with prolonged ICU stay and increased ICU mortality. The ICU mortality was 61.8% among the AKI group compared to 20% in those without AKI.

Limitations

Its retrospective nature and the limited number of patients are the two main limitations of this study. Also, it was conducted at a single center, which limits the generalizability of the results. The data were collected from the digital patient records. Not all laboratory tests were performed on all patients.

Conclusion

Our study indicates that AKI and renal replacement therapy are common in critically ill COVID-19 patients. SOFA score, ARDS, and shock were significantly higher among patients who developed AKI. The presence of AKI was associated with increased amounts of oxygen therapy and invasive mechanical ventilation needs. The severity of illness at ICU admission and ICU mortality are higher among those with AKI. Since AKI is seen in almost one in two patients and its development is associated with higher mortality, urine output, and creatinine values should be closely monitored in critically ill COVID-19 patients. It is recommended not to delay RRT therapy as soon as stage 2 AKI develops to preserve kidney function. In addition, optimal hemodynamic monitoring with appropriate fluid management and vasopressor drugs is required to ensure adequate renal perfusion.

References

1. WHO. Novel coronavirus – China. Jan 12, 2020. http://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/ (accessed Jan 19, 2020).
2. COVID-19 (SARS-CoV-2 INFECTION) guide. Republic of Turkey, Ministry of Health November 7th 2020, Ankara. https://covid19.saglik.gov.tr/TR-66301/covid-19-rehberi.html
3. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Mar;579(7798):270-3. doi: 10.1038/s41586-020-2012-7. Epub 2020 Feb 3. PMID: 32015507; PMCID: PMC7095418.
4. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med. 2020 Apr;14(2):185-92. doi: 10.1007/s11684-020-0754-0. Epub 2020 Mar 12. PMID: 32170560; PMCID: PMC7088738.
5. Kabbani N, Olds JL. Does COVID19 Infect the Brain? If So, Smokers Might Be at a Higher Risk. Mol Pharmacol. 2020 May;97(5):351-3. doi: 10.1124/molpharm.120.000014. Epub 2020 Apr 1. PMID: 32238438; PMCID: PMC7237865.
6. Jiang L, Zhu Y, Luo X, Wen Y, Du B, Wang M, Zhao Z, Yin Y, Zhu B, Xi X. Beijing Acute Kidney Injury Trial (BAKIT) workgroup. Epidemiology of acute kidney injury in intensive care units in Beijing: the multi-center BAKIT study. BMC Nephrol. 2019 Dec 16;20(1):468. doi: 10.1186/s12882-019-1660-z. PMID: 31842787; PMCID: PMC6915890.
7. Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med. 2015 Aug;41(8):1411-23. doi: 10.1007/s00134-015-3934-7. Epub 2015 Jul 11. PMID: 26162677.
8. Gabarre P, Dumas G, Dupont T, Darmon M, Azoulay E, Zafrani L. Acute kidney injury in critically ill patients with COVID-19. Intensive Care Med. 2020 Jul;46(7):1339-48. doi: 10.1007/s00134-020-06153-9. Epub 2020 Jun 12. PMID: 32533197; PMCID: PMC7290076.
9. Zamoner W, Santos CADS, Magalhães LE, de Oliveira PGS, Balbi AL, Ponce D. Acute Kidney Injury in COVID-19: 90 Days of the Pandemic in a Brazilian Public Hospital. Front Med (Lausanne). 2021 Feb 9;8:622577. doi: 10.3389/fmed.2021.622577. PMID: 33634152; PMCID: PMC7900413.
10. Cheng Y, Zhang N, Luo R, Zhang M, Wang Z, Dong L, et al. Risk Factors and Outcomes of Acute Kidney Injury in Critically Ill Patients with Coronavirus Disease 2019. Kidney Dis (Basel). 2021 Mar;7(2):111-9. doi: 10.1159/000512270. Epub 2020 Oct 26. PMID: 33821208; PMCID: PMC7649690.
11. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int. 2012;2:1–138.
12. Republic of Turkey Ministry of Health. General Directorate of Public Health. COVID-19 (SARS-CoV-2) infection guideline,Study of Scientific Board. Ankara, Turkey: Republic of Turkey Ministry of Health; 2020. https://hsgm.saglik.gov.tr/en/covid-19-i-ngilizce-dokumanlar/rehberler.html
13. World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (2019- nCoV) infection is suspected: interim guidance 13 March 2020. Geneva, Switzerland: WHO; 2020.
14. Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, et al. Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). Intensive Care Med. 2020 May;46(5):854-87. doi: 10.1007/s00134-020-06022-5. Epub 2020 Mar 28. PMID: 32222812; PMCID: PMC7101866.
15. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control. 1988 Jun;16(3):128-40. doi: 10.1016/0196-6553(88)90053-3. Erratum in: Am J Infect Control 1988 Aug;16(4):177. PMID: 2841893.
16. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Restrepo MI, Whitney CG. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019 Oct 1;200(7):e45-e67. doi: 10.1164/rccm.201908-1581ST. PMID: 31573350; PMCID: PMC6812437.
17. World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (2019- nCoV) infection is suspected: interim guidance 13 March 2020. Geneva, Switzerland: WHO; 2020.
18. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012 Jun 20;307(23):2526-33. doi: 10.1001/jama.2012.5669. PMID: 22797452.
19. McGonagle D, O'Donnell JS, Sharif K, Emery P, Bridgewood C. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. Lancet Rheumatol. 2020 Jul;2(7):e437-e445. doi: 10.1016/S2665-9913(20)30121-1. Epub 2020 May 7. PMID: 32835247; PMCID: PMC7252093.
20. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M. Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost. 2001 Nov;86(5):1327-30. PMID: 11816725.
21. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15;395(10223):507-13. doi: 10.1016/S0140-6736(20)30211-7. Epub 2020 Jan 30. PMID: 32007143; PMCID: PMC7135076.
22. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5. Epub 2020 Jan 24. Erratum in: Lancet. 2020 Jan 30 PMID: 31986264; PMCID: PMC7159299.
23. Cao M, Zhang D, Wang Y, et al. Clinical Features of Patients Infected with the 2019 Novel Coronavirus (COVID-19) in Shanghai, China. Preprint. medRxiv. 2020;2020.03.04.20030395. Published 2020 Mar 6. doi: 10.1101/2020.03.04.20030395
24. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. Intensive Care Med. 2020 Jun;46(6):1114-1116. doi: 10.1007/s00134-020-06026-1. Epub 2020 Mar 31. PMID: 32236644; PMCID: PMC7106051.
25. Nisula S, Kaukonen KM, Vaara ST, Korhonen AM, Poukkanen M, Karlsson S, et al. FINNAKI Study Group. Incidence, risk factors and 90-day mortality of patients with acute kidney injury in Finnish intensive care units: the FINNAKI study. Intensive Care Med. 2013 Mar;39(3):420-8. doi: 10.1007/s00134-012-2796-5. Epub 2013 Jan 5. Erratum in: Intensive Care Med. 2013 Apr;39(4):798. PMID: 23291734.

26. Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med. 2015 Aug;41(8):1411-23. doi: 10.1007/s00134-015-3934-7. Epub 2015 Jul 11. PMID: 26162677.
27. Jiang L, Zhu Y, Luo X, Wen Y, Du B, Wang M, et al. Acute Kidney Injury Trial (BAKIT) workgroup. Epidemiology of acute kidney injury in intensive care units in Beijing: the multi-center BAKIT study. BMC Nephrol. 2019 Dec 16;20(1):468. doi: 10.1186/s12882-019-1660-z. PMID: 31842787; PMCID: PMC6915890.
28. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. CRICS TRIGGERSEP Group (Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020 Jun;46(6):1089-98. doi: 10.1007/s00134-020-06062-x. Epub 2020 May 4. PMID: 32367170; PMCID: PMC7197634.
29. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996 Jul;22(7):707-10. doi: 10.1007/BF01709751. PMID: 8844239.
30. Vasilevskis EE, Pandharipande PP, Graves AJ, Shintani A, Tsuruta R, Ely EW, et al. Validity of a Modified Sequential Organ Failure Assessment Score Using the Richmond Agitation-Sedation Scale. Crit Care Med. 2016 Jan;44(1):138-46. doi: 10.1097/CCM.0000000000001375. PMID: 26457749; PMCID: PMC4748963.
31. Lambden S, Laterre PF, Levy MM, Francois B. The SOFA score-development, utility and challenges of accurate assessment in clinical trials. Crit Care. 2019 Nov 27;23(1):374. doi: 10.1186/s13054-019-2663-7. PMID: 31775846; PMCID: PMC6880479.
32. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, Melamed ML, et al; STOP-COVID Investigators. Factors Associated With Death in Critically Ill Patients With Coronavirus Disease 2019 in the US. JAMA Intern Med. 2020 Nov 1;180(11):1436-1447. doi: 10.1001/jamainternmed.2020.3596. Erratum in: JAMA Intern Med. 2020 Nov 1;180(11):1555. PMID: 32667668; PMCID: PMC7364338.
33. Raschke RA, Agarwal S, Rangan P, Heise CW, Curry SC. Discriminant Accuracy of the SOFA Score for Determining the Probable Mortality of Patients With COVID-19 Pneumonia Requiring Mechanical Ventilation. JAMA. 2021 Apr 13;325(14):1469-70. doi: 10.1001/jama.2021.1545. PMID: 33595630; PMCID: PMC7890534.
34. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, et al. LUNG SAFE Investigators; ESICM Trials Group. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. JAMA. 2016 Feb 23;315(8):788-800. doi: 10.1001/jama.2016.0291. Erratum in: JAMA. 2016 Jul 19;316(3):350. Erratum in: JAMA. 2016 Jul 19;316(3):350. PMID: 26903337.
35. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020 May;8(5):475-481. doi: 10.1016/S2213-2600(20)30079-5. Epub 2020 Feb 24. Erratum in: Lancet Respir Med. 2020 Apr;8(4):e26. PMID: 32105632; PMCID: PMC7102538.
36. Xu W, Sun NN, Gao HN, Chen ZY, Yang Y, Ju B, Tang LL. Risk factors analysis of COVID-19 patients with ARDS and prediction based on machine learning. Sci Rep. 2021 Feb 3;11(1):2933. doi: 10.1038/s41598-021-82492-x. PMID: 33536460; PMCID: PMC7858607.
37. Ahmed AR, Ebad CA, Stoneman S, Satti MM, Conlon PJ. Kidney injury in COVID-19. World J Nephrol. 2020 Nov 29;9(2):18-32. doi: 10.5527/wjn.v9.i2.18. PMID: 33312899; PMCID: PMC7701935.
38. Joannidis M, Forni LG, Klein SJ, Honore PM, Kashani K, Ostermann M, et al. Lung-kidney interactions in critically ill patients: consensus report of the Acute Disease Quality Initiative (ADQI) 21 Workgroup. Intensive Care Med. 2020 Apr;46(4):654-672. doi: 10.1007/s00134-019-05869-7. Epub 2019 Dec 9. PMID: 31820034; PMCID: PMC7103017.
39. Yap SC, Lee HT. Acute kidney injury and extrarenal organ dysfunction: new concepts and experimental evidence. Anesthesiology. 2012;116:1139-48. [PMID: 22415388 doi: 10.1097/ALN.0b013e31824f951b]
40. Andres-Hernando A, Dursun B, Altmann C, et al. Cytokine production increases and cytokine clearance decreases in mice with bilateral nephrectomy. Nephrol Dial Transplant 2012; 27: 4339-4347 [PMID: 22778179 doi: 10.1093/ndt/gfs256]
41. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 28;395(10229):1054-62. doi: 10.1016/S0140-6736(20)30566-3. Epub 2020 Mar 11. Erratum in: Lancet. 2020 Mar 28;395(10229):1038. Erratum in: Lancet. 2020 Mar 28;395(10229):1038. PMID: 32171076; PMCID: PMC7270627.
42. Peerapornratana S, Manrique-Caballero CL, Gómez H, et al. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int. 2019;96:1083-99. [PMID: 31443997 doi: 10.1016/j.kint.2019.05.026]
43. Chaibi K, Dao M, Pham T, et al. Severe Acute Kidney Injury in Patients with COVID-19 and Acute Respiratory Distress Syndrome [published correction appears in Am J Respir Crit Care Med. 2021 Jan 1;203(1):151.] Am J respir Crit Care Med. 2020;202(9):1299-301. doi: 10.1164/rccm.202005-1524LE.

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Evaluation of postmortem pathological changes in the lung in SARS-CoV-2 RT-PCR positive cases

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Ethics Committee Approval
This study was approved by the Turkish Ministry of Health, General Directorate of Health Services, Scientific Research Platform (Number: 2021-03-24T16_20_16, Date: 24.03.2021), and the Council of Forensic Medicine, Education and Scientific Research Commission (Number: 21589509/2021/333, Date: 30.03.2021).

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Abstract

Background/Aim: The most common cause of death in COVID-19 is acute respiratory distress syndrome. Diffuse alveolar damage is the histological characteristic and counterpart of acute respiratory distress syndrome. Histopathological findings, accompanied by immunohistochemical findings, can provide valuable information in the pathogenesis of Covid-19. We aimed to investigate the histopathological findings by supporting our results with immunohistochemical staining in SARS-CoV-2 positive autopsies.
Methods: A total of 101 autopsy cases with positive postmortem SARS-CoV-2 rt-PCR tests between May 2020-May 2021 were investigated in this retrospective cohort study. Cases with negative postmortem swab samples on rt-PCR and those with severe autolysis were excluded from the study. Pathological changes in the lung were examined with hematoxylin and eosin-stained preparations. Immunohistochemical assay with pancytokeratin, TTF-1, IL-6, CD68, CD3, CD8, and antibodies against the SARS-CoV-2 nucleocapsid protein were also performed for further evaluation.
Results: Diffuse alveolar damage findings were present in 58 (61.7%) out of 94 cases in our study. Seventeen (18.1%) showed findings compatible with the exudative phase, 37 (39.3%) were in the proliferative phase, and 4 (4.3%) were in the fibrotic phase of diffuse alveolar damage. Pulmonary perivascular lymphocytic infiltrates contained more CD3 (+) T lymphocytes than CD8 (+) T lymphocytes, immunohistochemically.
Conclusion: The finding of more CD3 positive T lymphocytes than the CD8 positive T lymphocytes in the perivascular lymphocytic infiltrate correlates with the hypothesis of the direct destruction of CD8 (+) T lymphocytes or through impairment of cellular immunity by SARS-CoV-2 induced mediators. Detection of immunohistochemical staining with IL-6 in COVID-19 supports the cytokine storm mentioned in the previous studies and the role of IL-6 in cytokine storm in SARS-CoV-2 infection. The limited number of immunohistochemical studies on SARS-CoV-2 increases the importance of our study, which evaluates IL-6, CD3, and CD8 expressions at the tissue level. Autopsy research is important and contributes to the development of protective, diagnostic, and therapeutic modalities.

Keywords: Autopsy, SARS-CoV-2, COVID-19, IL-6, CD3, CD8

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Introduction

Coronaviruses are enveloped, non-segmented, positive single-stranded RNA viruses that predominantly cause upper respiratory tract infections, which led to severe acute respiratory syndrome (SARS) outbreak in 2003 and the Middle East respiratory syndrome (MERS) outbreak in 2012 [1]. Coronavirus infections caused by SARS and MERS arose from bats that infected civet cats and dromedary camels, respectively, as secondary hosts [1]. SARS-CoV, which caused severe acute respiratory syndrome (SARS) infection, and MERS-CoV, which caused the Middle East respiratory syndrome (MERS), are members of the beta coronavirus group, just like SARS-CoV-2, and have more than 79.6% similarity to the SARS-CoV in genetic sequence [2, 3]. SARS-CoV-2 was quite similar to a bat coronavirus formerly detected in Rhinolophus affinis from the Yunnan County of China with a genome sequence identity similarity of over 96% [3].

Most individuals who were infected with SARS-CoV-2 recovered from the disease; however, particularly the elder persons and those with comorbidities became heavily ill [4]. The mortality rate among patients with severe symptoms was high, despite intensive care, and ranged between 7-15% [4, 5]. The overall mortality rate in the world is about 2% [5]. As of August 17, 2021, there were 208 million confirmed cases and more than 4.3 million deaths from COVID-19 in a total of 222 countries in the world [5].

Diffuse alveolar damage, the histomorphological counterpart of acute respiratory distress syndrome (ARDS), is thought to be responsible for most of the deaths in persons infected with SARS-CoV-2. However, some cases with fatal outcomes result from cardiac damage, shock, pulmonary embolism, thrombosis, and associated stroke [4].

Autopsy examinations are particularly important and considered the gold standard to understanding the pathophysiology of COVID-19 [6]. However, most studies focused on the clinical and molecular aspects of SARS-CoV-2 [6]. At the very beginning of the pandemic, data from autopsies were limited, insufficient, and remained inadequate for a long time because of suggestions to suspend postmortem examinations in patients with suspected COVID-19 infection. Postmortem examinations were thought to carry a high risk for autopsy workers and required an autopsy unit with proper biosecurity accessories [4]. Subsequent studies added valuable information about the pathophysiology of the COVID-19 disease and helped to understand the course of the disease.

In this comprehensive study, 101 patients who died due to COVID-19 disease and were autopsied at the Istanbul Forensic Medicine Institute, morgue department, were studied. SARS-CoV-2 positivity was detected with real-time reverse-transcriptase-polymerase-chain-reaction (rt-PCR) assay from pharyngeal/tracheal swabs or lung tissues. These cases were analyzed for their histopathological pulmonary findings. We aimed to compare the count of CD3 and CD8-positive perivascular T lymphocytes to assess their role in the pathogenesis of SARS-CoV-2 infection, and to investigate the tissue-level expression of IL-6 immunohistochemically, which has been reported to increase in bronchoalveolar fluid and plasma [7-9]. Also, the frequency and presence of megakaryocytes in the lung in COVID-19 infection were evaluated microscopically, and their relationship with acute lung injury and thrombosis was discussed. Our findings were evaluated in light of the current literature to reveal the pathological mechanisms of COVID-19. We think that the pulmonary findings in our autopsies will contribute to the literature since they were performed on a large series in one center and focused on morphological and immunohistochemical findings in the lung, which is the main tissue of acute injury in COVID-19.

Materials and methods

This study was authorized and approved by the Turkish Republic, Ministry of Health, General Directory of Health Services Scientific Research Platform ("2021-03-24T16_20_16"), and the Council of Forensic Medicine, Education and Scientific Research Commission ("21589509/2021/333-30.03.2021").

This retrospective cohort study was conducted on rt-PCR confirmed COVID-19 autopsies and the findings of 101 autopsy cases with positive postmortem SARS-CoV-2 rt-PCR assays performed at the Istanbul Council of Forensic Medicine between May 2020 and May 2021 were evaluated. Those with negative rt-PCR postmortem swab samples and 7 cases with severe autolysis were excluded from the study.

Autopsy technique and tissue sampling

Autopsy procedures were altered, and the autopsy technique was modified in the Morgue Department during the COVID-19 pandemic. All COVID-19 autopsy cases were examined by opening three cavities in the body as a rule. Organs other than the heart and central nervous system were not removed and investigated in their normal localization in the body. Tissue samples were obtained from the organs (heart, lung, liver, kidney, central nervous system) for histopathological examination. In addition to nasopharyngeal swabs, tracheal and lung swab samples were also taken from suspicious cases for COVID-19 infection. All cases were reviewed for macroscopic autopsy information. Age, gender, height, weight, and hospitalization information were noted if available.

Real-Time PCR

Postmortem SARS-CoV-2 rt-PCR assay, which is a nucleic acid amplification method that detects the viral RNA of COVID-19 was studied in the nasopharyngeal swab, deep tracheal swab, lung swabs, and paraffin blocks of tissues taken from the lungs of suspected cases. Nucleic acids were extracted on the QIASymphony (Qiagen / Germany) device using the QIAsymphony DSP Virus / Pathogen Midi kit. RealStar® SARS-CoV-2 RT-PCR Kit RUO (Altona Diagnostics, Hamburg, Germany) was used in the rt-PCR method and amplified in the Rotor-Gene (Qiagen / Germany) device according to the manufacturer's guide.

The nucleic acids were extracted from paraffin-embedded lung tissues which were fixated with 10% formalin solution. Sections of each lung block were cut by a rotary microtome device. Deparaffinization was performed with ethyl alcohol and xylene, after which the samples were incubated for

24 hours by adding proteinase K and ATL buffer solution [10-12].

Histopathological investigation

Histopathological changes detected in the lung were investigated in hematoxylin and eosin-stained sections with a "Nikon Eclipse Ni" research light microscope. In this study, demographic data such as age, gender, body mass index (BMI), event information, causes of death, and histopathological findings (alveolar edema, interstitial edema, interstitial inflammation, perivascular lymphocytic inflammation, the presence of megakaryocytes, acute bronchopneumonia, the presence of hyaline membrane, fibrin thrombus in the vascular lumen, and fibrin in the alveolar lumens, type II pneumocyte hyperplasia, alveolar hemorrhage, squamous metaplasia, and the presence of multinuclear cells in the alveolar lumen and infarct) were analyzed.

Immunohistochemical staining

BenchMark Ultra, Roche's fully automatic immunohistochemical slide staining system was used for the immunohistochemical procedures. The presence of SARS-CoV-2 was also revealed by antibodies against the SARS-CoV-2 nucleocapsid protein (E8R1L): A monoclonal mouse mAb (Cell signaling Technology, Danvers, Massachusetts, USA). Positive staining was defined as cytoplasmic and membranous staining. Additional immunohistochemical staining for Pancytokeratin (PanCK) (Dako FLEX Monoclonal Mouse Anti-Human Cytokeratin, Clone AE1/AE3, Ready to use), TTF-1 (Dako FLEX Monoclonal Mouse Anti-Thyroid Transcription Factor, Clone 8G7G3/1, Ready to use), IL-6 (Santa Cruz Biotechnology, Inc, Monoclonal Mouse antibody, dilution 1/100), CD68 (Dako FLEX Monoclonal Mouse Anti-Human CD-68, Clone KP1, Ready to use), CD3 (Dako FLEX Polyclonal Anti-Human CD-3, Ready to use), CD8 (Dako FLEX Monoclonal Mouse Anti-Human CD-8, Clone C8/144B, Ready to use) were performed on the lung tissues for further evaluation. PanCK and TTF-1 were used to differentiate the cells of pneumocyte origin from the megakaryocytes, CD68, for differentiation of histiocytes from megakaryocytes, IL-6, to investigate its role in the cytokine storm, and CD8 and CD3i for the assessment and comparison of the amount of CD3 and CD8 positive T cells in perivascular lymphocytic infiltrates.

Statistical analysis

The program used for statistical analysis was SPSS (Statistical Package for the Social Sciences) 2012 version 21. Descriptive statistical methods (standard deviation, average, median, frequency, minimum, maximum, ratio), and the Chi-Square test were used to compare the data. A *P*-value of <0.05 was considered significant.

Results

Of the 101 cases examined, 79.2% were male (n = 80) and 20.8% were female (n = 21). Their mean age was 56.1 (18.9) (range 7-98) years, mean weight, 82.7 (20.7) kilograms, mean height, 169.6 (10.2) cm, and mean body mass index, 28.8 (7.2) kg/m^2. The mean ages of the males and females were 55.7 (18.7) years and 58.10 (20.3) years, respectively, their mean BMIs, 28 (6.3) and 31.8 (9.6) kg/m2, respectively, their mean weights, 83.2 (19.2) and 80.8 (26.2) kg, respectively, and their mean heights, 172.4 (9.3) and 158.9 (5.3) cm, respectively. BMI was remarkably higher in females than in males (P<0.05). Postmortem SARS-CoV-2 rt-PCR assay was positive for SARS-CoV-2 in all cases. RT-PCR samples were taken from the nasopharyngeal swab, deep tracheal swab and, lung swabs during the autopsy, and paraffin blocks sections from cases histopathologically suspected for COVID-19 during the routine pathological investigation.

Seven out of 101 cases were excluded from the histopathological examination due to severe autolysis findings, which distorts morphology. Diffuse alveolar damage (DAD) findings were present in 58 (61.7%) out of 94 cases included in our study (Table 1). Seventeen cases (18.1%) with DAD findings were in the exudative phase, 37 (39.3%) were in the proliferative phase, and 4 (4.3%) were in the fibrotic phase. The histopathological findings are summarized in Table 2. Forty-three (45.7%) cases were hospitalized, 48 (58.1%) were not, and in 3 (3.2%) cases, there was no information about hospitalization. The time between the onset of the disease and death could not be determined precisely due to limitations in obtaining clinical information.

Table 1: Histopathological stages of diffuse alveolar damage of COVID-19 cases

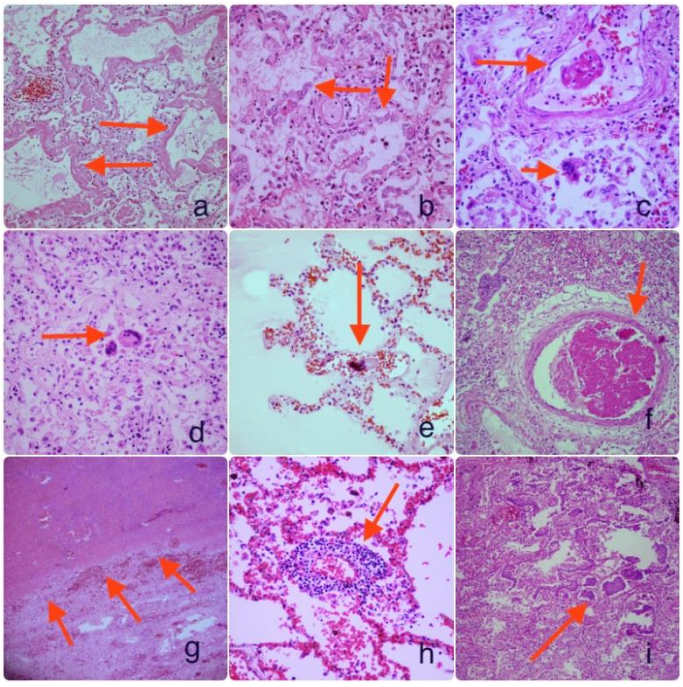
Diffuse Alveolar Damage	n	%
Absent	36	38.3%
Exudative stage	17	18.1%
Proliferative stage	37	39.3%
Fibrotic stage	4	4.3%
Total	94	100

Table 2: Histopathological findings in the lung

Histopathological Findings	n (%)
Hyaline Membrane	50 (53.2%)
Type II pneumocyte hyperplasia	41 (43.6%)
Alveolar edema	85 (90.4%)
Interstitial oedema	80 (85.1%)
Fibrin thrombus	49 (52.1%)
Perivascular Inflammation	63 (67%)
Alveolar fibrin	44 (46.8%)
Squamous metaplasia	33 (35.1%)
Alveolar hemorrhage	36 (38.3%)
Multinuclear cells	33 (35.1%)
Acute bronchopneumonia	60 (63.8%)
Interstitial inflammation	73 (77.7%)
Infarction	4 (4.3%)
Megakaryocytes	63 (67%)

There was alveolar edema in 85 (90.4%), interstitial edema in 80 (85.1%), interstitial inflammation in 73 (77.7%), perivascular lymphocytic inflammation (Figure 1h) and megakaryocytes in 63 (67%) (Figure 1e), and acute bronchopneumonia in 60 (63.8%). There were hyaline membranes in the alveolar walls in 50 (53.2%) (Figure 1a), fibrin thrombus in the vascular lumen in 49 (52.1%) (Figure 1c -long arrow- and 1f), fibrin in the alveolar lumens in 44 (46.8%), type II pneumocyte hyperplasia in 41 (43.6%) (Figure 1b), alveolar hemorrhage in 36 (38.3%), squamous metaplasia (Figure 1i) and multinuclear cells in alveolar lumens in 33 (35.1%) (Figure 1c -short arrow- and Figure 1d), and infarction (Figure 1g) in 4 (4.3%) cases.

Figure 1: Histopathological findings of the lung in COVID-19 cases (Hematoxylin and eosin stain -H&E-)



a: Exudative pattern with a hyaline membrane (arrows) x200. b: Proliferative phase of diffuse alveolar damage with type II pneumocyte hyperplasia (arrows)x400. c: Intra-alveolar multinuclear giant cell (short arrow), and at the top, a fibrin thrombus in the vascular lumen (long arrow) x400. d: Intra-alveolar multinuclear giant cell (arrow)x400. e: Megakaryocyte in the vascular lumen (arrow)x400 f: Fibrin thrombus in the vascular lumen (arrow) and associated squamous metaplasia x200. g: Pulmonary infarction (arrows)x200. h: Perivascular lymphocytic cuffing (arrow)x400. i: Squamous metaplasia (arrow)x200.

Hyaline membrane, a typical feature of DAD and especially the histological hallmark of the exudative phase, was found in 50 (53.2%) cases. Type II pneumocyte hyperplasia was present in 43 (45.7%). Exudative, proliferative, or fibrotic stage of DAD was not detected in the lungs of 36 cases. The histopathological pulmonary findings found in these cases, which do not have signs of diffuse alveolar damage, are shown in Table 3.

Table 3: Histopathological pulmonary findings of SARS-CoV-2 rt-PCR (+) and diffuse alveolar damage (-) 36 autopsy cases

Histopathological Findings	n (%)
Alveolar edema	29 (80.6%)
Megakaryocytes	18 (50.0%)
Interstitial edema	24 (66.7%)
Perivascular Inflammation	24 (66.7%)
Interstitial inflammation	23 (63.9%)
Alveolar hemorrhage	10 (27.8%)
Fibrin thrombus	9 (25.0%)
Acute bronchopneumonia	6 (16.7%)
Multinuclear cells	4 (11.1%)
Alveolar fibrin	3 (8.3%)
Squamous metaplasia	1 (2.8%)
Infarction	1 (2.8%)

COVID-19: Coronavirus disease of 2019, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, rt-PCR: reverse-transcriptase-polymerase-chain-reaction

Immunohistochemical staining revealed that pulmonary perivascular lymphocytic infiltrate contained more CD3 (+) T lymphocytes than CD8 (+) T lymphocytes. Cytoplasmic and membranous immunohistochemical staining of Interleukin-6 (IL-6) were found in the lung. In addition, CD68, Pancytokeratin (PanCK) and, Thyroid Transcription Factor-1 (TTF-1) were applied to the lung tissue for differentiation of histiocytes and epithelial cells from megakaryocytes immunohistochemically.

Discussion

Histopathological findings in most organs in COVID-19 infection are quite similar to those seen in SARS and MERS infections [13]. In the studies related to SARS-CoV-2, the lung, heart, and vascular systems are the most affected by the infection. Among these organs, histopathological findings are most common and severe in the lungs [13]. Pulmonary histopathological findings of COVID-19 cases were at different phases of diffuse alveolar damage, with associated type II pneumocyte hyperplasia, pneumocyte desquamation, intra-alveolar hemorrhage, fibroblast plugs, squamous metaplasia, interstitial thickening, patchy chronic inflammation, presence of megakaryocytes, hemophagocytosis, and vascular damage [4]. The histopathological findings obtained in our study are consistent with the findings of the previous studies.

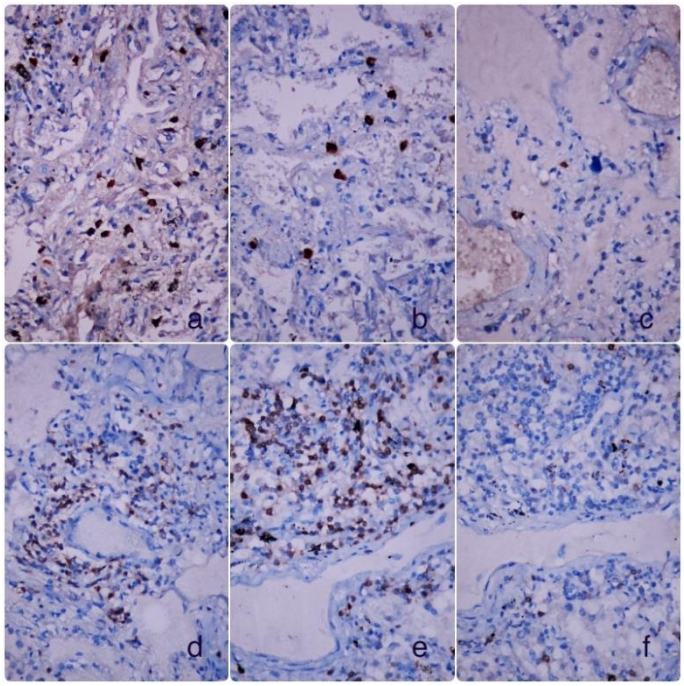
Normally, viruses enter the host cells and manipulate their cellular process to replicate and spread to the other cells [14]. Angiotensin-converting enzyme-2 (ACE2) is a membrane protein that is thought to be the host cell receptor for SARS-CoV-2 [14, 15]. It has been shown that COVID-19 patients have remarkably more ACE2-positive cells in their lungs [16]. Changes in the amount of ACE2-positive endothelial cell count play a crucial role in morphological changes seen in the vascular endothelial cells in COVID -19 patients [16].

Histopathologically, diffuse alveolar damage (DAD) is the morphologic equivalent of ARDS [17]. The lung responds quite similarly to most injuries regardless of the cause. The microscopic appearance depends on the time between the offending agent and the time of death, also on the extent and severity of the damage [17]. Histopathological findings of diffuse alveolar damage (DAD) found in COVID-19 are indistinguishable from DAD which develops due to other causes. The exudative (acute) phase of DAD is evident during the first week of the onset of disease and is characterized by inflammatory cell-mediated alveolar damage with alveolar edema and/or bleeding, congestion in capillary vessels, and hyaline membranes with or without microvascular thrombus. Hyaline membranes are the main morphologic characteristic of the exudative phase of diffuse alveolar damage and are most remarkable between 3-7 days after the onset of disease [17]. The subacute proliferative (organized) phase of DAD begins at the end of the first week and morphologically shows type II pneumocyte hyperplasia at the alveolar surface with associated alveolar wall thickening, myofibroblast proliferation, and reactive pneumocytes [4, 17]. Type II pneumocyte hyperplasia, which is characterized by prominent cytological reparative atypia such as cytomegaly, nucleomegaly, prominent nucleolus, clearing of nuclear chromatin, multinucleation, and squamous metaplasia, can be explained as a secondary response to the damage to the lung tissue caused by the virus or other offending agents [18]. The fibrotic (chronic) phase shows signs of honeycomb lung with fibrosis of the alveolar cavities, interstitium, and associated squamous metaplasia [4]. DAD does not always progress to the fibrotic (chronic) phase, sometimes it heals entirely without any sequelae. In studies, SARS-CoV-2 deaths were mostly detected at the exudative phase of diffuse alveolar damage compared to SARS-CoV-1 deaths, which indicates that the lungs in COVID-19 patients deteriorated faster, were more severely damaged, and caused earlier death [4]. However, in our study, 37 (39.3%) COVID-19 cases were in the proliferative phase and 17 (18.1%) were in the exudative phase of diffuse alveolar damage. The proliferative phase of diffuse alveolar damage indicates that these deceased persons were infected with the SARS-CoV-2 virus for more than 1 week.

More decedents at the proliferative phase than the exudative phase found in our study may be related to earlier diagnosis and hospitalization. We believe that the filiation process and starting treatment at an early stage of infection, which are strictly performed during the pandemic in our country, might extend survival. In addition, some of the cases that we investigated in the study were those that were hospitalized and received treatment. This explains the high number of cases in the proliferative stage in our study. Hereby, it is important to determine infected cases and start medical treatment as soon as possible to extend survival time.

A distinctive feature of COVID-19 is "cytokine storm syndrome", in which an increase in proinflammatory cytokines leads to endothelial cell destruction and the resultant increase in vascular permeability. This leads to fluid collection in the alveoli, causing hypoxia and, in critically ill cases, acute respiratory distress syndrome [19-21]. The pathogenesis of COVID-19 involves an inflammatory response involving a group of mediators, including IL-6. IL-6 is a proinflammatory cytokine that has pleiotropic functions which include autoimmunity, inflammation, and acute phase response [22, 23]. IL-6 alters the host defense through various immune-stimulating mechanisms such as altering antigen-dependent B lymphocyte differentiation, increasing IgG formation by B lymphocytes, and managing monocytes and their differentiation to macrophages [23]. IL-6 also activates complements and triggers coagulation [24]. Also, the level of serum SARS-CoV-2 nucleic acid is tightly related to exceedingly elevated levels of IL-6 [23]. In our study, cytoplasmic and membranous staining and the expression of IL-6 in the lung were detected in rt-PCR positive SARS-CoV-2 decedents (Figure 2a, 2b, 2c). However, while most previous studies investigated IL-6 in the bronchoalveolar lavage aspirate and plasma, in our study, this was also confirmed at the lung tissue level. Detection of immunohistochemical expression of IL-6 in the lung tissue in COVID-19 cases supports the cytokine storm mentioned in previous studies and the role of IL-6 in cytokine storm in SARS-CoV-2 infection. IL-6 was pointed out as an indicator of lung damage and may indicate morbidity and mortality. [7, 8]. The role of IL-6 in SARS-CoV-2 infection should be carefully evaluated to understand whether it is a pathogenic or protective effect. Further studies may be useful for therapeutic purposes, particularly for patients who are critically ill and resistant to supportive care.

Figure 2: a,b,c: Interleukin-6 (IL-6) immunohistochemistry in SARS-CoV-2 infected lung tissue, cytoplasmic and membranous staining x400, d,e: Higher count of CD3 (+) T lymphocytes at the site of perivascular lymphocytic cuffing in the lungs x400, CD3. f: Lower count of CD8 (+) T lymphocytes at the site of perivascular lymphocytic cuffing in the lungs x400, CD8.

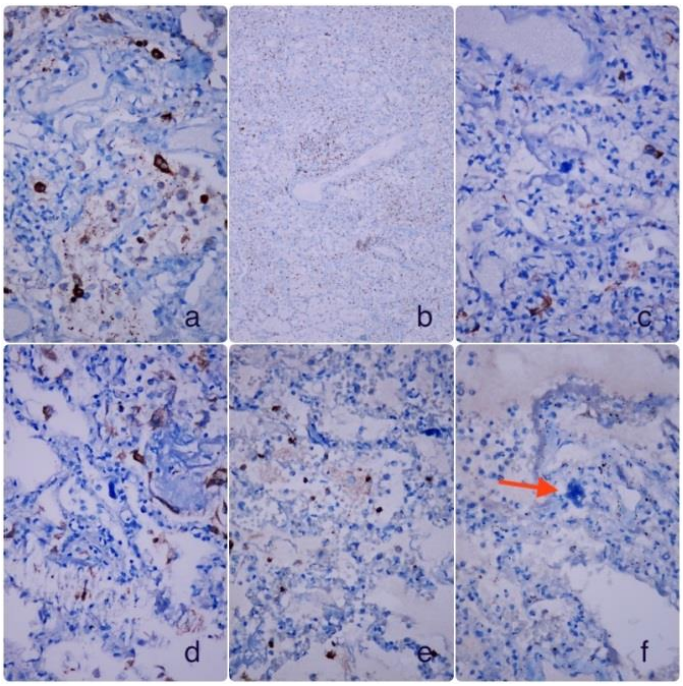


Lymphocytes and proinflammatory mediators such as IL-6 have a role in the antiviral activity, which aims to clear the virus [25]. Generally, viral infections stimulate T lymphocytes, which leads to rapid increases in CD8 (+) T lymphocytes. Lymphocytes were found mainly in the pulmonary perivascular areas and detected in 63 (67%) of 94 cases we evaluated. In our study, CD3 (+) T lymphocyte immunohistochemical staining was significantly higher than CD8 (+) T lymphocyte staining in the pulmonary perivascular areas, indicating a predominance of CD3 (+) T lymphocytes over CD8 (+) T lymphocytes in the perivascular lymphocytic infiltrate, in line with another study [26]. This finding supports the hypothesis of Huang JL et al. [27], who stated that the SARS-CoV-2 virus directly or indirectly destructs the CD8 (+) T lymphocytes through impairment of cellular immunity or SARS-CoV-2 induced-mediators, but the mechanism is not clear yet. However, an immunohistochemical study by Frisoni P et al. [24] reported that CD8 (+) T cells and CD20 (+) B cells were mainly found in the lung. They showed that the reason for this was that none of the patients in their study had received any treatment that would activate the immune system, for they died in an out-of-hospital setting [24]. In our study, 45.7% (n=43) of the cases were hospitalized. The immunohistochemical staining patterns of CD3 and CD8 T lymphocytes in the pulmonary perivascular areas are demonstrated in Figures 2d, 2e, and 2f.

Megakaryocytes are normally found in the lung and partially act in platelet homeostasis [28]. Megakaryocytes and their platelet production have attracted attention in recent decades [28]. Many studies showed that these platelets are not characteristic but play role in acute lung injury, which may be related to the activation of the coagulation cascade [28-30]. However, some studies indicate that their number also increases in the lung in diffuse alveolar damage, shock, sepsis, and burns [28-32]. The SARS-CoV-2 infection causes the release of megakaryocytes which may enter the capillaries and embolize to

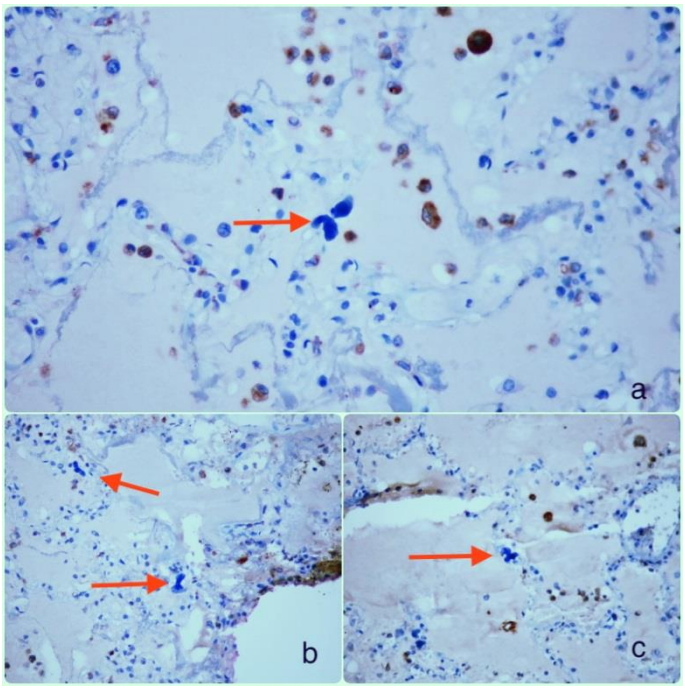
the other organs [33]. If the peripheral demand of platelet increases, the number of megakaryocytes rises in the bone marrow. It was thought that SARS-CoV-2 could drastically modify the human coagulation system as well as trigger fibrin thrombus formation in the distal pulmonary arteries by increasing megakaryocytes [33]. Interstitial megakaryocytes are common, especially in patients with macroscopic pulmonary embolism [26, 34]. These findings show us that COVID-19 infection, together with severe acute lung damage, affects the coagulation system, causing it to turn into a fatal infection by promoting coagulation. Borzcuk et al. [15] reported the presence of platelets and/or fibrin microthrombi in 84% of SARS-CoV-2 cases. In our study, fibrin thrombus was detected in 49 (52.1%) cases and the presence of megakaryocytes, in 63 (67%) cases. Activation of the coagulation cascade and triggering of fibrin thrombus formation in COVID-19, which benefits from preventive anticoagulant and antiplatelet treatment, may play role in mortality. SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb recognizes endogenous levels of total SARS-CoV-2 nucleocapsid protein and does not react with nucleocapsid proteins from SARS and MERS coronaviruses. In our study, SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb was used in some cases, and cytoplasmic and membranous staining was observed in the infected cells (Figure 3a, 3b, 3c, 3d, 3e, 3f). Megakaryocytes were not stained with SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb (Figure 3f), CD68 (Figure 4a, 4b, 4c), Pancytokeratin (Figure 5a, 5b, 5c), and TTF-1 (Figure 5d, 5e, 5f). CD68, Pancytokeratin (PanCK), and Thyroid Transcription Factor-1 (TTF-1) immunohistochemical staining were used for differentiation of histiocytes and epithelial cells from megakaryocytes, considering that it may be of pneumocyte origin due to the viral cytopathic effect.

Figure 3: SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb immunoreactivity



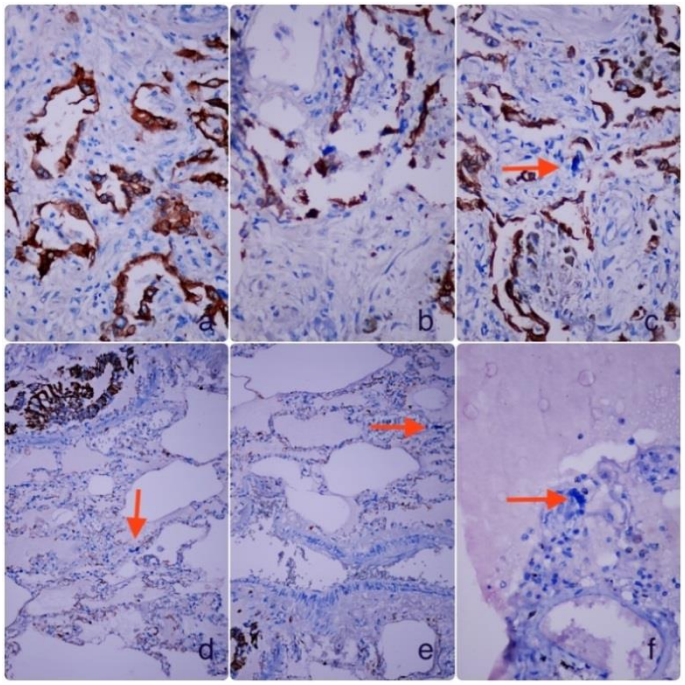
a,c,d,e: Antibodies against SARS-CoV-2 nucleocapsid protein were used to detect the infected cells. x400. b: Antibodies against SARS-CoV-2 nucleocapsid protein were used to detect the infected cells. x200. f: No staining was observed in megakaryocytes (Arrow) x 400.

Figure 4: CD68 immunohistochemical staining



a,b,c: CD68 staining was not observed in the megakaryocytes (arrows)x 400.

Figure 5: A: Pancytokeratin (PanCK) (a,b,c) and TTF-1 (d,e,f) immunohistochemical staining



a,b: Pancytokeratin (PanCK) immunoreactivity of Type II pneumocytes x400. c: Pancytokeratin (PanCK) immunoreactivity was not detected in the megakaryocytes (arrow)x400. d,e,f: TTF-1 immunoreactivity was not detected in the megakaryocytes (arrows)x400.)

Das et al. [35] stated that the presence of multinuclear syncytial cells and smudge cells in the light microscopic examination may give some clues to the viral etiologic agent, but without characteristic intranuclear or intracytoplasmic inclusions, the etiologic agent could not be assessed clearly. In addition, various histopathological findings in viral infections were evaluated and interstitial inflammation was reported as the most common among these morphologic findings. Although interstitial inflammation has no directive and predictive effect on the etiology of the viral agents, it is the most common finding in the presence of viral infection [35]. In our study, no intranuclear or intracytoplasmic inclusions were detected in 94 SARS-CoV-2 positive cases by light microscopy, in line with the current studies [35-38]. In addition, SARS-CoV-2-specific

histopathological, viral cytopathic changes, which differ from other viral infections that cause acute lung injury, were not observed in our study.

Limitations

Although the histopathological findings of the lungs were evaluated in a large series in this study, that we could not reach clinical information, such as concomitant diseases and the duration of SARS-CoV-2 positivity, limited our comprehensive evaluation. The lack of information on the period between the onset of the disease and death may also be considered a study restriction. Since our study was conducted in forensic autopsies, although all our cases had positive rt-PCR assay results, the causes of death in some cases were due to forensic causes. In addition, due to the delays in the supply of immunohistochemical markers during the pandemic, SARS-CoV-2 Nucleocapsid Protein (E8R1L) Mouse mAB, Pancytokeratin (PanCK), TTF-1, IL-6, CD68, CD3, and CD8 immunohistochemical assays could be performed in a limited number of cases. Lastly, an electron microscopic evaluation could not be performed for further investigation of the ultrastructural level.

Conclusion

Our study is important for the evaluation and verification of histopathological findings in the lungs of cases with positive SARS-CoV-2 rt-PCR tests. Our findings reveal that DAD findings are the predominant pathology in the lung in autopsies; however, in some cases, only minor findings were seen in the lung without associated DAD findings. Perivascular lymphocytic infiltrates in the lung contained more CD3 positive T lymphocytes than CD8 positive T lymphocytes, which correlates with the hypothesis of direct destruction of CD8 (+) T lymphocytes or through impairment of cellular immunity by SARS-CoV-2 related mediators.

Immunohistochemical staining with IL-6 in the lung in COVID-19 cases supports the role of IL-6 in cytokine storm and may require further investigation.

The number of megakaryocytes is increased in the lungs, which correlates with reports which mention that SARS-CoV-2 infection results in megakaryocyte release and promotes coagulation and vascular thrombosis, as in most other causes of acute lung injury. Also, no intranuclear or intracytoplasmic inclusions were seen in any SARS-CoV-2 rt-PCR positive cases by light microscopy.

Autopsies are highly important in understanding the pathogenesis of SARS-CoV-2 infection and contribute to the development of diagnostic and therapeutic modalities for infected patients. We thought that the pulmonary findings we describe will contribute to helping better perceive the SARS-CoV-2 pathogenesis and could be of guidance to the development of new treatment strategies and modalities.

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References

1. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. Histopathology. 2020;77(2):198-209. doi: 10.1111/his.14134.
2. Martines RB, Ritter JM, Matkovic E, Gary J, Bollweg BC, Bullock H, et al. Pathology and Pathogenesis of SARS-CoV-2 Associated with Fatal Coronavirus Disease, United States. Emerg Infect Dis. 2020;26(9):2005-15. doi: 10.3201/eid2609.202095.
3. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3. doi: 10.1038/s41586-020-2012-7.
4. Satturwar S, Fowkes M, Farver C, Wilson AM, Eccher A, Girolami I, et al. Postmortem Findings Associated With SARS-CoV-2: Systematic Review and Meta-analysis. Am J Surg Pathol. 2021;45(5):587-603. doi: 10.1097/PAS.0000000000001650.
5. Worldometers (2021). COVID-19 coronavirus pandemic [Online]. Website: www.worldometers.info/coronavirus/ [Accessed 17 08 2021].
6. Bryce C, Grimes Z, Pujadas E, Ahuja S, Beasley MB, Albrecht R, et al. Pathophysiology of SARS-CoV-2: the Mount Sinai COVID-19 autopsy experience. Mod Pathol. 2021;34:1456–67. doi:10.1038/s41379-021-00793-y.
7. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Crit Care Med. 2005;33(1):1-6;discussion 230-2. doi: 10.1097/01.ccm.0000149854.61192.dc.
8. Kim GW, Lee NR, Pi RH, Lim YS, Lee YM, Lee JM, et al. IL-6 inhibitors for treatment of rheumatoid arthritis: past, present, and future. Arch Pharm Res. 2015;38(5): 575-84. doi: 10.1007/s12272-015-0569-8.
9. Stapleton RD, Suratt BT, Neff MJ, Wurfel MM, Ware LB, Ruzinski JT, et al. Bronchoalveolar fluid and plasma inflammatory biomarkers in contemporary ARDS patients. Biomarkers. 2019;24(4):352–59. doi:10.1080/1354750X.2019.1581840.
10. Chan PK, Chan DP, To KF, Yu MY, Cheung JL, Cheng AF. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. J Clin Pathol. 2001;54(5):401-3. doi: 10.1136/jcp.54.5.401.
11. Arslan MN, Büyük Y, Ziyade N, Elgörmüş N, Şirin G, Çoban İ, et al. COVID-19 autopsies of Istanbul. Ir J Med Sci. 2021;23:1–13. doi: 10.1007/s11845-021-02602-6.
12. Bugra A, Das T, Arslan MN, Ziyade N, Buyuk Y. Postmortem pathological changes in extrapulmonary organs in SARS-CoV-2 rt-PCR-positive cases: a single-center experience. Ir J Med Sci. 2021;7:1–11. doi: 10.1007/s11845-021-02638-8.
13. Vasquez-Bonilla WO, Orozco R, Argueta V, Sierra M, Zambrano LI, Muñoz-Lara F, et al. A review of the main histopathological findings in coronavirus disease 2019. Hum Pathol. 2020;105:74–83. doi: 10.1016/j.humpath.2020.07.023.
14. Gencer S, Lacy M, Atzler D, an der Vorst EPC, Döring Y, Weber C. Immunoinflammatory, Thrombohaemostatic, and Cardiovascular Mechanisms in COVID-19. Thromb Haemost. 2020;120(12):1629-41. doi: 10.1055/s-0040-1718735.
15. Borczuk AC, Salvatore SP, Seshan SV, Patel SS, Bussel JB, Mostyka M. COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City. Mod Pathol. 2020;33(11):2156-68. doi: 10.1038/s41379-020-00661-1.
16. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383:120–8. doi: 10.1056/NEJMoa2015432.
17. Cheung OY, Graziano P, Leslie KO. Acute Lung Injury. In: Leslie KO, Wick MR, eds. Practical Pulmonary Pathology: A Diagnostic Approach, 3rd edn. Philadelphia, PA, USA: Elsevier; 2018. pp 125-46.
18. Franks TJ, Chong PY, Chui P, Galvin JR, Lourens RM, Reid AH, et al. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. Hum Pathol. 2003;34(8):743-8. doi: 10.1016/s0046-8177(03)00367-8.
19. Brune K, Frank J, Schwingshackl A, Finigan J, Sidhaye VK. Pulmonary epithelial barrier function: some new players and mechanisms. Am J Physiol Lung Cell Mol Physiol. 2015;08(8):L731-45. doi: 10.1152/ajplung.00309.2014.
20. Mehta P, McAuley DF, Brown M, Finigan J, Sidhaye VK, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-34. doi: 10.1016/S0140-6736(20)30628-0.
21. Calabrese F, Pezzuto F, Fortarezza F, Hofman P, Kern I, Panizo A, et al. Pulmonary pathology and COVID-19: lessons from autopsy. The experience of European Pulmonary Pathologists. Virchows Arch. 2020;477(3):359-72. doi: 10.1007/s00428-020-02886-6.
22. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg Microbes Infect. 2020;9(1):1123-30. doi: 10.1080/22221751.2020.1770129.
23. Gubernatorova EO, Gorshkova EA, Polinova AI, Drutskaya MS. IL-6: Relevance for immunopathology of SARS-CoV-2. Cytokine Growth Factor Rev. 2020;53:13-24. doi: 10.1016/j.cytogfr.2020.05.009.
24. Frisoni P, Neri M, D'Errico S, Alfieri L, Bonuccelli D, Cingolani M, et al. Cytokine storm and histopathological findings in 60 cases of COVID-19-related death: from viral load research to immunohistochemical quantification of major players IL-1β, IL-6, IL-15 and TNF-α. Forensic Sci Med Pathol. 2021;31:1–15. doi: 10.1007/s12024-021-00414-9.
25. Mor F, Cohen IR. IL-2 rescues antigen-specific T cells from radiation or dexamethasone-induced apoptosis. Correlation with induction of Bcl-2. J Immunol. 1996;156(2):515-22.
26. Rapkiewicz AV, Mai X, Carsons SE, Pittaluga S, Kleiner DE, Berger JS, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: A case series. EClinicalMedicine. 2020;24:100434. doi: 10.1016/j.eclinm.2020.100434.
27. Huang JL, Huang J, Duan ZH, Wei J, Min J, Luo XH, et al. Th2 predominance and CD8+ memory T cell depletion in patients with severe acute respiratory syndrome. Microbes Infect. 2005;7(3):427-36. doi: 10.1016/j.micinf.2004.11.017.
28. Valdivia-Mazeyra MF, Salas C, Nieves-Alonso JM, Martín-Fragueiro L, Bárcena C, Muñoz-Hernández P, et al. Increased number of pulmonary megakaryocytes in COVID-19 patients with diffuse alveolar damage: an autopsy study with clinical correlation and review of the literature. Virchows Arch. 2021;478(3):487-96. doi: 10.1007/s00428-020-02926-1.
29. Washington AV, Esponda O, Gibson A. Platelet biology of the rapidly failing lung. Br J Haematol. 2020;188(5):641-51. doi: 10.1111/bjh.16315.
30. Yadav H, Kor DJ. Platelets in the pathogenesis of acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol. 2015;309(9):L915-23. doi: 10.1152/ajplung.00266.2015.
31. Mandal RV, Mark EJ, Kradin RL. Megakaryocytes and platelet homeostasis in diffuse alveolar damage. Exp Mol Pathol. 2007;83(3):327-31. doi: 10.1016/j.yexmp.2007.08.005.
32. Wells S, Sissons M, Hasleton PS. Quantitation of pulmonary megakaryocytes and fibrin thrombi in patients dying from burns. Histopathology. 1984;8(3): 517-27. doi: 10.1111/j.1365-2559.1984.tb02361.x.
33. Tombolini A, Scendoni R. SARS-CoV-2-related deaths in routine forensic autopsy practice: histopathological patterns. Int J Legal Med. 2020;134(6): 2205-8. doi: 10.1007/s00414-020-02354-5.
34. Jackson NR, Zeigler K, Torrez M, Makino Y, Adolphi NL, Lathrop S, et al. New Mexico's COVID-19 Experience. Am J Forensic Med Pathol. 2020;42(1):1-8. doi: 10.1097/PAF.0000000000000664.
35. Daş T, Sargan A, Yağmur G, Yildirim M, Topal CS, Gürler AS, et al. Viral Pneumonias in Forensic Autopsies: Evaluation and Classification of Histopathologic Changes With Microbiologic Correlation. Am J Forensic Med Pathol. 2016;37(4):255-63. doi: 10.1097/PAF.0000000000000261.

36. Lacy JM, Brooks EG, Akers J, Armstrong D, Decker L, Gonzalez A, et al. COVID-19: Postmortem Diagnostic and Biosafety Considerations. Am J Forensic Med Pathol. 2020;41(3):143-51. doi: 10.1097/PAF.0000000000000567.
37. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8(4):420-2. doi: 10.1016/S2213-2600(20)30076-X.
38. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer. J Thorac Oncol. 2020;15(5):700-4. doi: 10.1016/j.jtho.2020.02.010.

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Impact of knowledge, attitude and anxiety levels about COVID-19 on the quality of life in pregnant women

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Ethics Committee Approval
This study was approved by the local ethics committee of Yeni Yüzyıl University Private Gaziosmanpaşa Hospital (Date: 31.03.2021 Number: 08.04.2021).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Depression and anxiety during pregnancy can lead to pregnancy-related maternal or fetal complications if they are severe or persistent. Therefore, it is important to identify pregnant women who are significantly affected by the COVID-19 pandemic, take the necessary measures or implement rehabilitation programs. This study aimed to determine the feelings, thoughts, attitudes, beliefs, concerns and levels of general depression and anxiety in pregnant women caused by the COVID-19 pandemic and examine the factors that cause mental health disorders.

Methods: A total of 85 pregnant women who were followed up in the Gynecology and Obstetrics clinics of our hospital and who were never infected with COVID-19 were included in this cross-sectional study. Pregnant women were asked to fill out a questionnaire about the COVID-19 pandemic, and the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and the Short Form 12-Item Health Survey (SF-12) questionnaires.

Results: According to the BDI, 9.4% of the pregnant women had moderate depression, while none had severe depression. According to BAI, 12.9% had moderate anxiety, and 4.7% had severe anxiety. BDI (P=0.001) and BAI (P=0.022) scores were significantly higher, and SF-12 physical role (P=0.004) and SF-12 social functioning (P=0.02) scores were significantly lower in those with a high level of anxiety for their unborn baby.

Conclusion: Our findings show that depression and anxiety levels in pregnant women have been high during the COVID-19 pandemic, and that pregnant women are concerned about their own and their babies' health. Anxiety and depression levels affect the attitude and belief of pregnant women, and all these mental health deteriorations decrease their quality of life.

Keywords: Pregnant, COVID-19, Beck Depression Inventory, Beck Anxiety Inventory, Short Form-12 Health Scale

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Introduction

In addition to the clinical picture, clinical course, prognosis and complications caused by the Coronavirus Disease-2019 (COVID-19), the effects of the pandemic process itself are of great importance in terms of socioeconomic and public health. News about the disease precautions and restrictions regarding the pandemic, difficulties in accessing health facilities, uncertainties and doubts about vaccines affect the mental health of people negatively [1, 2].

Numerous factors such as physiological, hormonal and social changes that occur during pregnancy affect the psychology of a pregnant woman in many ways. The events and restrictions experienced during the COVID-19 pandemic may negatively affect the psychology of pregnant women who have already become more psychologically sensitive [3, 4]. Studies have shown that both the concerns of pregnant women about COVID-19 and their general depression and anxiety levels can significantly change during the pandemic process [5-7].

Depression and anxiety during pregnancy can lead to pregnancy-related, maternal or fetal complications if they are severe or persistent [8]. Therefore, it is important to identify those who are significantly affected by the COVID-19 pandemic, take the necessary measures or implement rehabilitation programs [3, 5, 8]. This study aimed to determine the feelings, thoughts, attitudes, beliefs, concerns and the levels of general depression and anxiety in pregnant women caused by the COVID-19 pandemic and examine the factors that cause mental health disorders.

Materials and methods

This cross-sectional study was conducted in a private university hospital between January-March 2021.

Participants

A total of 85 pregnant women who were followed up in the Gynecology and Obstetrics clinics of our hospital, who were never infected with COVID-19 were included in the study with randomization. Those who did not want to participate, those with a history of psychiatric disease, and those with additional serious pathologies were excluded from the study, considering that they might cause bias in the analysis. Based on our power analysis, a minimum of 84 subjects provided a power of 0.95.

Questionnaires

Four separate questionnaires were filled out by the participants:

COVID-19 pandemic questionnaire

This questionnaire, prepared by us, consists of 24 questions that can be answered with "yes-no" or "no/never-a little/sometimes-a lot", and inquires the changes in attitudes, emotions, concerns and habits during the COVID-19 pandemic, and the participants' thoughts and beliefs about COVID-19. Responses were not scored, each question was evaluated and analyzed separately.

Beck Depression Inventory (BDI)

This scale is a questionnaire consisting of 21 questions whose responses are gradually scored as "none" (0 points), "some" (1 point), "moderate" (2 points) and "severe" (3 points). The total score ranges between 0-63 and accordingly, the levels of depression are determined as follows: 0–9: Minimal, 10-18: Mild, 19-29: Moderate, and 30-63: Severe [9, 10].

Beck Anxiety Inventory (BAI)

This scale is a questionnaire consisting of 21 questions whose responses are gradually scored as "none" (0 points), "some" (1 point), "moderate" (2 points) and "severe" (3 points). The total score ranges between 0-63 and accordingly, the anxiety levels are determined as follows: 0–7: Minimal, 8-15: Mild, 16-25: Moderate, and 26-63: High (severe) [11, 12].

Short Form 12-Item Health Survey (SF-12)

This scale is a questionnaire consisting of 12 questions measuring the quality of life of the individual in the last four weeks. The questionnaire consists of general health, physical functionality, physical role, emotional role, bodily pain, mental health and social functionality subcomponents. Each subcomponent is interpreted by evaluating the graded responses given within itself by calculating the percentage of the possible total score [13, 14].

Statistical analysis

All statistical analyses in the study were performed with the SPSS 25.0 software (IBM SPSS, Chicago, IL, USA). Descriptive data were given as numbers and percentages. In terms of categorical variables, comparisons between the groups were made with the Pearson's Chi Square test and the Fisher's Exact Test. Whether continuous variables are suitable for normal distribution was confirmed by the Kolmogorov-Smirnov Test. The differences between the groups in terms of continuous variables were analyzed using Mann-Whitney U test. The results were evaluated within a 95% confidence interval, and $P<0.05$ values were considered significant. Bonferroni correction was performed where appropriate.

Ethic

This study was approved by the local ethics committee of Yeni Yüzyıl University Private Gaziosmanpaşa Hospital (Date: 31.03.2021 Number: 08.04.2021).

Results

Among all, 67.1% of the pregnant women were 30 years old and younger, 88.2% were in their first pregnancy. According to the BDI, 55.3% of the pregnant women had minimal depression, 35.3% had mild depression, 9.4% had moderate depression, and none had severe depression. According to BAI, 52.9% of pregnant women had minimal anxiety, 29.4% had mild anxiety, 12.9% had moderate anxiety, and 4.7% had severe anxiety (Table 1).

The mean age of the pregnant women was 29.1 (5.0) years (median: 29; min-max: 18-42). The mean week of gestation was 22.6 (10.8) weeks (median: 24; min-max: 5-39) (Table 2).

The median BDI score was 9 (min-max: 0-25), and the median BAI score was 7 (min-max: 0-44). The median values of SF-12 subcomponents varied between 40-75% (Table 2).

In the COVID-19 questionnaire, the questions that pregnant women most frequently answered as "a lot" were wearing a mask outside (95.3%), washing hands frequently (91.8%) and paying attention to social distance (83.5%). This questionnaire revealed that general anxiety increased by 97.6%, anxiety about the baby increased by 95.3%, anxiety about the

pregnancy increased by 82.4%, and anxiety about being admitted to a hospital increased by 90.6%. A total of 10.6% of the pregnant women did not believe that the pandemic would end, and 22.4% did not believe in the news about coronavirus vaccines (Table 3).

Table 1: General distributions

	n	%
Age groups (year)		
≤30	57	67.1
>30	28	32.9
Age groups (year)		
≤35	76	89.4
>35	9	10.6
Parity		
1	75	88.2
≥2	10	11.8
Trimester		
1st trimester	26	30.6
2nd trimester	28	32.9
3rd trimester	31	36.5
Beck Depression Inventory level		
Minimal depression	47	55.3
Mild depression	30	35.3
Moderate depression	8	9.4
Beck Anxiety Inventory level		
Minimal anxiety	45	52.9
Mild anxiety	25	29.4
Moderate anxiety	11	12.9
Severe anxiety	4	4.7

Table 2: The mean and median values of some variables

	Mean	SD	Median	Minimum	Maximum
Age (years)	29.1	5.0	29.0	18	42
Parity	1.5	0.9	1.0	1	4
Gestational week	22.6	10.8	24.0	5	39
Beck Depression Inventory score	9.5	5.7	9.0	0	25
Beck Anxiety Inventory score	9.0	8.0	7.0	0	44
SF-12 general health score (%)	52.7	18.0	60.0	20.0	100
SF-12 physical functioning score (%)	72.6	22.4	75.0	50.0	100
SF-12 role emotional score (%)	80.3	21.2	75.0	50.0	100
SF-12 bodily pain score (%)	42.8	19.8	40.0	20.0	100
SF-12 mental health score (%)	57.9	15.4	61.1	16.7	88.9
SF-12 social functioning score (%)	70.4	24.6	60.0	20.0	100

SD: Standard Deviation

Table 3: Responses to the questions of the COVID-19 questionnaire

No		No		Yes			
		n	%	n	%		
1	Are there people in your close environment diagnosed with COVID-19?	60	70.6	25	29.4		
2	Have you admitted to a hospital with COVID-19 - like symptoms?	74	87.1	11	12.9		
3	Have you been laid off due to COVID-19?	82	96.5	3	3.5		
		No / Never		A little / sometimes		Much	
		n	%	n	%	n	%
4	Has there been any change in socializing with your friends during the pandemic?	9	10.6	37	43.5	39	45.9
5	Have you quarantined yourself?	30	35.3	22	25.9	33	38.8
6	Are you paying attention to social distance?	1	1.2	13	15.3	71	83.5
7	Do you wash your hands more often?	0	0.0	7	8.2	78	91.8
8	Are you using hand sanitizer?	2	2.4	25	29.4	58	68.2
9	Do you always wear a mask outside?	0	0.0	4	4.7	81	95.3
10	Has there been any change in your smoking?	67	78.8	5	5.9	13	15.3
11	Have you received extra vitamin supplements for the epidemic?	54	63.6	24	28.2	7	8.2
12	Have you stocked up food?	40	47.1	39	45.9	6	7.1
13	Did your general anxiety increase in the pandemic?	2	2.4	54	63.5	29	34.1
14	Are you worried about going to a hospital?	8	9.4	53	62.4	24	28.2
15	Have your anxiety about yourself increased in the pandemic?	15	17.6	53	62.4	17	20.0
16	Have you worried about your unborn baby?	4	4.7	34	40.0	47	55.3
17	Do you have any concerns about your current children?	8	27.6	7	24.1	14	48.3
18	Do you have concerns about your relatives over the age of 65?	6	7.1	32	37.6	47	55.3
19	Are you considering breastfeeding without a mask?	40	47.1	35	41.2	10	11.8
20	Do you have any concerns about your baby after birth?	9	10.6	38	44.7	38	44.7
21	Does COVID-19 cause congenital anomalies?	38	44.7	39	45.9	8	9.4
22	Does COVID-19 cause preterm birth?	41	48.2	40	47.1	4	4.7
23	Do you believe this epidemic will end?	9	10.6	61	71.8	15	17.6
24	Do you believe the news about the coronavirus vaccine?	19	22.4	60	70.6	6	7.1

The minimal depression rate, according to BDI, was significantly higher in those who had no relatives who contracted the COVID-19 disease (P=0.032). The moderate depression rate was significantly higher in those who wore a mask outside with low/moderate frequency (P=0.012). The rate of receiving extra vitamin support was significantly lower in pregnant women in the third trimester (P=0.009). The rate of those who were worried about their elderly relatives was significantly lower among those with multiparity (P=0.008). The rate of concern about the baby after birth was significantly lower in those over the age of 30 years (P=0.026). SF-12 emotional score was significantly higher in those whose relatives did not contract COVID-19 (P=0.009). BAI score was significantly higher among those who quarantined themselves (P=0.049). SF-12 mental health score was significantly higher in patients who washed their hands more frequently during the pandemic (P=0.04). SF-12 general health score was significantly lower among those who always wore masks outside (P=0.049). BDI (P=0.001) and BAI (P=0.022) scores were significantly higher, and SF-12 physical role (P=0.004) and SF-12 social functioning (P=0.02) scores were significantly lower among those with heightened anxiety about the baby after birth. The BDI score was significantly lower in those who had concerns about their current child (P=0.048). The BDI score was significantly higher in those who did not find the measures of the Ministry of Health sufficient (P=0.002), and the SF-12 general health score was significantly lower in those who found the measures sufficient (P=0.034).

SF-12 general health (P=0.006) and SF-12 bodily pain scores (P=0.011) were significantly higher, and SF-12 emotional role (P=0.014), SF-12 mental health (P=0.003) and SF-12 social functioning (P=0.01) scores were significantly lower in those with moderate or high-level depression according to the BDI score. SF-12 general health (P=0.028) and SF-12 bodily pain scores (P=0.004) were significantly higher, and SF-12 physical role (P=0.015), SF-12 emotional role (P=0.039), SF-12 mental health (P=0.004), and SF-12 social functioning (P=0.011) scores were significantly lower in those with moderate or high-level anxiety according to their BAI scores (Table 4).

SF-12 social functionality score was significantly lower in the second trimester (P=0.043). Apart from that, age groups, number of pregnancies, and trimester groups were similar in terms of the median BDI, BAI, and SF-12 subcomponents scores (P>0.05 for each) (Table 4).

The rate of those with moderate/high level anxiety according to the BAI score was significantly higher among the pregnant women aged 30 years and younger (P=0.017). The rate of pregnant women in the third trimester with moderate depression was significantly lower, according to the BDI score (P=0.044) (Table 5).

Table 4: Comparisons between BDI levels, BAI levels, age, parity and trimester groups in terms of median BDI, BAI and SF-12 scores

	BDI score	BAI score	SF-12 General health	SF-12 Physical functioning score	SF-12 Role physical score	SF-12 Role emotional score	SF-12 Bodily pain score	SF-12 Mental health score	SF-Social functioning score
BDI levels	-	<0.001*	0.006*	NS	NS	0.014**	0.011*	0.003**	0.01**
BAI levels	0.001*	-	0.028*	NS	0.015**	0.039**	0.004*	0.004**	0.011**
Age (≤30 years vs. >30)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Parity (1 vs. >1)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Trimester (1 vs. 2 vs. 3)	NS	NS	NS	NS	NS	NS	NS	NS	0.043***

* Significantly high in the moderate/severe group, ** Significantly low in the moderate/severe group, *** Significantly lower in the 2nd trimester. BDI: Beck Depression Inventory, BAI: Beck Anxiety Inventory, SF-12: Short Form 12-Item Health Survey, NS: Not significant.

Table 5: Comparisons between age, parity and trimester groups in terms of BDI and BAI level distributions

	BDI levels (Mild vs. moderate vs. severe)	BDI levels (Mild/moderate vs. severe)	BAI levels (Minimal vs. mild vs. moderate vs. severe)	BAI levels (Minimal/mild vs. moderate/severe)
Age (≤30 years vs. >30)	NS	NS	NS	0.017*
Parity (1 vs. >1)	NS	NS	NS	NS
Trimester (1 vs. 2 vs. 3)	0.044**	NS	NS	NS

*At the age of 30 years and under, the ratio of moderate/severe is significantly high, ** The moderate rate is significantly low in the 3rd trimester. BDI: Beck Depression Inventory, BAI: Beck Anxiety Inventory, NS: Not significant

Discussion

The COVID-19 pandemic causes fear and anxiety about transmission, severe clinical picture and poor prognosis in individuals who have not yet contracted the disease. In addition, social isolation and various related measures negatively affect people's mental health. Wearing masks and hygiene measures also reduce the quality of life of people [1, 2]. These adverse situations may affect the psychological status of pregnant women who are going through more physiological and hormonal changes compared to the normal population [3, 4]. It has been reported that stress and anxiety can lead to complications in pregnant women such as preeclampsia, depression, nausea, preterm birth, low-birth weighted baby and low APGAR score [8]. Therefore, for the pregnancies to progress more healthily during the pandemic, it is necessary to monitor the psychological status of pregnant women more closely, diagnose depression or anxiety, and take the necessary measures against possible negative effects [3, 5, 8].

It has been shown that depression levels of the pregnant women increased during the COVID-19 pandemic compared to before [5-7, 15]. Durankus et al. [16] reported that the pandemic had a significant effect on the psychology of pregnant women. Farrell et al. [17] found the depression rate in pregnant women to be 39% during the pandemic process. In our study, 55.3% of the pregnant women had minimal depression, 35.3% had mild depression, and 9.4% had moderate depression, while none had severe depression. The median BDI score was 9 (14.3%; according to the possible maximum score of 63), and the maximum BDI score was 25 (39.7% according to the possible maximum score). These findings show that, in general, depression levels in pregnant women are not very high during the pandemic process, but the presence of depression is significant.

The anxiety levels of pregnant women also increased during the COVID-19 pandemic process compared to before [4-7, 15]. Dagklis et al. [18] reported a high/very high anxiety rate of 17% during the pandemic. Farrell et al. [17] found the rate of anxiety in pregnant women to be 34% during the pandemic process. Salehi et al. [19] found that general mental health is associated with fear and anxiety levels against COVID-19. In the present study, 52.9% of pregnant women had minimal anxiety, 29.4% had mild anxiety, 12.9% had moderate anxiety, and 4.7% had severe anxiety. The median BAI score was 7 (11.1% according to the possible maximum score of 63), and the maximum BAI score was 44 (69.8% according to the possible maximum score). These findings show that anxiety is at significant rates during the pandemic in pregnant women, the level of anxiety in pregnant women is not high in general, the rate anxiety in pregnant women varies greatly, and that the rate of pregnant women with moderate/high level anxiety is not very low.

The level of anxiety about themselves and their babies increased among the pregnant women during the pandemic [5-7, 20, 21]. Yassa et al. [22] reported the rate of pregnant women who think they are weaker and more susceptible to COVID-19 due to pregnancy as 52%. Lebel et al. [7] and Kajdy et al. [20] found that pregnant women were particularly worried about their and their babies' health, which increased their general anxiety levels. In the COVID-19 questionnaire conducted in the present study, the general anxiety increased by 97.6%, the anxiety about the baby increased by 95.3%, the anxiety about the pregnant women increased by 82.4%, and the anxiety about being admitted to a hospital increased by 90.6%. These findings show that the pandemic makes pregnant women more concerned about themselves and their babies.

Young pregnant women have higher levels of maternal anxiety [5]. In our study, the rate of concerns about the baby after birth was significantly lower in pregnant women over 30 years of age. Corbett et al. [23] showed that the rate of concern about themselves increased from 17% before the pandemic to 51% during the pandemic among pregnant women. These researchers also reported the rate of pregnant women with anxiety about their babies regarding the pandemic as 64%. Durankus et al. [16] reported that there is a relationship between social isolation and the psychology of pregnant women in the pandemic. In our study, BDI and BAI scores were significantly higher, and SF-12 physical role and SF-12 social functioning scores were significantly lower in those with high levels of anxiety about the baby after birth. These findings, on one side, show that pregnant women whose concerns about themselves and their babies have increased due to COVID-19 have higher levels of overall depression and anxiety. They may also indicate that the pregnant women with high overall depression and/or anxiety levels are becoming more sensitive to anxiety about themselves and their babies due to COVID-19.

In this study, minimal depression rate and SF-12 emotional score were significantly higher in those with COVID-19 in their relatives. These findings show that the overall depression level increases and the emotional quality of life significantly decreases in pregnant women with relatives who contracted the disease.

Among all our patients, 10.6% did not believe that the pandemic would end, and 22.4% did not believe in the news about the coronavirus vaccines. These findings show that the rate of hopelessness about the end of the pandemic and belief in vaccines are not low in pregnant women, and that these beliefs increase with overall depression and anxiety. Pregnant women with high overall depression and anxiety levels are more sensitive to hopelessness about the ending of the pandemic and the effectiveness of vaccines.

Regarding the COVID-19 questionnaire formed in this study, the questions that pregnant women most frequently answered as "a lot" were wearing a mask outside (95.3%), washing hands frequently (91.8%) and paying attention to social distance (83.5%). These findings show that pregnant apply personal measures on COVID-19 in a high rate. The moderate depression rate was significantly higher in those who wore a

mask outside with low/moderate frequency. SF-12 general health score was significantly lower in those who always wore masks outside. The tendency to wearing a mask decreases in pregnant women who have a high level of depression and increases in those with low levels of general health scores.

Corbett et al. [23] reported the rate of self-isolation as 35% among pregnant women during the pandemic. We found this rate to be 38.8%. In addition, we found that the BAI score was significantly higher in those that were quarantining themselves. These findings show that the level of anxiety against COVID-19 is higher in pregnant women with high levels of overall anxiety, and that they therefore protect themselves more. However, with the increase in age, this concern and self-protection decreases.

Yassa et al. [22] reported the additional vitamin receiving rate in late-term pregnant women as 31%, while we found this rate to be 36.4%. We also found that the rate of extra vitamin support was significantly low among pregnant women in their third trimester. This finding may show that the pregnant women in advanced weeks, in the early-term of the pandemic, do not feel the need to take extra vitamins because of COVID-19.

The BDI score was significantly low in those with concerns about their current children. The more depressed pregnant women are more concerned about their children contracting COVID-19.

The BDI score was significantly higher in those who did not find the measures of the Ministry of Health sufficient, and the SF-12 general health score was significantly lower in those who found the measures sufficient. The level of belief in the measures taken among the pregnant women who are more depressed is lower, but pregnant women with poor overall health status believe in these measures.

The anxiety levels of pregnant women are independent of age, parity and trimester during the pandemic [18]. However, some studies reported that primipara or younger women were more sensitive to anxiety [6, 24]. In this study, age groups, number of pregnancies, and trimester groups were similar in terms of the median BDI, BAI, and SF-12 subcomponents scores. The levels of overall depression, anxiety, and the quality of life in the last period are not directly related to the age, parity and trimester in pregnant women.

SF-12 general health and SF-12 bodily pain scores were significantly higher, and SF-12 emotional role, SF-12 mental health and SF-12 social functioning scores were significantly lower in our patients with moderate or high-level depression according to the BDI score. In addition, SF-12 general health and SF-12 bodily pain scores were significantly higher, and SF-12 physical role, SF-12 emotional role, SF-12 mental health, and SF-12 social functioning scores were significantly lower in those with moderate or high-level anxiety according to their BAI scores. The three general scale findings used in our study have overlapped each other, which show that our analyses made about the pregnant women are healthy.

Limitations

There are some limitations to our study. We could not compare our results with the pre-pandemic period, thus, could not analyze the changes in the levels of pandemic-related anxiety and depression.

Conclusion

Our findings show that depression and anxiety levels in pregnant women are high during the COVID-19 pandemic, and that pregnant women are concerned about health of themselves and their babies. Anxiety and depression levels affect the attitude and belief of pregnant women, and all these mental health deteriorations decrease the quality of life of pregnant women.

References

1. Stamu-O'Brien C, Carniciu S, Halvorsen E, Jafferany M. Psychological aspects of COVID-19. J Cosmet Dermatol. 2020;19:2169-73.
2. Rahman J, Muralidharan A, Quazi SJ, Saleem H, Khan S. Neurological and Psychological Effects of Coronavirus (COVID-19): An Overview of the Current Era Pandemic. Cureus. 2020;12:e8460.
3. Kotlar B, Gerson E, Petrillo S, Langer A, Tiemeier H. The impact of the COVID-19 pandemic on maternal and perinatal health: a scoping review. Reprod Health. 2021;18:10.
4. Hessami K, Romanelli C, Chiurazzi M, Cozzolino M. COVID-19 pandemic and maternal mental health: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2020 [Epub ahead of print] doi: 10.1080/14767058.2020.1843155.
5. Berthelot N, Lemieux R, Garon-Bissonnette J, Drouin-Maziade C, Martel É, Maziade M. Uptrend in distress and psychiatric symptomatology in pregnant women during the coronavirus disease 2019 pandemic. Acta Obstet Gynecol Scand. 2020;99:848-55.
6. Wu Y, Zhang C, Liu H, et al. Perinatal depressive and anxiety symptoms of pregnant women during the coronavirus disease 2019 outbreak in China. Am J Obstet Gynecol. 2020;223:240.e1-240.e9.
7. Lebel C, MacKinnon A, Bagshawe M, Tomfohr-Madsen L, Giesbrecht G. Elevated depression and anxiety symptoms among pregnant individuals during the COVID-19 pandemic. J Affect Disord. 2020;277:5-13.
8. Rashidi Fakari F, Simbar M. Coronavirus Pandemic and Worries during Pregnancy; a Letter to Editor. Arch Acad Emerg Med. 2020;8:e21.
9. Jackson-Koku G. Beck Depression Inventory. Occup Med (Lond). 2016;66:174-5.
10. [Wang YP, Gorenstein C. Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. Braz J Psychiatry. 2013;35:416-31.
11. Julian LJ. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). Arthritis Care Res (Hoboken) 2011;63:467-72.
12. Smarr KL, Keefer AL. Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). Arthritis Care Res (Hoboken). 2011;63:454-66.
13. Huo T, Guo Y, Shenkman E, Muller K. Assessing the reliability of the short form 12 (SF-12) health survey in adults with mental health conditions: a report from the wellness incentive and navigation (WIN) study. Health Qual Life Outcomes. 2018;16:34.
14. Busija L, Pausenberger E, Haines TP, Haymes S, Buchbinder R, Osborne RH. Adult measures of general health and health-related quality of life: Medical Outcomes Study Short Form 36-Item (SF-36) and Short Form 12-Item (SF-12) Health Surveys, Nottingham Health Profile (NHP), Sickness Impact Profile (SIP), Medical Outcomes Study Short Form 6D (SF-6D), Health Utilities Index Mark 3 (HUI3), Quality of Well-Being Scale (QWB), and Assessment of Quality of Life (AQoL). Arthritis Care Res (Hoboken). 2011;63:S383-412.
15. Ayaz R, Hocaoğlu M, Günay T, Yardımcı OD, Turgut A, Karateke A. Anxiety and depression symptoms in the same pregnant women before and during the COVID-19 pandemic. J Perinat Med. 2020;48:965-70.
16. Durankuş F, Aksu E. Effects of the COVID-19 pandemic on anxiety and depressive symptoms in pregnant women: a preliminary study. J Matern Fetal Neonatal Med. 2020 [Epub ahead of print] doi: 10.1080/14767058.2020.1763946.
17. Farrell T, Reagu S, Mohan S, Elmidany R, Qaddoura F, Ahmed EE, et al. The impact of the COVID-19 pandemic on the perinatal mental health of women. J Perinat Med. 2020;48:971-6.
18. Dagklis T, Tsakiridis I, Mamopoulos A, Athanasiadis A, Papazisis G. Anxiety During Pregnancy in the Era of the COVID-19 Pandemic. https://ssrn.com/abstract=3588542.doi: 10.2139/ssrn.3588542
19. Salehi L, Rahimzadeh M, Molaei E, Zaheri H, Esmaelzadeh-Saeieh S. The relationship among fear and anxiety of COVID-19, pregnancy experience, and mental health disorder in pregnant women: A structural equation model. Brain Behav. 2020;10:e01835.
20. Kajdy A, Feduniw S, Ajdacka U, et al. Risk factors for anxiety and depression among pregnant women during the COVID-19 pandemic: A web-based cross-sectional survey. Medicine (Baltimore). 2020;99:e21279.
21. Moyer CA, Compton SD, Kaselitz E, Muzik M. Pregnancy-related anxiety during COVID-19: a nationwide survey of 2740 pregnant women. Arch Womens Ment Health. 2020;23:757-65.
22. Yassa M, Birol P, Yirmibes C, et al. Near-term pregnant women's attitude toward, concern about and knowledge of the COVID-19 pandemic. J Matern Fetal Neonatal Med. 2020;33:3827-34.
23. Corbett GA, Milne SJ, Hehir MP, Lindow SW, O'connell MP. Health anxiety and behavioural changes of pregnant women during the COVID-19 pandemic. Eur J Obstet Gynecol Reprod Biol. 2020;249:96-7.
24. Effati-Daryani F, Zarei S, Mohammadi A, et al. Depression, stress, anxiety and their predictors in Iranian pregnant women during the outbreak of COVID-19. BMC Psychol 2020;8:99.

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Does muscle stiffness predict early-onset knee osteoarthritis?

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Required permissions to carry out the study were obtained from Hacettepe University Clinical Research Ethics Committee and T.C. Ministry of Health Medical Device and Medicine Agency with the decision number 15.03.2018, 2018 / 04-39 (KA-17112).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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Abstract

Background/Aim: The precise evaluation of the surrounding soft tissues and documenting it by clinical measurements are extremely important for detecting early-stage knee osteoarthritis (OA). Shear wave ultrasound elastography is the latest technology that directly measures the mechanical properties of a tissue, including muscle stiffness. This study was planned to investigate the relationship between Rectus Femoris (RF) and Biceps Femoris (BF) muscle stiffness and the relevant symptoms observed in OA.
Methods: Thirty-one patients including 21 females and 10 males between 45-67 years of age, diagnosed with early-stage knee OA and 10 healthy individuals were included in this prospective case-control study. Both RF and BF muscle stiffness were evaluated by shear wave elastography (SWE), and the knee range of motion was calculated by a goniometer. The pain intensity was measured by the Visual Analogue Scale (VAS), knee function, with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and functional performance, using the 6-minute walk test and the step-climbing test.
Results: There was a positive correlation between BF muscle stiffness and WOMAC stiffness (P=0.014, r=0.446). There was no difference in Rectus Femoris and Biceps Femoris muscle stiffness between the osteoarthritis patients and healthy individuals (P=0.11 and P=0.10, respectively).
Conclusion: It can be concluded that the symptom of stiffness seen in early-stage knee OA is caused by BF muscle spasm related to the flexor reflex.

Keywords: Biceps femoris, Muscle stiffness, Osteoarthritis, Rectus femoris, Shear wave elastography

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Introduction

Early knee osteoarthritis (OA), also known as "gonarthrosis," may cause loss of strength and flexibility in the Rectus Femoris (RF) and Biceps Femoris (BF) [1, 2], as well as an increase in stiffness in these muscles [3, 4]. This loss can be compensatory [5] to protect excessive loads in accompanying joint structures, including the joint cartilage [6]. Shear Wave Elastography (SWE) was previously used to assess the thickness and structural changes of joint cartilage [7, 8]. Shear wave elastography (SWE) is a non-invasive, reliable, and repeatable method that predicts elasticity by measuring the velocity of the speed of tissues [9]. Limited studies evaluated the skeletal muscles of the knee joint using this technology [10-12]. Skeletal muscles and connective tissues are important dynamic and static stabilizers of the knee joint that prevent injuries and progression of the disease, respectively [13]. Two studies revealed that the Quadriceps Femoris (QF) tendon plays a decisive role in the knee flexion range of motion and severity of OA. The stiffness of the QF tendon also affects gait in gonarthrosis, and studies show it plays a decisive role in the knee flexion range of motion and the severity of OA [11, 12]. Structural and functional changes of the QF tendon, but not the other connective tissues of the knee joint, were previously evaluated. None of these studies were conducted in early-stage gonarthrosis. We researched whether SWE could determine structural changes in the QF and the BF muscles in patients with early-stage gonarthrosis and whether there is a relationship between SWE values, function, pain, and knee range of motion, and early-stage knee OA.

Materials and methods

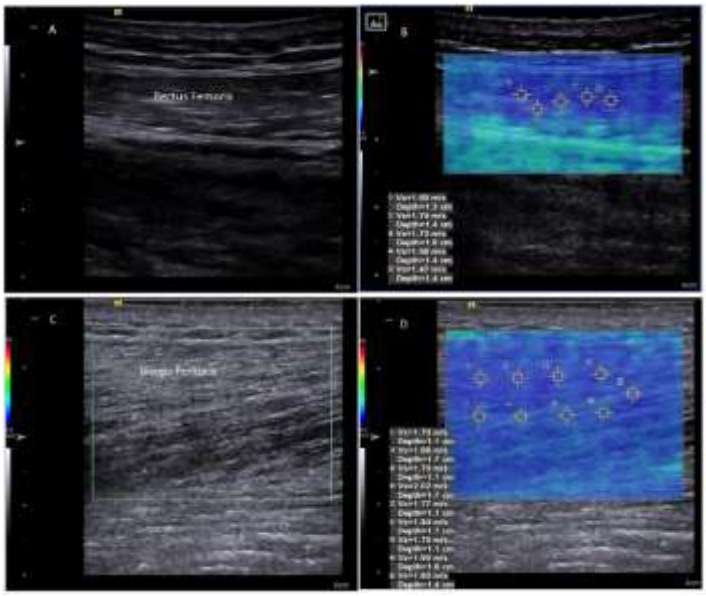
In this prospective case-control study, the G*Power statistical analysis software was used to calculate the minimum sample size for the Spearmen Correlation test, based on the effect size of a similar previous study [10]. The minimum total sample size was 30 individuals. The association of RF and BF muscle stiffness with pain levels, range of motion, knee function, and functional performance was examined using the appropriate statistical methods. In the second G*Power analysis, Quadriceps Femoris muscle stiffness was the main outcome. Bidirectional hypothesis design was used to achieve an 80% working power. A minimum of 20 adults was needed for each group, for a significant difference of 50% and 5% type 1 error. Initially, the study began with a total of 40 osteoarthritis patients and 20 healthy individuals; however, it was completed with 31 patients and 10 healthy individuals who were suitable for orthopedic examination, radiological and magnetic resonance evaluations. It was highly difficult to find healthy adults between 45-60 years of age without any orthopedic problems in the knee. Therefore, the study evaluated 31 (21 females, 10 males) osteoarthritis patients and 10 healthy individuals admitted to Beytepe Hospital, Hacettepe University. Patients were diagnosed with Stage 1–Stage 2 knee OA according to the Kellgren-Lawrence classification system based on prior examinations performed by the relevant physician, and the absence of any previous surgery or concomitant ligament or meniscus injuries. Individuals who voluntarily agreed to participate in the study and signed the informed consent form were included. Age (years), height (m), and weight (kg) of the patients were recorded, and Body Mass Index (BMI) (kg/m²) was calculated by dividing the total body weight by the square of height.

Pain intensity was evaluated with the Visual Analogue Scale (VAS) measured at rest and during activity, in which the patient marked the intensity of their pain on a 10 cm-long line containing marked indicators on either side ranging from '0', indicating no pain, to '10', indicating unbearable pain.

The knee flexion range of motion was evaluated with a goniometer in the prone position, which is a widely used, valid, and reliable method [14].

RF and BF Shear Wave velocity were evaluated by a single physician using SWE, an ultrasonography-based imaging method that shows the soft tissue changes in the early stages of the disease. SWE provides numerical information on the stiffness and flexibility of both the superficial and the deep soft tissues. Since the measurements are independent of the examiner and repeatable, SWE is considered a valid and reliable method for evaluating the stiffness of the skeletal muscles [15]. Shear wave elastography of RF and BF muscles was performed with the Acuson S3000 ultrasound system (Siemens Medical Solutions, Mountain View, CA) equipped with a 9L4 (4–9-MHz) linear array ultrasound transducer. The RF SWE measurement was performed in the supine position on the distal one-third of the anterior superior iliac spine and patella. The ultrasound transducer was placed longitudinally on the muscle fibers with the knee flexed at 30 degrees [15]. The biceps femoris long head SWE measurement was performed in the prone position on the distal one-fourth point between the posterior superior iliac spine and the fibular head. The ultrasound transducer was placed longitudinally on the muscle fibers with the knee flexed at 30-45 degrees [16]. The mean muscle stiffness was calculated from the radio frequency data using customized software, Virtual Touch Tissue Imaging Quantification, Siemens Medical Solutions. The measurement was repeated thrice for each muscle, the average of the three measurements was taken and recorded with a calculator in meters per second (m/s). Images of muscle stiffness measurements are depicted in Figure 1.

Figure 1: A: 2D sonogram of the rectus femoris muscle. B: SWE images of the rectus femoris muscle C: 2D sonogram of the biceps femoris muscle D: SWE images of the biceps femoris muscle. Squares marked on B and D are region of interest from where SWE measures were obtained



Knee function was evaluated by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire scores, the 6-minute walk test, and the step-climbing test. WOMAC questionnaire evaluates function in the knee and hip osteoarthritis under three headings of pain, stiffness, and function, and its validity and reliability studies on hip and knee OA were performed both in Turkey and abroad [17, 18].

Other assessment measures, such as the 6-minute walk test and the step-climbing test, are reliable and easily applicable tests for evaluating functional performance and mobility [19]. In the 6-minute walk test, the maximum distance the patient can travel in a fixed corridor for 6 min is recorded in meters. The step-climbing test involves asking the patient to climb up and down the 10-step ladder as quickly and safely as possible without holding on and the elapsed time is recorded in seconds [20].

Statistical analysis

Statistical analysis was conducted with the SPSS version 21 (Armonk, NY: IBM Corp). The G*Power statistical analysis software was used to calculate the sample size for the Spearmen Correlation test based on the effect size of a similar study [10], which revealed a minimum sample size of thirty individuals. The association between RF and BF muscle stiffness and pain levels, range of motion, knee function as well as functional performance was examined using the appropriate statistical methods. The normality of the data distribution was determined with the Shapiro-Wilk test and histogram evaluations. The association of the data variables with each other was evaluated with the Spearman rank correlation test. A *P*-value of less than 0.05 was considered significant.

Results

The mean age and the mean body mass index of 31 patients (21 females, 10 males) were 54.58 (1.27) years, and 30.60 (4.61) kg/m^2, respectively. The mean pain intensity was 5.77 (2.56) cm (2–10) at rest and 5.00 (4.29) cm (0–10) during activity. The mean knee flexion range of motion, step climbing test scores and 6-minute walk test scores of all individuals included in the study were 119.83 (15.02) (91–140) degrees, 12.31(4.96) (5.70–25.00) seconds, and 449.22 (95.28) (287–748) meters, respectively. The WOMAC pain, stiffness, function, and total scores were 7.31(3.01) (2–12) points, 2.34 (2.51) (0–7) points, 27.51 (10.35) (45–10) points, and 36.62 (12.84) (63–12) points, respectively. The mean RF and BF Shear wave velocity values of all patients were 1.84 (0.25) (1.43–2.42) m/s and 1.71 (0.32) (1.14–2.32) m/s, respectively (Table 1). There was no difference in muscle stiffness between the osteoarthritis patients and the control group (*P*>0.05) (Table 2). RF Shear Wave velocity and rest pain, activity pain, range of motion, step climbing test, 6-minute walk test, WOMAC pain, stiffness, function, and total scores in early-stage knee osteoarthritis were not related *(P*>0.05) (Table 3, 4).

Although BF muscle stiffness was not correlated with rest pain, activity pain, joint range of motion, step climbing test, the 6-minute walk test, or the WOMAC pain, function, and total scores (*P*>0.05), it was positively correlated with the WOMAC stiffness score (*P*=0.014, r=0.446) (Table 4).

Table 1: Detailed data on individuals with knee osteoarthritis

		Early Knee OA Mean (SD) (Min-Max)
Pain intensity level	Activity (cm)	5.77 (2.6) (2–10)
	Rest (cm)	5 (4.29) (0–10)
Knee Flexion Range of Motion (degree)		119.83 (15.02) (91–140)
Step Climbing Score (sec)		12.31 (4.96) (5.70–25.00)
6-minute walk score (m)		449.22 (95.28) (287–748)
WOMAC (score)	WOMAC Pain (points)	7.31 (3.01) (2–12)
	WOMAC stiffness (points)	2.34 (2.51) (0–7)
	WOMAC Function (points)	27.51 (10.35) (45–10)
	WOMAC Total (points)	36.62 (12.84) (63–12)
RF Muscle Shear wave velocity (m/sec)		1.84 (0.25) (1.43–2.42)
BF Muscle Sear wave velocity (m/sec)		1.71 (0.32) (1.14–2.32)

OA: Osteoarthritis, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, SD: Standard deviation, Min: Minimum, Max.: Maximum, sec: second, m: meter, RF: Rectus femoris, BF: Biceps femoris, cm: centimeter

Table 2: Comparison of muscle shear wave velocity between individuals with early-stage knee OA and healthy individuals

		RF Muscle Shear Wave velocity (m/sec)	BF Muscle Shear Wave velocity (m/sec)
Early Knee OA	Mean Rank	22.68	19.29
Healthy Group	Mean Rank	15.80	26.30
z		-1.57	-1.61
P-value		0.11	0.10

OA: Osteoarthritis, RF: Rectus Femoris, BF: Biceps Femoris, Mann-Whitney Test*, p<0.05 was considered statistically significant, m: meter, sec: second

Table 3: The relationship of muscle stiffness with pain, range of motion, and knee functional performance

		Pain in Rest (0–10 cm)	Pain at Activity (0–10 cm)	Range of Motion (degree)	Stair Climbing Test (sec)	6-Minute Walk Test (meter)
RF Muscle Shear wave velocity (m/sec)	*P*	0.090	0.488	0.134	0.442	0.641
	r	-0.309	-0.129	0.275	-0.143	-0.087
BF Muscle Shear wave velocity (m/sec)	*P*	0.213	0.327	0.339	0.761	0.134
	r	-0.230	-0.182	-0.178	-0.057	0.275

RF: Rectus Femoris, BF: Biceps Femoris, Spearman Correlation Test*, p<0.05 was considered statistically significant, m: meter, sec: second, cm: centimeter

Table 4: The relationship of muscle stiffness with knee function

		WOMAC Pain (Point)	WOMAC Stiffness (Point)	WOMAC Function (Point)	WOMAC Total (Point)
Mean (SD) (Min-Max)		7.31 (3.01) (2–12)	2.34 (2.51) (0–7)	27.51 (10.35) (45–10)	36.62 (12.84) (63–12)
Rectus Femoris Muscle Shear Wave velocity (m/sec)	*P*	0.139	0.101	0.178	0.093
	r	0.277	0.305	0.253	0.312
Biceps Femoris Muscle Shear Wave velocity (m/sec)	*P*	*0.79*	0.01*	0.82	0.88
	r	-0.05	0.44	-0.04	0.02

Spearman Correlation Test, *p<0.05 was considered statistically significant. SD: Standard deviation, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, m: meter, sec: second

Discussion

This is the first study to investigate and explore the importance of muscle stiffness in early-stage knee OA. Many studies emphasized the importance of RF muscle strength in patients with knee OA [21-24] and reported that RF muscle strength decreased as the severity of the disease increased [24]. In addition to decreased muscle strength, pain, loss of function, and range of motion are the most common problems encountered by the patients in knee OA [5, 24, 25]. Although many factors cause these symptoms in knee OA, muscle stiffness may cause a gradual decrease in the intrinsic shock absorption feature; thus, resulting in decreased knee function in early-stage knee OA [2]. Muscle stiffness is an important parameter for joint stability and control of movements [26, 27]. The initial degeneration that starts in the cartilage tissue gradually reaches the bone tissue [28]; therefore, musculoskeletal problems may also be responsible for pain and loss of function in early knee OA [29]. For this reason, it is imperative to evaluate the surrounding soft tissues in early knee OA. In this study, the WOMAC stiffness parameter was correlated with Biceps Femoris muscle stiffness. According to the results of our study, we think that stiffness seen

in OA is related to the flexor reflex. In early-stage knee OA, unlike advanced-stage knee OA, stiffness and other symptoms are not continuous, though symptoms that increase with activity levels are present [6]. The flexor reflex that protects the knee joint may be responsible for symptoms that increase with activity in knee injury [29]. In the case of the flexor reflex, flexor muscles are activated, and extensor muscles are inhibited to protect the knee joint. This increase in activation of the flexor muscles can increase muscle stiffness by causing nutritional deficiency in that area due to overuse. Additionally, in the presence of pain, gamma motor neuron-muscle spindle sensitivity and reflex activity may be impaired due to the activity of group III and group IV afferent nociceptors. Gamma motor neuron stimulation can increase muscle stiffness by causing an increase in muscle spindle activity [29-31]. Many studies evaluated joint stiffness rather than muscle stiffness in individuals with early-stage knee OA, and to date, no study has evaluated the changes associated with muscle stiffness in early knee OA [32, 33]. However, previous studies reported that increased muscle activation in musculoskeletal problems increases muscle stiffness [34, 35]. According to our study results, RF and BF muscle stiffness was not associated with knee pain, range of motion, and knee function, as assessed by step-climbing test and 6-minute walk test. RF muscle stiffness was also not associated with knee function according to the WOMAC index. However, in their study conducted on individuals with neurological diseases, Lee et al., reported that a decrease in knee joint range of motion and knee function was due to the deterioration of the mechanical properties of the muscle [36]. Since RF muscle stiffness in early knee OA does not deteriorate enough to negatively affect knee function, such a relationship was not found in our results. We believe that the symptom of joint stiffness seen in early-stage knee OA, also frequently studied in the literature [30, 32, 33, 37], is caused by BF muscle stiffness. In the study of Langan et al. [38] on healthy individuals, BF muscle stiffness was associated with isotonic muscle performance, evaluated with the Biodex System. BF muscle stiffness may lead to improved dynamic muscle performance, which in turn may have implications for injury prevention. However, this study has not incorporated any performance-based tests, such as the step test. Since SWE is a recent evaluation method, there are no past literary insights that have evaluated the mechanical properties of the muscle in early-stage knee OA except for a few studies about muscle flexibility [39-41]. A study evaluating the muscle flexibility in osteoarthritis by measuring the passive knee extension range of motion reported that muscle flexibility decreased in patients with osteoarthritis as compared to patients without [40]. Various studies that evaluated how the mechanical properties of the muscle affect the knee joint reported that RF and H muscle stiffness decreases with age and is crucial for the control of knee joint movement [10, 26, 42]. In a study evaluating muscle stiffness after ACL surgery, muscle stiffness was associated with muscle strength [43]. As seen in the previous studies, muscle stiffness can be interpreted as muscle spasms as well as muscle strength. In our study, muscle stiffness was interpreted as a spasm because it was associated with stiffness. More studies are needed to correlate muscle stiffness with symptoms and function in early OA.

No correlation was found between pain and muscle stiffness, which was another evaluation parameter in our study. The increase in muscle stiffness is an intrinsic compensatory mechanism to reduce pain. Studies report that an increase in pain is generally associated with muscle stiffness [30, 44]. However, this relationship was not demonstrated because the pain associated with early knee OA increases with activities that put a load on the knee joint. Also, muscle stiffness was evaluated in the resting position of the muscle. In shear wave ultrasonography, muscle stiffness was evaluated passively at the time of relaxation of the muscle as suggested by many studies in the literature [15, 45, 46]. Additional follow-up studies should be planned accordingly to reveal the relationship between muscle flexibility and pain in early knee OA by involving the measurements of muscle stiffness during both rest and submaximal contractions. In our study, no correlation was found between muscle stiffness and pain in patients with early-stage knee OA. Even though RF muscle stiffness was not associated with function and range of motion, BF muscle stiffness was associated with WOMAC stiffness. To sum up, a decrease in BF muscle flexibility is directly associated with joint stiffness, as observed in early-stage OA.

Conclusion

We think that the inclusion of flexibility exercises for the Hamstring muscle group in the rehabilitation program might minimize the symptoms associated with stiffness. Further evaluation of muscle flexibility, as well as muscle strength in early-stage knee OA, may also reduce the rate of progression of the disease by reducing the risk of injury. Future studies examining the changes in muscle stiffness in all stages of osteoarthritis will enable us to understand the muscle stiffness seen in osteoarthritis better.

Limitations

According to the results of radiological magnetic resonance and orthopedic examination, it was very difficult to find a healthy individual without any orthopedic problems and complaints in the knee, so we could not reach a higher number of healthy individuals for comparison.

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References

1. van der Esch M, Holla JF, van der Leeden M, Knol DL, Lems WF, Roorda LD, et al. Decrease of muscle strength is associated with increase of activity limitations in early knee osteoarthritis: 3-year results from the cohort hip and cohort knee study. Arch Phys Med Rehabil. 2014;95(10):1962-8. doi: 10.1016/j.apmr.2014.06.007
2. Dıraçoğlu D, Başkent A, Yağcı İ, Oezcakar L, Aydın R. Isokinetic strength measurements in early knee ostedarthritis. Acta Reumatol Port. 2009;34(1):72-7.
3. Tuna S, Çelik B, Balcı N. The effect of physical therapy and exercise on pain and functional capacity according to the radiological grade of knee osteoarthritis. J Back Musculoskelet Rehabil. 2021 Jul 30. doi: 10.3233/BMR-200287. [Epub ahead of print].
4. Ahmad MA, Hamid MSA, Yusof A. Effects of low-level and high-intensity laser therapy as adjunctive to rehabilitation exercise on pain, stiffness and function in knee osteoarthritis: a systematic review and meta-analysis. Physiotherapy. 26 March 2021. doi: 10.1016/j.physio.2021.03.011. [Epub ahead of print].
5. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2012;20(3):401-6. doi: 10.1007/s00167-011-1743-2
6. Favero M, Ramonda R, Goldring MB, Goldring SR, Punzi L. Early knee osteoarthritis. RMD Open. 2015;1:e000062. doi: 10.1136/rmdopen-2015-000062

7. Wang L, Chen S, An K-N, Yang H-L, Luo Z-P. Theoretical prediction of ultrasound elastography for detection of early osteoarthritis. Sci World J. 2013;2013. doi: 10.1155/2013/565717
8. Yokuş A, Toprak M, Arslan H, Toprak N, Akdeniz H, Gündüz AM. Evaluation of distal femoral cartilage by B-mode ultrasonography and shear wave elastography in patients with knee osteoarthritis: a preliminary study. Acta Radiol. 2021;62(4):510-4. doi: 10.1177/0284185120930642
9. Brandenburg JE, Eby SF, Song P, Zhao H, Brault JS, Chen S, et al. Ultrasound elastography: the new frontier in direct measurement of muscle stiffness. Arch Phys Med Rehabil. 2014;95(11):2207-19. doi: 10.1016/j.apmr.2014.07.007
10. Alfuraih AM, Tan AL, O'Connor P, Emery P, Wakefield RJ. The effect of ageing on shear wave elastography muscle stiffness in adults. Aging Clin Exp Res. 2019;31(12):1755-63. doi: 10.1007/s40520-019-01139-0
11. Ebihara B, Mutsuzaki H, Fukaya T, Iwai K. Interpretation of causal relationship between quadriceps tendon Young's modulus and gait speed by structural equation modeling in patients with severe knee osteoarthritis. J Orthop Surg (Hong Kong). 2021;29(2):1-7. doi: 10.1177/23094990211034003
12. Ebihara B, Fukaya T, Mutsuzaki H. Relationship between quadriceps tendon young's modulus and maximum knee flexion angle in the swing phase of gait in patients with severe knee osteoarthritis. Medicina (Kaunas). 2020;56(9):437. doi: 10.3390/medicina56090437
13. Wu J, Qian Z, Liang W, Liu J, Ren L, Ren L. In vivo assessment of material properties of muscles and connective tissues around the knee joint based on shear wave elastography. J Mech Behav Biomed Mater. 2020;109:103829. doi: 10.1016/j.jmbbm.2020.103829
14. Gogia PP, Braatz JH, Rose SJ, Norton BJ. Reliability and validity of goniometric measurements at the knee. Phys Ther. 1987;67(2):192-5. doi: 10.1093/ptj/67.2.192
15. Taş S, Onur MR, Yılmaz S, Soylu AR, Korkusuz F. Shear wave elastography is a reliable and repeatable method for measuring the elastic modulus of the rectus femoris muscle and patellar tendon. J Ultrasound Med. 2017;36(3):565-70. doi: 10.7863/ultra.16.03032
16. Mendes B, Firmino T, Oliveira R, Neto T, Infante J, Vaz JR, et al. Hamstring stiffness pattern during contraction in healthy individuals: analysis by ultrasound-based shear wave elastography. Eur J Appl Physiol. 2018;118(11):2403-15. doi: 10.1007/s00421-018-3967-z
17. Tüzün E, Eker L, Aytar A, Daşkapan A, Bayramoğlu M. Acceptability, reliability, validity and responsiveness of the Turkish version of WOMAC osteoarthritis index. Osteoarthr Cartil. 2005;13(1):28-33. doi: 10.1016/j.joca.2004.10.010
18. Roos MK, LS Lohmander, EM. WOMAC osteoarthritis index: reliability, validity, and responsiveness in patients with arthroscopically assessed osteoarthritis. Scand J Rheumatol. 1999;28(4):210-5. doi: 10.1080/03009749950155562
19. Harada ND, Chiu V, Stewart AL. Mobility-related function in older adults: assessment with a 6-minute walk test. Arch Phys Med Rehabil. 1999;80(7):837-41. doi: 10.1016/s0003-9993(99)90236-8
20. Tolk J, Janssen R, Prinsen C, Latijnhouwers D, van der Steen M, Bierma-Zeinstra S, et al. The OARSI core set of performance-based measures for knee osteoarthritis is reliable but not valid and responsive. Knee Surg Sports Traumatol Arthrosc. 2019;27(9):2898-909. doi: 10.1007/s00167-017-4789-y
21. Dursun E. Diz ekleminde osteoartriti olan hastalarda egzersiz programının etkinliği [dissertation]. Ankara, Türkiye: Hacettepe Üniversitesi; 2007.
22. Hassan B, Doherty S, Mockett S, Doherty M. Effect of pain reduction on postural sway, proprioception, and quadriceps strength in subjects with knee osteoarthritis. Ann Rheum Dis. 2002;61(5):422-8. doi: 10.1136/ard.61.5.422
23. Bennell KL, Kyriakides M, Metcalf B, Egerton T, Wrigley TV, Hodges PW, et al. Neuromuscular versus quadriceps strengthening exercise in patients with medial knee osteoarthritis and varus malalignment: a randomized controlled trial. Arthritis Rheumatol. 2014;66(4):950-9. doi: 10.1002/art.38317
24. Wright LJ, Zautra AJ, Going S. Adaptation to early knee osteoarthritis: the role of risk, resilience, and disease severity on pain and physical functioning. Ann Behav Med. 2008;36(1):70-80. doi: 10.1007/s12160-008-9048-5
25. Osthoff A-KR, Niedermann K, Braun J, Adams J, Brodin N, Dagfinrud H, et al. 2018 EULAR recommendations for physical activity in people with inflammatory arthritis and osteoarthritis. Ann Rheum Dis. 2018;77(9):1251-60. doi: 10.1136/annrheumdis-2018-213585
26. Johansson H. Role of knee ligaments in proprioception and regulation of muscle stiffness. Journal of Electromyography and Kinesiology. 1991;1(3):158-79. doi: 10.1016/1050-6411(91)90032-Z
27. Ettema G, Huijing P. Skeletal muscle stiffness in static and dynamic contractions. Journal of biomechanics. 1994;27(11):1361-8. doi: 10.1016/0021-9290(94)90045-0
28. Madry H, Kon E, Condello V, Peretti GM, Steinwachs M, Seil R, et al. Early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2016;24(6):1753-62. doi: 10.1007/s00167-016-4068-3
29. Johansson H, Sojka P. Pathophysiological mechanisms involved in genesis and spread of muscular tension in occupational muscle pain and in chronic musculoskeletal pain syndromes: a hypothesis. Med Hypotheses. 1991;35(3):196-203. doi: 10.1016/0306-9877(91)90233-o
30. Masaki M, Ikezoe T, Yanase K, Ji X, Umehara J, Aoyama J, et al. Association of pain history and current pain with sagittal spinal alignment and muscle stiffness and muscle mass of the back muscles in middle-aged and elderly women. Clin Spine Surg. 2019;32(7):E346-E52. doi: 10.1097/BSD.0000000000000793
31. Houk JC. Regulation of stiffness by skeletomotor reflexes. Annu Rev Physiol. 1979;41(1):99-114. doi: 10.1146/annurev.ph.41.030179.000531
32. Serrão PR, Gramani-Say K, Lessi GC, Mattiello SM. Knee extensor torque of men with early degrees of osteoarthritis is associated with pain, stiffness and function. Braz J Phys Ther. 2012;16:289-94. doi: 10.1590/s1413-35552012005000031
33. Gustafson JA, Anderton W, Sowa GA, Piva SR, Farrokhi S. Dynamic knee joint stiffness and contralateral knee joint loading during prolonged walking in patients with unilateral knee osteoarthritis. Gait Posture. 2019;68:44-9. doi: 10.1016/j.gaitpost.2018.10.032
34. Kuo W-H, Jian D-W, Wang T-G, Wang Y-C. Neck muscle stiffness quantified by sonoelastography is correlated with body mass index and chronic neck pain symptoms. Ultrasound Med Biol. 2013;39(8):1356-61. doi: 10.1016/j.ultrasmedbio.2012.11.015
35. Ishikawa H, Muraki T, Morise S, Sekiguchi Y, Yamamoto N, Itoi E, et al. Changes in stiffness of the dorsal scapular muscles before and after computer work: a comparison between individuals with and without neck and shoulder complaints. Eur J Appl Physiol. 2017;117(1):179-87. doi: 10.1007/s00421-016-3510-z
36. Lee SS, Spear S, Rymer WZ. Quantifying changes in material properties of stroke-impaired muscle. Clin Biomech (Bristol, Avon). 2015;30(3):269-75. doi: 10.1016/j.clinbiomech.2015.01.004
37. Englund M, Niu J, Guermazi A, Roemer F, Hunter D, Lynch J, et al. Effect of meniscal damage on the development of frequent knee pain, aching, or stiffness. Arthritis Rheumatol. 2007;56(12):4048-54. doi: 10.1002/art.23071
38. Langan SP, Murphy T, Johnson WM, Carreker JD, Riemann BL. The influence of active hamstring stiffness on markers of isotonic muscle performance. Sports. 2021;9(5):70. doi: 10.3390/sports9050070
39. Soundarya N. A comparative study on the effectiveness of PNF stretching versus static stretching on Pain and Hamstring flexibility in osteoarthritis knee patients. Int J Res Pharm Sci. 2019;10(3):1789-94. doi:10.26452/ijrps.v10i3.1312
40. Reid DA, McNair PJ. Effects of an acute hamstring stretch in people with and without osteoarthritis of the knee. Physiotherapy. 2010;96(1):14-21. doi: 10.1016/j.physio.2009.06.010
41. Weng MC, Lee CL, Chen CH, Hsu JJ, Lee WD, Huang MH, et al. Effects of different stretching techniques on the outcomes of isokinetic exercise in patients with knee osteoarthritis. Kaohsiung J Med Sci. 2009;25(6):306-15. doi: 10.1016/S1607-551X(09)70521-2
42. Saito A, Wakasa M, Kimoto M, Ishikawa T, Tsugaruya M, Kume Y, et al. Age-related changes in muscle elasticity and thickness of the lower extremities are associated with physical functions among community-dwelling older women. Geriatr Gerontol Int. 2019;19(1):61-5. doi: 10.1111/ggi.13567
43. He X, Huang WY, Leong HT, Qiu JH, Ma CC, Fu S-C, et al. Decreased passive muscle stiffness of vastus medialis is associated with poorer quadriceps strength and knee function after anterior cruciate ligament reconstruction. Clin Biomech (Bristol, Avon). 2021;82:105289. doi: 10.1016/j.clinbiomech.2021.105289
44. Masaki M, Aoyama T, Murakami T, Yanase K, Ji X, Tateuchi H, et al. Association of low back pain with muscle stiffness and muscle mass of the lumbar back muscles, and sagittal spinal alignment in young and middle-aged medical workers. Clin Biomech (Bristol, Avon). 2017;49:128-33. doi: 10.1016/j.clinbiomech.2017.09.008
45. Ballyns JJ, Turo D, Otto P, Shah JP, Hammond J, Gebreab T, et al. Office-based elastographic technique for quantifying mechanical properties of skeletal muscle. J Ultrasound Med. 2012;31(8):1209-19. doi: 10.7863/jum.2012.31.8.1209
46. Shinohara M, Sabra K, Gennisson JL, Fink M, Tanter M. Real-time visualization of muscle stiffness distribution with ultrasound shear wave imaging during muscle contraction. Muscle Nerve. 2010;42(3):438-41. doi: 10.1002/mus.21723.

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Effects of cranial nerve blockage in patients with chronic migraine resistant to first-line treatment

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Ethics Committee Approval
The study was approved by the local Ethics Committee of Derince Training and Research Hospital (no:2019/18).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Migraine is a common, disabling neurological disorder and cranial nerve blocks (CNB) are used in the treatment of headaches. This study aimed to compare the effectiveness of a CNB with conventional medical treatment in patients with chronic migraine resistant to first-line treatment.
Methods: This retrospective cohort study included 102 patients with chronic migraine resistant to first-line treatment who were treated in our outpatient clinic. The patients were divided into two groups as those who underwent CNB (n=67) and the control group, who were only treated with conventional drugs (n=35). Bilateral CNB was performed on the patients at baseline and in the second week. The patients' Visual Analogue Scale (VAS) scores, number of days in pain, and the number of analgesics taken were recorded at baseline and the second month.
Results: The second-month VAS scores and the number of days in pain were significantly lower than baseline in both the CNB and control groups ($P<0.01$, and $P<0.05$, respectively). However, while the number of analgesics taken in the 2nd month was lower in the CNB group, it was similar in the control group. ($P<0.01$, $P=0.33$). No significant difference was found between the groups in terms of the number of days in pain in the second month ($P=0.09$). The second month's VAS scores and the number of analgesics taken were significantly lower in the CNB group compared to the control group ($P=0.01$, $P<0.01$).
Conclusion: Our findings indicated that the CNB was more effective than conventional treatment in patients with chronic migraine resistant to first-line treatment.

Keywords: Chronic migraine, Nerve block, Great occipital nerve, GON, SON

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Introduction

Migraine is a quite common condition and causes workforce loss, which increases its cost to society. The prevalence of migraine in Europe and America is 17.6% in women and 5.7% in men [1]. In Turkey, the one-year prevalence of migraine is 16.4% [2]. Various medical treatment options, such as beta-blockers, calcium channel blockers, antiepileptic drugs, tricyclic antidepressants, selective serotonin reuptake inhibitors, and botulinum toxin are available to reduce the frequency of attacks in patients with migraine. Among these, Divalproex, topiramate, metoprolol, propranolol, and timolol are recommended as first-line treatments [3]. However, the long-term use of multiple drugs is required in resistant patients, most of which cannot receive regular medical treatment because of the limited effects and many intolerable side effects of these treatments. One study conducted with episodic migraine patients reported that only 28.3% of the patients were regularly using medical treatment [4].

For several decades, headaches were treated with cranial nerve blocks (CNB). The most common CNB for headache treatment is the great occipital nerve (GON) block, although peripheral nerve block applications, such as blocks of the lesser occipital nerve (LON) and the supraorbital (SON) or the supratrochlear (STN) branches of the trigeminal nerve, are also used [5]. Some publications report that the GON block is effective in cervicogenic, tension-type, and migraine headaches [6-10].

In this study, we aimed to evaluate the effectiveness of CNB in patients with chronic migraine unresponsive to first-line treatment.

Materials and methods

This retrospective study included 102 patients with chronic migraine who were admitted to Derince Training and Research Hospital between 2018-June 2019. The study was approved by the Health Sciences University Kocaeli Derince Training and Research Hospital Clinical Research Ethics Committee (13.06.2019 - no: 2019/18) and conducted per the Declaration of Helsinki. All participants were informed about the study and written informed consent was obtained from each participant. The diagnosis of chronic migraine was made according to the criteria of the International Headache Classification Committee (ICHD-3). Patients who had headaches for at least 15 days a month for the last 3 months, had 8 or more typical migraine attacks per month with or without aura for the last 3 months and who did not respond to the first-line treatment were included in the study. Patients with needle phobia, those who did not show up for the second injection two weeks later, and patients at risk of allergies were excluded from the study. Among the 118 migraine patients who underwent CNB in our clinic between 2018 and June 2019, 67 patients who met the inclusion criteria were included in the study group. Thirty-five patients who were followed up in our clinic with medical treatment and met the inclusion criteria were included as the control group. To avoid bias in patient selection, CNB was recommended to all patients, and patients who did not accept were followed up with medical treatment.

Bilateral GON, LON, and SON blocks were performed on the patients in the CNB group at the beginning and in the second week. The injection was administered after wiping the area with an antiseptic solution. The GON block was achieved by an injection of 1.5 ml 2% lidocaine, 2 cm lateral and 2 cm inferior to the occipital protuberance. The LON block was attained by an injection of 1.5 ml 2% lidocaine, 2/3 lateral to the line between the occipital protuberance and the mastoid. The SON block was administered from the medial to the outer part of the eyebrow, 2 cm from the frontal notch at the mid-pupillary level, in the supraorbital arch. All injections were made with a 27 G needle.

After the injection, the patients were followed up for ten minutes for early side effects. The same protocol was repeated two weeks later. The primary outcomes were a decrease in pain score and the days with pain in a month. The secondary outcome was the number of analgesics taken per month.

A Visual Analog Scale (VAS) score was used to assess the severity of pain. The number of days in pain and the number of analgesics taken in the last month were recorded. The patients' VAS scores, the number of days in pain, and the number of analgesics taken in the second month after the first injection were also recorded.

Statistical analysis

All data were analyzed using SPSS 21.0 (IBM Corp.; Armonk, NY, USA). All descriptive data were presented as mean (SD). Parametric tests were used for normally distributed data and nonparametric tests, for non-normally distributed data. A paired sample t-test was used in the analysis of dependent groups, while Student's t-test was used for parametric data in independent groups. The Mann-Whitney U test was used to evaluate non-parametric data. The Chi-square test was used to compare categorical variables. A *P*-value of <0.05 was considered statistically significant.

Results

In total, 102 patients were included in the study, with 67 patients in the CNB group, and 35 patients in the control group. The mean ages of the patients in the CNB and control groups were 41.2 (10.6) (range, 22–68) years and 39.1 (13.1) (range, 20–65) years, respectively. The CNB group included 61 female and 6 male patients, while the control group consisted of 30 female and 5 male patients.

In the CNB group, the mean VAS scores, number of analgesics taken last month, and the number of days in pain in the last month were 8.1 (0.7), 21.9 (9.6), and 23.4 (6.5), respectively. In the control group, the same values were 8.0 (0.8), 13.8 (2.5), and 16.0 (1.3), respectively. The two groups were similar in terms of age and gender distribution ($P=0.37$, and $P=0.41$, respectively).

The number of days in pain in the last month and the number of analgesics taken were significantly higher in the CNB group than in the control group ($P<0.01$ for all). No significant difference was found between the baseline VAS scores of the CNB and control groups ($P=0.05$). The baseline characteristics of the study and the control groups are shown in Table 1.

Table 1: Baseline characteristics of the study and control groups

	CNB group	Control group	*P*-value*
Age (years)	41.2 (10.6)	39.1 (13.1)	0.37
Gender (Female/Male)	61 / 6	30 / 5	0.41
VAS	8.1 (0.7)	8.0(0.8)	0.05
The number of days in pain (the last month)	23.4 (6.5)	16 (1.39)	<0.01
The number of analgesics taken (the last month)	21.9 (9.6)	13.8 (2.5)	<0.01

* Independent Samples T-test, VAS: Visual Analog Scale, CNB: Cranial Nerve Block

The second-month VAS scores, the number of days in pain, and the number of analgesics taken were significantly lower than those at baseline both in the CNB group (P<0.01 for all), and the control group (P<0.01, and P<0.02, respectively). However, no significant difference was detected between the number of analgesics taken in the control group (P=0.33) (Table 2).

Table 2: Comparison of the primary and secondary outcomes between the groups

	VAS	VAS (2nd month)	*P*-value*
CNB group	8.1 (0.7)	5.3 (2.4)	<0.01
Control group	8.0 (0.8)	6.5 (1.8)	<0.01
	The number of days in pain	The number of days in pain (2nd month)	
CNB group	23.4 (6.5)	9.2 (10.3)	<0.01
Control group	16 (1.3)	12.6 (8.8)	0.02
	The number of analgesics taken	The number of analgesics taken (2nd month)	
CNB group	21.9 (9.6)	6.7 (8.5)	<0.01
Control group	13.8 (2.5)	12.3 (9.0)	0.33

* Paired Samples T-test, VAS: Visual Analog Scale, CNB: Cranial Nerve Block

The number of days in pain in the second month was similar between the two groups (P=0.09). However, second-month VAS scores and the number of analgesics taken were significantly lower in the CNB group than in the control group (P=0.01 and <0.01, respectively) (Table 3).

Table 3: Comparison of the CNB and control group 2nd month data

2nd-month data	CNB group	Control group	*P*-value*
VAS	5.3 (2.4)	6.5 (1.8)	0.01
The number of days in pain	9.2 (10.3)	12.6 (8,8)	0.09
The number of analgesics taken	6.7 (8.5)	12.3 (9.0)	<0.01

* Independent Samples T-test, VAS: Visual Analog Scale, CNB: Cranial Nerve Block

Discussion

Our study shows that the CNB was more effective than conventional treatments in patients with chronic migraine unresponsive to first-line treatment. Conflicting results were published regarding the efficacy of CNBs in the treatment of migraine, but most of these studies deemed CNBs as effective as migraine treatments. The treatment protocol and the local agents used for injection vary between the studies, and additional steroid injections were used in some. No consensus has been reached on whether CNB should be performed bilaterally or unilaterally. Differences in terms of the choice of nerve for the block and the frequency of administration also exist [8, 11-14].

Caputi et al. [11] reported that repeated GON and SON blocks with bupivacaine injection reduced the frequency of migraine attacks, the duration of pain, and the pain intensity in 85% of patients for 6 months. Repeated injections (5 to 10 injections) were also significantly more effective for reduction in pain severity and frequency, even in patients who did not benefit from the first injection. In our study, a significant reduction was also achieved in the frequency and severity of pain, although only two injections were administered to the patients.

A study that evaluated GON blocks with weekly bupivacaine injections for one month as a migraine treatment reported statistically significant decreases in the patients' MIDAS and VAS scores and the number of attacks [14]. Gul et al. [12] compared a bupivacaine injection group and a placebo-controlled group and reported significant decreases in the VAS scores and the number of days in pain in the last month in both groups in the first month, but only in the treatment group in the second and third months, and they deemed GON blocks as effective. Inan et al. [13] found that the VAS score, number of days in pain in the last month, and the pain duration in chronic migraine sufferers were significantly lower in patients who underwent GON blocks with bupivacaine injection compared to the placebo group. Another study comparing two groups who received only a GON block or a GON block and medical treatment showed decreases in the intensity and duration of pain in both groups in the third month, but no significant difference in the frequency of pain and the duration of the attack between the two groups. The authors emphasized that the GON block alone is effective without medical therapy [8]. In our study, while the number of analgesics taken in the CNB group was higher at the beginning, it was lower in the second month. Although there was no difference between the VAS scores, it was lower in the CNB group in the second month, and while the number of days in pain in the CNB group was higher, there was no difference between the two groups in the second month. These findings can be interpreted in two different ways as the number of days in pain and the number of analgesics taken were higher in the study group at the beginning, and the numerical decrease after the treatment was higher, or that there was a greater decrease due to CNB effectiveness.

A comparison of two groups that were administered a GON block with a mixture of lidocaine and bupivacaine and additionally injected with triamcinolone revealed no difference in terms of efficacy in the group that was given additional steroids [15]. Some publications in the literature report that the use of steroids in addition to local anesthetics does not have any additional benefit; however, other cases have shown a significant benefit after a block with a steroid injection alone [16]. We did not give steroid injections in addition to lidocaine injections because no consensus exists regarding this issue.

A comparison of unilateral and bilateral GON blocks revealed no significant difference, but the study emphasized that the unilateral GON block is also an effective treatment [17]. In our study, we administered the block bilaterally and found the treatment effective.

No significant side effects were reported due to nerve block treatments. Local pain at the injection site, nausea, dizziness, and presyncope attack were rarely observed [12]. We recommend monitoring the patient for 30 minutes after the injection. In our study, no side effects were seen.

In some studies, nerve blocks were generally performed with bupivacaine, lidocaine, or both. The doses also differed, but an injection of 1.5 ml of 0.5% bupivacaine has been effective in previous studies [12, 13, 18]. The GON block was also effective in a study that used a 1 ml injection of 2% lidocaine [19]. The studies that used 80 mg prednisolone and 20 mg triamcinolone in addition to a local anesthetic injection reported that steroid injections did not contribute to the efficacy [15]. In our study, we used 1.5 ml of 2% lidocaine for all injections.

A review of the literature reveals studies in which a single injection was performed, as well as studies in which repeated injections were performed [8, 13, 14, 17-21]. A study reported that patients who did not respond after a single injection would subsequently respond to treatment after repeated GON blocks [22]. In our study, although we applied only two repeated injections, we found the treatment effective. Increasing the number of repeated injections may further increase the effectiveness.

The advantage of our study is that, in addition to the GON block, concurrent SON and LON blocks were also performed, and the number of patients included in the study was relatively high compared to other studies in the literature.

Limitations

The main limitations of our study are its retrospective nature, that the number of patients in the control group was smaller than that in the study group, and the short follow-up period.

Conclusion

Our results show that the CNB was more effective than conventional treatment in patients with chronic migraine who do not respond to first-line treatment. CNB is a cheap, easy-to-apply, and effective treatment for chronic migraine patients. Therefore, we think that it should be used more frequently in daily practice. Randomized controlled studies should be conducted to compare patients who receive a GON block alone and those who receive GON, SON, and LON blocks performed together as cranial nerve blocks.

References

1. Stewart WF, Shechter A, Rasmussen B. Migraine prevalence. A review of population-based studies. Neurology. 1994;44(6 Suppl 4):S17-23.
2. Ertas M, Baykan B, Orhan EK, Zarifoglu M, Karli N, Saip S, et al. One-year prevalence and the impact of migraine and tension-type headache in Turkey: a nationwide home-based study in adults. J Headache Pain. 2012;13(2):147-57. doi: 10.1007/s10194-011-0414-5
3. Ha H, Gonzalez A. Migraine Headache Prophylaxis. American family physician. 2019;99(1):17-24.
4. Blumenfeld AM, Bloudek LM, Becker WJ, Buse DC, Varon SF, Maglinte GA, et al. Patterns of use and reasons for discontinuation of prophylactic medications for episodic migraine and chronic migraine: Results from the second International Burden of Migraine Study (IBMS-II). Headache: The Journal of Head and Face Pain. 2013;53(4):644-55. doi: 10.1111/head.12055
5. Blumenfeld A, Ashkenazi A, Napchan U, Bender SD, Klein BC, Berliner R, et al. Expert Consensus Recommendations for the Performance of Peripheral Nerve Blocks for Headaches – A Narrative Review. Headache: The Journal of Head and Face Pain. 2013;53(3):437-46. doi: 10.1111/head.12053
6. Terzi T, Karakurum B, Üçler S, İnan LE, Tulunay C. Greater occipital nerve blockade in migraine, tension-type headache and cervicogenic headache. J Headache Pain. 2002;3(3):137-41. doi: 10.1007/s101940200031
7. Ashkenazi A, Young WB. The Effects of Greater Occipital Nerve Block and Trigger Point Injection on Brush Allodynia and Pain in Migraine. Headache: The Journal of Head and Face Pain. 2005;45(4):350-4. doi: 10.1111/j.1526-4610.2005.05073.x
8. Inan N, Inan LE, Coşkun Ö, Tunç T, Ilhan M. Effectiveness of Greater Occipital Nerve Blocks in Migraine Prophylaxis. Noro Psikiyatr Ars. 2016;53(1):45-8. doi: 10.5152/npa.2015.10003
9. Tang Y, Kang J, Zhang Y, Zhang X. Influence of greater occipital nerve block on pain severity in migraine patients: A systematic review and meta-analysis. The American Journal of Emergency Medicine. 2017;35(11):1750-4. doi: 10.1016/j.ajem.2017.08.027
10. Zhang H, Yang X, Lin Y, Chen L, Ye H. The efficacy of greater occipital nerve block for the treatment of migraine: A systematic review and meta-analysis. Clinical Neurology and Neurosurgery. 2018;165:129-33. doi: 10.1016/j.clineuro.2017.12.026
11. Caputi CA, Firetto V. Therapeutic Blockade of Greater Occipital and Supraorbital Nerves in Migraine Patients. Headache: The Journal of Head and Face Pain. 1997;37(3):174-9. doi: 10.1046/j.1526-4610.1997.3703174.x
12. Gul HL, Ozon AO, Karadas O, Koc G, Inan LE. The efficacy of greater occipital nerve blockade in chronic migraine: A placebo-controlled study. Acta Neurologica Scandinavica. 2017;136(2):138-44. doi: 10.1111/ane.12716
13. Inan LE, Inan N, Karadaş Ö, Gül HL, Erdemoğlu AK, Türkel Y, et al. Greater occipital nerve blockade for the treatment of chronic migraine: a randomized, multicenter, double-blind, and placebo-controlled study. Acta Neurologica Scandinavica. 2015;132(4):270-7. doi: 10.1111/ane.12393
14. Okmen K, Dagistan Y, Dagistan E, Kaplan N, Cancan E. Efficacy of the greater occipital nerve block in recurrent migraine type headaches. Neurologia i Neurochirurgia Polska. 2016;50(3):151-4. doi: 10.1016/j.pjnns.2016.01.015
15. Ashkenazi A, Matro R, Shaw JW, Abbas MA, Silberstein SD. Greater occipital nerve block using local anaesthetics alone or with triamcinolone for transformed migraine: a randomised comparative study. Journal of Neurology, Neurosurgery & Psychiatry. 2008;79(4):415. doi: 10.1136/jnnp.2007.124420
16. Tobin J, Flitman S. Treatment of migraine with occipital nerve blocks using only corticosteroids. Headache. 2011;51(1):155-9. doi: 10.1111/j.1526-4610.2010.01801.x
17. Ünal-Artık HA, İnan LE, Ataç-Uçar C, Yoldaş TK. Do bilateral and unilateral greater occipital nerve block effectiveness differ in chronic migraine patients? Neurological Sciences. 2017;38(6):949-54. doi: 10.1007/s10072-017-2861-5
18. Deniz Palamar M, Derya Uluduz M, Sabahattin Saip M, Gul Erden M. Ultrasound-guided greater occipital nerve block: an efficient technique in chronic refractory migraine without aura? Pain Physician. 2015;18:153-62. doi: 10.36076/ppj/2015.18.153
19. Kashipazha D, Nakhostin-Mortazavi A, Mohammadianinejad SE, Bahadoram M, Zandifar S, Tarahomi S. Preventive effect of greater occipital nerve block on severity and frequency of migraine headache. Global journal of health science. 2014;6(6):209. doi: 10.5539/gjhs.v6n6p209
20. Dilli E, Halker R, Vargas B, Hentz J, Radam T, Rogers R, et al. Occipital nerve block for the short-term preventive treatment of migraine: A randomized, double-blinded, placebo-controlled study. Cephalalgia. 2014;35(11):959-68. doi: 10.1177/0333102414561872
21. Takmaz SA, İnan N, Uçler S, Yazar MA, İnan L, Başar H. Greater occipital nerve block in migraine headache: preliminary results of 10 patients. The journal of the Turkish Society of Algology. 2008;20(1):47-50.
22. Piñero MR, Carrillo PM, Hueso MP, de la Cruz Rodríguez C, Mesonero LL, Peral AG. Pericranial nerve blockade as a preventive treatment for migraine: experience in 60 patients. Neurología (English Edition). 2016;31(7):445-51. doi: 10.1016/j.nrl.2014.10.001.

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Clinical and laboratory characteristics of patients with COVID-19 followed up due to acute ischemic stroke

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Ethics Committee Approval
Approval was obtained from the Ethics Committee of Meram Faculty of Medicine, Konya Necmettin Erbakan University (20/11/2020, 2897) and the Ministry of Health for this retrospective study.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: As coronavirus infectious disease 2019 (COVID-19) spreads worldwide, there is increasing evidence of an association between COVID-19 and vascular diseases. However, there are limited data on the clinical characteristics, stroke mechanisms, and prognosis of stroke patients with COVID-19. We aimed to evaluate the clinical and laboratory features and prognosis of patients with COVID-19 who were followed up due to acute ischemic stroke.

Methods: Fifty-six patients with a confirmed diagnosis of COVID-19 and acute ischemic stroke were included in this retrospective study. The demographic characteristics, medical history, symptoms, clinical, laboratory and imaging findings of the patients were evaluated retrospectively. The patients were divided into two groups according to the modified Rankin Scale (mRS) score in the first month, as those with good or poor prognosis.

Results: There were forty (71%) males, 16 (29%) females, and their overall mean age was 69.21 (8.77) (55-90) years. Fifty (89.2%) of 56 patients had pneumonia findings in chest computed tomography. The mortality rate was 35.7% (n=10) and 26 patients (46.4%) had a poor prognosis according to the mRS scores. Increased C-reactive protein and D-dimer levels were associated with mortality in the COVID-19 positive acute ischemic stroke patients (P=0.035, P=0.023).

Conclusion: The COVID-19–associated coagulopathy increases mortality and grossly affects the course of the infectious process. Inflammation markers may be associated with poor prognosis in stroke patients with COVID-19.

Keywords: COVID-19, SARS-CoV-2, Stroke, D-dimer, Mortality

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Introduction

Coronavirus infectious disease 2019 (COVID-19), caused by the new type of coronavirus 2 (SARS-CoV-2), leads to acute severe respiratory failure syndrome and has been spreading rapidly all over the world since December 2019. There is increasing evidence of an association between COVID-19 and cerebrovascular disease and other types of vascular disease [1].

In retrospective studies, the incidence of stroke in hospitalized COVID-19 patients ranges between 2.5% and 6%, with an increased frequency [2,3]. The clinical course of COVID-19 is more severe in older patients, male patients, patients with comorbidities such as hypertension, diabetes, heart disease and obesity, all of which are also considered as risk factors for stroke [4]. This increase in the incidence of stroke may be due to the increase in risk factors or to the progression of acute severe respiratory failure syndrome, multiple organ dysfunction syndrome, coagulopathy similar to that which occurs in sepsis, diffuse intravascular coagulation or cardiac effects in the process.

Many opinions can be put forward regarding the pathophysiologic mechanisms of stroke development in the course of COVID-19 infection. The uncontrollable cytokine storm seen in patients with severe infections can lead to multiple organ dysfunction. In particular, the activation of the microthrombotic pathway by destructive pathologic mechanisms mediated by the endothelial system can cause stroke. The markers of thrombosis tendency and inflammation such as D-dimer, fibrinogen, and C-reactive protein (CRP), and inflammatory cytokine levels such as tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2) receptor and IL-6 have been observed to increase in infected patients [5].

In post-mortem examinations of infected patients, lymphocytic endotheliitis was shown in many organs, including the lungs, heart, kidneys, small intestine, and liver, and ischemic events, due to direct endothelial damage and diffuse endothelial inflammation [6]. Two main pathways may be affected as a result of endothelial damage. Systemic inflammatory response syndrome (SIRS) can be triggered by cytokine release (e.g. IL-1, IL-6, TNF-α) and nitric oxide release as a result of inflammatory pathway activation. In the activation of the microthrombotic pathway, von Willebrand factor multimers (vWFM) are formed by platelet activation in the first stage. Non-destructible vWFMs cause formation of microthrombi together with activated thrombocytes in target organs. As a result, vascular microthrombotic disease develops due to endothelial damage [7].

It is thought that direct viral infection of endothelial cells through angiotensin-converting enzyme 2 (ACE-2) receptors may contribute to the host inflammatory response. Histopathologic analysis of the central nervous system (CNS) is required to determine whether the CNS vasculitis associated with SARS-CoV-2 within the wide clinical spectrum of COVID-19 can result from lymphocytic endotheliitis.

With the cytokine storm that also occurs in COVID-19, widespread microvascular thrombosis is observed with prothrombotic activation, and D-dimer levels are high in these patients [8]. Patients may also experience severe cardiac involvement leading to acute myocarditis and heart failure, which may be a risk factor especially for cardioembolic strokes [9]. In addition to these, severe hypoxia associated with acute respiratory distress syndrome developing during the course of COVID-19 negatively affects cerebral autoregulation, leading to intracerebral bleeding, cerebral vasodilation, and edema [10].

Given that there is a limited number of studies in the literature on acute ischemic stroke in patients with COVID-19, it is important to add case series to the literature. In this study, we aimed to evaluate the clinical and laboratory features and prognosis of patients with COVID-19 who were followed up due to acute ischemic stroke.

Materials and methods

Fifty-six patients with acute ischemic stroke and COVID-19 who were followed up between March and October 2020 in a pandemic hospital were included in this single-center retrospective study. All patients with COVID-19 were diagnosed according to the World Health Organization (WHO) guidelines, and patients who were positive for SARS-CoV-2 with real-time reverse transcription-polymerase chain reaction (rRT-PCR) in throat swabs were included.

The demographic features, medical history, symptoms, clinical, laboratory and imaging findings of the patients were evaluated from electronic medical records, retrospectively. The diagnosis of acute ischemic stroke was confirmed using brain computed tomography (CT), magnetic resonance imaging (MRI) and clinical symptoms. All neurologic symptoms were examined and approved by neurologists. Ischemic stroke types were classified according to the Trial of Org 10 172 in Acute Stroke Treatment (TOAST) classification [11]. The patients were divided into two groups according to modified Rankin Scale (mRS) scores in the first month as those having a good or poor prognosis. A mRS score of 3-6 was considered as poor prognosis. National Institute of Health Stroke Scale (NIHSS) scores were recorded at the time of admission. Patients were also grouped according to 30-day mortality.

Approval was obtained from the Local Ethics Committee (Protocol No: 2020-2897) and the Ministry of Health.

Statistical analysis

The normality of distribution of continuous variables was tested with the Shapiro-Wilk test. Student's t-test (for normal data) and the Mann-Whitney U test (for non-normal data) were used to compare two independent groups. The Chi-square test was used to investigate relationships between two categorical variables. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 24.0, and *P*-values <0.05 were considered statistically significant.

Results

Fifty-six patients with positive COVID-19 nasopharyngeal swab PCR tests who were followed up for acute ischemic stroke were included in this study. Forty (71%) of the patients were male, 16 (29%) were female, and the mean age was 69.21 (8.77) (55-90) years. The mean NIHSS score of the patients was 11.5 (1-24). Thirty-two (57.1%) patients had hypertension, 22 (39.3%) had diabetes mellitus, 10 (17.9) had atrial fibrillation, four (7.1%) had valvular heart disease, four

(7.1%) had a history of stroke, and 10 (17.9%) were smokers (Table 1).

Table 1: Demographic and clinical characteristics of patients with COVID-19 and acute ischemic stroke in the good and poor prognosis groups compared according to mRS scores

Characteristic	Total n=56	Good Prognosis (mRS 0-2) (n=26)	Poor Prognosis (mRS 3-6) (n=30)	*P*-Value
Demographics and risk factors				
Age, Mean	69.21(8.770)	70.54 (9.62)	68.07 (8.12)	0.467
Sex,-Males	40 (71.4%)	16 (61.5%)	24 (80%)	0.281
-Females	16 (28.6%)	10 (38.5%)	6 (20%)	
Current smoking	10 (17.9%)	4 (15.4%)	6 (20%)	0.339
Hypertension	32 (57.1%)	14 (53.8%)	18 (60%)	0.743
Diabetes mellitus	22 (39.3%)	8 (30.8%)	14 (46.7%)	0.390
Hyperlipidemia	18 (32.1%)	8 (30.8%)	10 (33.3%)	0.885
Atrial fibrillation	10 (17.9%)	4 (15.4%)	6 (20%)	0.755
Valvular heart disease	10 (17.9)	4 (15.4)	6 (20)	0.755
Previous stroke	4 (7.1%)	0 (0%)	4 (13.3%)	0.172
Stroke Classification (TOAST)	n (%)	n (%)	n (%)	
Large Vessel Disease	22 (39.3%)	12 (33.4%)	10 (50)	0.096
Small Vessel Disease	8 (14.3%)	8 (22.2%)	0 (0%)	
Cardio-Embolic	14 (25%)	6 (16.7%)	8 (40%)	
Stroke of Determined Origin	0 (0%)			
Stroke of Undetermined Origin	12 (21.4%)	10 (27.8%)	2 (10%)	
NIHSS on admission median	11.5 (8.04)	4.62 (3.55)	17.47 (5.63)	0.001*
Mortality, n(%)	20 (66.7)	0 (0 %)	20 (66.7 %)	0.001*

NIHSS: National Institute of Heath Stroke Scale, mRS: Modified Rankin Score

Lymphopenia was detected in 60.7% of the patients. CRP levels were increased in 89.2%, ferritin levels in 25%, D-dimer levels in 82.1%, lactate dehydrogenase (LDH) levels in 25%, and fibrinogen levels, in 32.1%. Thrombocytopenia was detected in 25%. No significant difference was observed between the good and poor prognosis groups in terms of laboratory parameters, but a statistically significant correlation was observed between mortality and increased CRP and D-dimer levels (P=0.035, P=0.023) (Table 2).

Table 2: Relationship between laboratory parameters and mortality in patients with COVID-19 and acute ischemic stroke

Laboratory Findings	30-day mortality		*P*-value
	No (n=18) Mean (SD)	Yes (n=10) Mean (SD)	
White blood cell, ×103/uL	8.48 (3.24)	11.27 (9.19)	0.851
Neutrophil, ×103/uL	6.81 (3.03)	7.5 (7.22)	0.556
Lymphocyte , ×103/uL	0.95 (0.43)	3.18 (6.83)	0.572
Monocyte, ×103/uL	0.69 (0.42)	0.59 (0.44)	0.410
Platelet ×103/uL	266.99 (118.91)	195.91 (66.1)	0.083
C-reactive protein (CRP), mg/L	80.71 (70.94)	90.9 (45.85)	0.035
Ferritin, ng/mL	416.61 (470.97)	501.61 (335.67)	0.609
D-dimer, ng/ml	1206.34 (1253.5)	7234.55 (12374.42)	0.023
Lactate Dehydrogenase (LDH), U/L	112.12 (333.8)	208.87 (274.06)	0.057
Fibrinogen, mg/dL	541.5 (119.78)	381.2 (210.44)	0.146
Prothrombin time (PT), s	13.58 (2.39)	14.68 (2.84)	0.230
Troponin, ng/mL	112.12 (333.8)	208.87 (274.06)	0.057

* Significant at 0.05 level, Student t or Mann Whitney u test

The median duration from the first symptoms of SARS-CoV-2 infection to acute ischemic stroke was 9 (range, 1-20) days. According to the TOAST classification, 22 of the 56 patients with ischemic stroke had large vessel atherosclerosis, eight had small vessel occlusion, and 14 had cardioembolism. The etiology was not clear in 12 patients (stroke of undetermined etiology).

Twenty-eight patients needed intensive care. The choice of treatment for ischemic stroke (antiaggregant / anticoagulant / i.v. tissue plasminogen activator) was determined by the treatment team, who comprehensively reviewed the clinical syndrome, laboratory findings, and time of presentation. Intravenous thrombolytic therapy was administered to five patients who had an acute ischemic stroke during hospitalization and mechanical thrombectomy was given to two patients. An anticoagulant dose of low-molecular-weight (LMW) heparin (enoxaparin) was administered to 10 patients with suspected cardioembolism, 42 patients received antiaggregants and deep vein thrombosis prophylaxis-dose enoxaparin.

There was evidence of pneumonia in the chest CTs of 50 (89.2%) patients. Mortality was observed in 20 patients (35.7%) and poor prognosis, in 26 patients (46.4%). Acute ischemic stroke was observed in 10 patients who were hospitalized for COVID-19 treatment, and 46 patients were hospitalized due to the association of acute ischemic stroke and COVID-19. There was a significant correlation between NIHSS scores at admission and mortality (P=0.001).

Discussion

Patients with severe symptoms of COVID-19 may be at risk for thromboembolic events resulting from COVID-19-associated coagulopathy. SARS-CoV-2 can damage endothelial cells and activate inflammatory and thrombotic pathways [6]. Endothelial cell infection or monocyte activation, upregulation of tissue factors, and the release of microparticles that activate the thrombotic pathway and cause microangiopathy are also valid for SARS-CoV-2, as in other viruses [12, 13]. It is suggested that monocyte activation forms part of the secondary hemophagocytic lymphohistiocytosis identified in severe COVID-19 [14]. Endothelial dysfunction can lead to microvascular and macrovascular complications in the brain, as well as in systemic events [15].

Studies reported that patients hospitalized due to COVID-19 showed increased coagulation activity with increased D-dimer concentrations [16,17]. In a retrospective study, D-dimer levels were higher in patients with cerebrovascular disease and COVID-19 than patients with COVID-19 without cerebrovascular disease [18]. In our study, D-dimer levels were higher among the non-survivors (p = 0.023). In a case series of 221 patients with COVID-19 published by Li et al., 11 patients had acute ischemic stroke, one patient had sinus vein thrombosis, and one patient had hemorrhagic stroke (15). Thrombocytopenia increased fibrinogen, and D-dimer levels in these patients were associated with a coagulopathy secondary to COVID-19.

Ischemic infarction areas seen in COVID-19 tend to occur in large vessel territories, and more often in multiple vessel territories [19]. In this study, 36 of 56 patients had large vessel atherosclerosis, 14 had small vessel occlusion and 18 had cardioembolism. In New York City, five stroke patients with COVID-19 who were aged under 50 years had large vessel occlusion (mean NIHSS: 17) [20]. This case series suggested that young patients might also be at risk for ischemic stroke. In our case series, there were no patients aged under 50 years.

A pro-inflammatory immune response develops during COVID-19 and the cytokine storm that develops in some patients gets ahead of the damage caused by the infection. Hypercoagulability is such an example [21]. Coexistence of COVID-19 with a severe headache and cerebral sinus vein thrombosis was reported in a 59-year-old patient with a history of obesity, smoking, hypertension, and diabetes [3]. Sinus vein thrombosis was not encountered in our case series.

In a study involving 184 COVID-19 patients in an intensive care unit (ICU) in Germany, the incidence of thrombosis was 31%, and arterial ischemic cerebrovascular disease was found in three patients. It is recommended that thrombosis prophylaxis should be strictly administered in all

patients with COVID-19 admitted to the ICU [22]. In our study, LMW heparin prophylaxis was given to all patients.

In a multivariate analysis of a retrospective series of 440 patients with severe COVID-19, age, prolongation of prothrombin time, increased D-dimer, and thrombocytopenia were associated with mortality [23]. In our study, mortality was correlated with high CRP and D-dimer levels and thrombocytopenia, but no significant difference was found in terms of age and other variables. In our study, 40 (71%) of 56 patients had severe COVID-19 pneumonia findings, which suggested that the possibility of acute ischemic stroke was higher in patients with severe infections, similar to the literature [24].

Limitations

This study had some limitations. It was a single-center study. We had a limited number of patients and we were not able to perform advanced etiologic examinations in every patient. However, it may be beneficial to share our clinical experience because there are a limited number of small case series studies on acute ischemic stroke in COVID-19. More data are needed in this area to determine the contribution of processes involved in the pathogenesis of stroke in patients with COVID-19. Multicenter clinical studies with a larger number of patients are needed.

Conclusion

Acute ischemic stroke is not uncommon in patients with COVID-19, particularly those who are severely infected and those with pre-existing vascular risk factors. COVID-19–associated coagulopathy increases mortality and has important effects on the course of the infectious process. Inflammation markers may be associated with poor prognosis in patients with stroke and COVID-19. However, the data in the literature are limited. In this period, the accumulation of knowledge, which will increase with the meticulous monitoring and recording of patients with a multi-faceted perspective, will contribute to understanding the underlying pathophysiologic mechanisms and determining the appropriate clinical approach.

References

1. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 2020 Jun 1;77(6):683-90. doi: 10.1001/jamaneurol.2020.1127.
2. Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, et al. Neurologic Features in Severe SARS-CoV-2 Infection. N Engl J Med. 2020 Jun 4;382(23):2268-70. doi: 10.1056/NEJMc2008597.
3. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al.Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020 Jul;191:145-7. doi: 10.1016/j.thromres.2020.04.013.
4. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15;395(10223):507-13.doi: 10.1016/S0140-6736(20)30211-7.
5. Ridker PM. Anticytokine Agents: Targeting Interleukin Signaling Pathways for the Treatment of Atherothrombosis. Circ Res. 2019 Feb;124(3):437-50. doi: 10.1161/CIRCRESAHA.118.313129.
6. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS,et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020 May 2;395(10234):1417-8. doi: 10.1016/S0140-6736(20)30937-5.
7. Chang JC. Sepsis and septic shock: endothelial molecular pathogenesis associated with vascular microthrombotic disease. Thromb J. 2019 May 30;17:10. doi: 10.1186/s12959-019-0198-4.
8. Wang HY, Li XL, Yan ZR, Sun XP, Han J, Zhang BW. Potential neurological symptoms of COVID-19. Ther Adv Neurol Disord. 2020 Mar 28;13:1756286420917830. doi: 10.1177/1756286420917830.
9. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol. 2020 May;17(5):259-60. doi: 10.1038/s41569-020-0360-5.
10. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun. 2020 Jul;87:18-22. doi: 10.1016/j.bbi.2020.03.031.
11. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993 Jan;24(1):35-41. doi: 10.1161/01.str.24.1.35.
12. Lopes da Silva R. Viral-associated thrombotic microangiopathies. Hematol Oncol Stem Cell Ther. 2011;4(2):51-9. doi: 10.5144/1658-3876.2011.51.
13. Brisse E, Wouters CH, Andrei G, Matthys P. How Viruses Contribute to the Pathogenesis of Hemophagocytic Lymphohistiocytosis. Front Immunol. 2017 Sep 7;8:1102. doi: 10.3389/fimmu.2017.01102.
14. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020 Mar 28;395(10229):1033-4. doi: 10.1016/S0140-6736(20)30628-0.
15. Li Y, Li M, Wang M, Zhou Y, Chang J, Xian Y, et al. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. Stroke Vasc Neurol. 2020 Sep;5(3):279-84. doi: 10.1136/svn-2020-000431.
16. Lee SG, Fralick M, Sholzberg M. Coagulopathy associated with COVID-19. CMAJ. 2020 May 25;192(21):E583. doi: 10.1503/cmaj.200685.
17. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, Clark C, Iba T. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020 May;18(5):1023-6. doi: 10.1111/jth.14810.
18. Yaghi S, Ishida K, Torres J, Mac Grory B, Raz E, Humbert K, et al. SARS-CoV-2 and Stroke in a New York Healthcare System. Stroke. 2020 Jul;51(7):2002-11. doi: 10.1161/STROKEAHA.120.030335.
19. Beyrouti R, Adams ME, Benjamin L, Cohen H, Farmer SF, Goh YY, et al. Characteristics of ischaemic stroke associated with COVID-19. J Neurol Neurosurg Psychiatry. 2020 Aug;91(8):889-91. doi: 10.1136/jnnp-2020-323586.
20. Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, et al. Large-Vessel Stroke as a Presenting Feature of Covid-19 in the Young. N Engl J Med. 2020 May 14;382(20):e60. doi: 10.1056/NEJMc2009787.
21. Magdi M, Rahil A. Severe Immune Thrombocytopenia Complicated by Intracerebral Haemorrhage Associated with Coronavirus Infection: A Case Report and Literature Review. Eur J Case Rep Intern Med. 2019 Jul 12;6(7):001155. doi: 10.12890/2019_001155.
22. Hughes C, Nichols T, Pike M, Subbe C, Elghenzai S. Cerebral Venous Sinus Thrombosis as a Presentation of COVID-19. Eur J Case Rep Intern Med. 2020 Apr 29;7(5):001691. doi: 10.12890/2020_001691.
23. Wang J, Hajizadeh N, Moore EE, McIntyre RC, Moore PK, Veress LA, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A case series. J Thromb Haemost. 2020 Jul;18(7):1752-5. doi: 10.1111/jth.14828.
24. Goldberg MF, Goldberg MF, Cerejo R, Tayal AH. Cerebrovascular Disease in COVID-19. AJNR Am J Neuroradiol. 2020 Jul;41(7):1170-2. doi: 10.3174/ajnr.A6588.

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The effect of dual stimulation on ploidy rates in patients with poor ovarian response

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Ethics Committee Approval
Ethics committee approval for the study was obtained from the Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (ATADEK) with the decision number 2021-01/14.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: It is difficult to obtain healthy oocytes in poor ovarian response patients with conventional treatment methods. This study aimed to find out which agent is most effective at inducing ovulation in one menstrual cyle. We aimed to examine the effects of follicular and luteal stimulation in patients with poor ovarian response on oocyte count, blastocyst, euploid embryo, and pregnancy rates.
Methods: A total of 134 patients were included in this retrospective cohort study, and the rates of ploidy in the embryos obtained by follicular and luteal stimulation were evaluated. All cases were treated with the antagonist protocol beforehand. The research was conducted by examining the data of patients who underwent Dual stimulation (Duostim) between 2015 and 2017 in the IVF Clinic of Acıbadem University Atakent Hospital retrospectively.
Results: While ploidy rates in FS and LS were significant in terms of age and AMH values ($P<0.05$ for all), they did not differ with BMI values ($P>0.05$ for all). The rate of aneuploid embryo development in follicular phase was 81.8%, while that in luteal phase was 18.2%, and the rates of euploid embryo development in follicular and luteal treatments were 34.6% and 18.2%, respectively. The pregnancy rate with euploid embryos by LPS was significantly higher compared that by FPS ($P<0.05$).
Conclusion: Our study concludes that follicles entering the anovulatory phase in the follicular phase can be saved by LS, so that healthier embryos can be obtained.

Keywords: Duostim, Poor-responder patients, Double ovarian stimulation, Reduce ovarian reserve

Introduction

The worldwide prevalence of infertility ranges between 2.5-10.5% in women [1]. Some of these women must undergo assisted reproductive techniques, and the poor prognosis group consists of those with advanced age and poor ovarian response. The rate of poor ovarian response ranges between 5.6- 35.1% among infertile women. It may be necessary to implement a special treatment protocol in these patients to obtain high-quality oocytes and increase the pregnancy rate [2, 3]. Although there is still no clear protocol, transferring the embryos with a freeze-thaw protocol to use a hormonally unstimulated endometrium may be beneficial in increasing pregnancy rates [4, 5].

A treatment plan, known as the POSEIDON group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number), is suggested to determine the correct oocyte quantity and sufficiency [6]. It was concluded that a healthy pregnancy can be achieved after finding a healthy embryo by a good blastocyst culture and euploidy study [7, 8].

The most difficult patient group in assisted reproductive techniques is those with weak ovarian response. Numerous and high-quality embryos cannot be obtained in these patients with standard ovarian stimulations. Luteal phase stimulants and embryo freezing technology come into play at this point [9].

For this reason, follicular and luteal phase stimulants (dual stimulation, duostim) administered in the same menstrual cycle in addition to luteal phase stimulation are used in the treatment of patients with weak ovarian capacity, especially in cancer patients who want to urgently preserve their reproductive function [10, 11].

Duostim protocols are less preferred than traditional treatments due to time consumption and high cost. Cycle cancellation is also more frequent with dual stimulation [12, 13].

For patients undergoing Duostim, ESHRE identified patients with poor ovarian response using the Bologna criteria [14]. Accordingly, at least two of the following three criteria must be met:

1. Advanced maternal age (40 years or older) and any other risk factors for poor ovarian response
2. Obtaining 3 or fewer oocytes with a previously conventional stimulation protocol
3. Abnormal ovarian reserve test (AFC<5-7 or AMH<0.5-1.1 ng/ml)

Before these criteria, different definitions of weak ovarian response were made [12, 15, 16].

Compared to conventional treatment, dual stimulation is somewhat superior to the traditional method in terms of the number of eggs, mature eggs, and blastocysts obtained. After two phases of stimulation, higher oocytes and embryos were obtained in the luteal phase [17-23]. Additionally, the number of normal karyotype blastocysts were higher [24].

Choosing a personalized treatment that will increase success and reduce complications is the most significant measure in ovarian stimulation. Selection of the right stimulation agent, the use of agonists or antagonists to reduce LH (luteal hormone) peak, hCG or agonist trigger use to obtain a mature oocyte, fresh or frozen embryo transfer, and whether the embryo is selected with pregenetic diagnosis or morphological characteristics are the key steps. The main goal in all is to keep the ovarian response at a maximum in patients with a weak response [25].

There may be more than one follicular wave in a human ovarian cycle, which eradicates conventional stimulation protocols [26].

Dual stimulation, that is, duostim follicular phase stimulation (FPS) that complements luteal phase stimulation (LPS), is implemented to preserve fertility in patients with a low ovarian reserve and advanced age [19, 27]. In addition, duostim can be used in all patients to increase the number of mature oocytes and increase the cumulative birth rate. It also shortens the time to obtain euploid blastocyst [28, 29].

Cimadomo et al. [30] compared follicular and luteal phase stimulations in the same cycle to evaluate the efficacy of treatment.

In this article, we aimed to examine the effects of follicular and luteal stimulation in patients with poor ovarian response on oocyte count, blastocyst, euploid embryo, and pregnancy rates. We intended to provide an unbiased perspective on the number of oocytes and healthy blastocysts obtained after the treatment by evaluating the number of healthy embryos after two stimulations given in one cycle.

Materials and methods

This retrospective cohort study evaluated the data of patients who underwent Dual stimulation (Duostim) between 2015 and 2017 in the IVF Clinic of Acıbadem University Atakent Hospital. Ethics committee approval for the study was obtained from the Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (ATADEK) with the decision number 2021-01/14. There were 134 patients in the study, and the rates of ploidy in the embryos obtained by stimulatory and luteal stimulation were evaluated. The patients consisted of women who had previously used an antagonist protocol due to poor ovarian response, from whom insufficient oocytes were collected, or blastocysts or a genetically healthy embryo could not be obtained.

Inclusion criteria

Being under the age of 41 years, having a menstrual cycle length between 21 and 35 days, having an indication for starting treatment with at least 300 IU, having both ovaries in place and not having undergone ovarian surgery, having received antagonist protocol treatment with failure to obtain a euploid embryo, FSH level not exceeding 15 IU/ml and LH level not exceeding 12 IU/ml.

Exclusion criteria

Presence of follicles larger than 10 mm before treatment, endometriosis stage 3 or 4, and concomitant uterine pathology (adenomyosis, submucous myoma, Asherman syndrome), and having an azoospermic partner.

Stimulation protocol

Dual stimulation was performed to all patients who were treated. After screening and baseline evaluation of the ovaries at gynecological examination, fixed-dose recombinant FSH (rec-FSH) (300 IU/day; Gonal-F, Merck-Serono, Germany; Puregon, MSD, USA) was administered for 4 days. Follicular growth was monitored on day 5 and then every 2 days. A gonadotropin releasing hormone antagonist (GnRH antagonist) (Cetrorelix, Cetrotide, Merck-Serono; Ganirelix, Orgalutran, MSD) was administered daily (in a single subcutaneous dose)

after identification of a prominent follicle ≥13-14 mm in diameter and until the day of the ovulation trigger. A bolus dose of 0.5 ml buserelin (Suprefact, Hoechst Marion Roussel, Germany) was administered when at least two follicles reached a diameter of ≥17-18 mm. Oocyte retrieval was performed 35 hours after the trigger. Approximately 5 days after the first oocyte retrieval, that is, when complete luteinization was achieved, recombinant LH (rec-LH) (300 IU/day; Luveris, Merck- Serono) was started with the same protocol and daily dose as for FPS, regardless of the number of antral follicles counted in the scan. Oocyte collection was performed with the same pick-up protocol. After oocyte collection, intracytoplasmic sperm injection was performed, and an embryo culture medium was created. Oocytes were collected from the follicles by transvaginal ultrasound-guided aspiration and cultured for 2-3 hours in a culture medium (CSCM, Irvine Scientific, Australia) at 37°C, 5% CO_2, and 5% O_2. Then, peeling and fertilization were performed in a Hepes-buffered medium (Irvine Scientific). Fertilization was assessed 16-20 hours after intracytoplasmic sperm injection by the presence of two equally sized pronuclei. Embryo culture was performed in a single 25 µl microdrop CSCM in a benchtop incubator (MINC, Cook Medical, USA) in a controlled humidified atmosphere until the fully expanded blastocyst stage (Day 5).

After laser-assisted zona dehiscence, a trophectoderm biopsy was performed. Biopsies were obtained from all embryos that developed as viable blastocysts, regardless of their morphological quality and/or full expansion days. After trophectoderm biopsy, collapsed blastocysts were vitrified with Cryotop devices and solutions (Kitazato BioPharma Co., Japan).

Embryo vitrification protocol

Vitrification was performed using the Cryotop device and solutions (Kitazato BioPharma Co., Japan). Initial equilibration was carried out in 7.5% ethylene glycol and 7.5% dimethyl sulfoxide at room temperature for 12-15 minutes. Embryos were then transferred for 1 minute into 15% ethylene glycol, 15% dimethyl-sulfoxide, and 0.5 M sucrose, then placed on the Cryotop film strip as a single small drop. Excess solution was removed leaving only a thin layer around each embryo and the Cryotop was submerged in liquid nitrogen. The strip was capped, and the sample was stored by immersion in liquid nitrogen.

Embryo thawing protocol

On warming, the Cryotops were removed from liquid nitrogen and the Cryotop's filmstrip was rapidly immersed in 1 ml of 37°C warming solution containing 1.0 M sucrose for 1 minute, then the oocytes and embryos were transferred to a room temperature solution containing 0.5 M sucrose and incubated for 3 minutes. After two consecutive washings in a basic medium at room temperature for 6 minutes each, the embryos were placed in a 1 ml culture medium (Cleavage medium, Sage).

Pregenetic diagnostic analysis

Trophectoderm biopsy was performed on the 5th embryos [31]. All biopsy procedures were performed in 10 ml of HEPES buffered medium (Quinn's Advantage, Cooper Surgical) coated with pre-equilibrated mineral oil. The laser was used to help drill a 10–20-micron hole into the embryo's outer wall. 5-10 trophectoderm cells were then aspirated into a trophectoderm biopsy pipette (research instrument), and the cells were removed from the embryo body with the help of the laser. All embryos were frozen by vitrification after the biopsy and sent to the genetics laboratory for chromosome analysis.

Embryo transfer

After the detection of euploid embryos and spontaneous follicle development, which began on the 7th day of the cycle, the patients were called to the outpatient clinic every 2 days for vaginal ultrasonography and LH measurements. Embryo transfers were performed with the natural cycle transfer on the 7th day of ovulation. Dydrogesterone 10 mg was administered 3 times a day for luteal support.

Statistical analysis

Descriptive statistics were used to define continuous variables (mean, standard deviation, minimum, median, maximum). Frequencies (n) and percentages (%) were used to define the categorical variables. Independent and non-normally distributed continuous variables were compared with the Mann-Whitney U test, and two independent and normally distributed continuous variables were compared with the student's t-test. Chi-Square (or Fisher Exact test, where appropriate) was used to examine the relationship between the categorical variables.

The statistical significance level was set at 0.05 and SPSS 24.0 program was used for all statistical analyses.

Results

One hundred and thirty-four patients who had previously failed treatment with the antagonist protocol were evaluated retrospectively. In the FPS and LPS stimulation steps of the dual stimulation performed in the same cycle, AMH, BMI, age-related ploidy ratios, in addition to the ploidy rates of the two treatments were comparatively evaluated (Tables 1 and 2).

Table 1: Comparisons according to FSP

	FSP 0 (n=96)		FSP 1 (n=37)		
	Mean (SD)	Med. (Min-Max)	Mean (SD)	Med. (Min-Max)	*P*-value
Age	36(4)	38 (26-40)	34(4)	35 (27-40)	0.001
AMH	0.98(0.58)	0.98 (0.01-2.34)	1.27(0.5)	1.23 (0.14-2.4)	0.004
BMI	26(4)	26 (19-37)	28(4)	28 (20-33)	0.058
FSH blood level	9.04(2.25)	9 (5-14)	7.97(1.98)	8 (5-13)	0.008
LH blood level	9.35(1.82)	9 (6-13)	8.57(1.52)	8 (6-12)	0.025
Afc	5.64(2.19)	6 (1-10)	6.81(1.65)	7 (4-12)	0.009
E2 blood level	31.3(8.64)	31.5 (17-54)	33.1(7.65)	34 (17-49)	0.232
FSP oocyte count	1.82(1.2)	2 (0-6)	2.38(1.01)	2 (0-5)	0.011
LPS oocyte count	2.64(1.44)	2 (0-7)	3.11(1.02)	3 (1-5)	0.024

Mann-Whitney U test, FSP: Follicular stimulation protocol, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, Afc: Antral Follicle Count, E2: Estradiol

Table 2: Comparisons according to LSP

	LSP 0 (n=55)		LSP 1 (n=78)		
	Mean (SD)	Med. (Min-Max)	Mean (SD)	Med. (Min-Max)	*P*-value
Age	36(4)	38 (26-40)	35(4)	36 (27-40)	0.017
AMH	0.91(0.55)	0.9 (0.01-2.34)	1.17(0.57)	1.2 (0.1-2.4)	0.003
BMI	26(4)	27 (19-35)	27(4)	27 (19-37)	0.847
FSH blood level	9.18(2.18)	9 (5-14)	8.44(2.21)	8 (5-14)	0.026
LH blood level	9.49(1.73)	9 (7-13)	8.88(1.77)	9 (6-13)	0.071
Afc	5.64(2.34)	6 (1-10)	6.19(1.91)	6 (2-12)	0.264
E2 blood level	30.6(9)	31 (17-49)	32.63(7.87)	33 (18-54)	0.117
FSP oocyte count	1.84(1.23)	2 (0-6)	2.08(1.13)	2 (0-5)	0.283
LSP oocyte count	2.56(1.34)	2 (0-6)	2.91(1.34)	3 (1-7)	0.165

Mann-Whitney U test, FSP: Follicular stimulation protocol, LSP: Luteal stimulation protocol, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, Afc: Antral Follicle Count, E2: Estradiol

While ploidy rates in FPS and LPS were significant in terms of age and AMH values (P<0.05), they did not differ with BMI values. However, the rate of aneuploid embryo development in FPS was 81.8% while that in LPS was 18.2%, and the rates of euploid embryo development in FPS and LPS treatments were 34.6% and 18.2%, respectively. The pregnancy

rate with euploid embryos by LPS was significantly higher compared to that by FPS (P<0.05) (Table 3).

Table 3: The comparisons according to LSP and FSP

		LSP				
		0		1		
		n	%	n	%	*P*-value
FSP	0	45	81.8	51	65.4	0.049
	1	10	18.2	27	34.6	

Fisher's Exact test, FSP: Follicular stimulation protocol, LSP: Luteal stimulation protocol

The main goal was to compare the rates of euploid embryos after FPS and LPS. To find the cut-offs of age, AMH, FSH blood level, LH blood level, AFC, E2 (estradiol) blood level, FSP oocyte number, LPS and oocyte number, ROC analysis was performed with FSP=0, and LSP=1, which revealed no significant differences in the areas under the curve (AUC) (P>0.05). The cut-offs were uninterpretable.

Discussion

Dual stimulation aims to obtain a higher number of oocytes compared to FPS. However, the main reason may also be the synchronized follicular development due to hormonal levels in the LPS period [30]. This study aimed to show the specific step of dual stimulation in which more euploid embryos can be obtained in patients with poor ovarian response and to determine whether the euploid embryo ratio is affected by AMH, BMI, and age. All our patients had a weak response, were previously treated with the antagonist protocol, and they could not get pregnant. According to the poor ovarian criteria, the baby birth rate increases in women with controlled ovarian stimulation when stimulation is performed at most 3 times. However, these patients usually drop the process because they are tired of the treatment [34].

While the unsuccessful result is the first reason for discontinuation of treatment, the second reason is financial factors [35, 36]. In dual stimulation, using a double stimulation agent in one cycle and obtaining more embryos prevents the abandonment of the process and increases the chance of obtaining euploid embryos. Only 9% of patients continue controlled ovarian stimulation, which is low [36]. In our clinic, patients receive two-cycle treatments in dual stimulation although they pay the single cycle cost. We perform dual stimulation in patients who meet the Bologna criteria and had failed controlled ovarian stimulation, and a ploidy scan to achieve a successful pregnancy.

Embryo quality was better in luteal stimulation than in follicular phase stimulation in the previous studies, as in ours [17-19, 22]. However, the live birth rates between luteal and follicular phase stimulation protocols were similar in studies with large series [37, 38].

In LPS, the reason for a good quality oocyte is a possible exacerbation because of the GnRH agonist stimulation used in FPS. This anovulatory wave creates a downregulation of AMH expression and increases the number of follicles with a diameter of 3-4 mm in LPS. Of course, the effect of endocrine and paracrine factors should also be confirmed, because, in some studies of Luo et al. [39], there was no significant difference between embryo quality and ploidy rates of patients given FPS and LPS.

High levels of estrogen and progesterone during the luteal phase may induce a more synchronous follicular development and promote FSH receptors in the granulosa cells [30].

The possibility of obtaining a euploid blastocyst in either of the two phases of the ovarian cycle suggests that non-dominant follicles may become more prominent and develop randomly. In other words, the dominant follicle is not a competent follicle for a good embryo. This perspective may enable us to focus on wave theories in follicle development and provide a different understanding of ovarian physiology. Such an interesting topic could also trigger future studies.

To evaluate the ovarian, clinical, and even postnatal outcomes of LS, not only patients with a poor ovarian response but rather wider case series should be investigated. In this regard, Chen et al. [38] compared LS with the traditional method in 2015 and reported that there was no difference in terms of birth data and congenital anomalies. However, this study was also performed retrospectively.

LH selection in dual stimulation is intended to support steroidogenesis and folliculogenesis [40, 41]. LH increases androgen production and the stimulation of preantral/antral follicles as well as FSH receptor expression in granulosa cells [42]. All these features are important in patients with advanced maternal age, decreased androgen sensitivity due to age, and endogenously deficient androgen. In this group of patients, the ovarian response to exogenous FSH is also extremely weak [43]. Although a therapeutic LH dose is not recommended in antagonist protocols involving controlled ovarian stimulation, adding LH to the treatment in patients with poor response achieves a higher chance of success with low r-FSH doses [44]. Despite all this, dual stimulation is still debated by the scientific community.

The development of follicles was monitored in many animal models before being studied in women. Dual stimulation is deemed suitable for the treatment of patients with a poor response in terms of both ovulatory and anovulatory fluctuations. It is mostly recommended for patients who need to preserve fertility for medical reasons. The advantage of dual stimulation is obtaining more eggs and embryos in a single cycle. Especially in LS, higher blastocyst and euploidy rates compared to those obtained in FS supported the use of the dual protocol in patients who will experience ovarian failure due to medical reasons and who have time constraints [25]. However, more research is needed on this subject.

Limitation

For more generalizable results, larger study groups and more parameters are needed. However, we aimed to determine the stage of treatment at which a euploid embryo can be obtained. Perhaps the same rates of euploidy could be achieved in randomized sequential controlled hyperstimulation. The retrospective design was another limitation, and the duostim protocol should have had a matched control group. Another limitation is that it has already been proven that the success rate is higher in frozen embryos [32, 33]. More data on luteal phase stimulation alone are needed.

Conclusion

Our study emphasizes that follicles entering the anovulatory phase in the follicular phase can be saved by LPS. Although there is no comparable difference in number, oocyte

retrieval in the LPS phase provides clinical benefits for the patient. It also encourages clinicians to undertake additional clinical and laboratory studies that could radically change the approach to ovarian stimulation in the future.

References

1. Polat M, Mumusoglu S, Yarali Ozbek I, Bozdag G, Yarali H. Double or dual stimulation in poor ovarian responders: where do we stand? Ther Adv Reprod Health. 2021;15:1-15. doi: 10.1177/26334941211024172.
2. Briggs R, Kovacs G, MacLachlan V, Motteram C, Baker HW. Can you ever collect too many oocytes? Hum Reprod. 2015;30(1):81-7. doi: 10.1093/humrep/deu272.
3. Drakopoulos P, Blockeel C, Stoop D, Camus M, de Vos M, Tournaye H, et al. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? Hum Reprod. 2016;31(2):370-6. doi: 10.1093/humrep/dev316.
4. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. Hum Reprod. 2011;26(10):2593-7. doi: 10.1093/humrep/der251.
5. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. Hum Reprod Update. 2014;20(6):808-21. doi: 10.1093/humupd/dmu027.
6. Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number), Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril. 2016;105(6):1452-3. doi: 10.1016/j.fertnstert.2016.02.005.
7. Dahdouh EM, Balayla J, García-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. Fertil Steril. 2015;104(6):1503-12. doi: 10.1016/j.fertnstert.2015.08.038.
8. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2016; 30(6):CD002118. doi: 10.1002/14651858.CD002118.
9. Labarta E. DuoStim: a new strategy proposed for women with poor ovarian response. Fertil Steril. 2020; 113:76–7.
10. Massin N. New stimulation regimens: endogenous and exogenous progesterone use to block the LH surge during ovarian stimulation for IVF. Hum Reprod Update. 2017; 23:211–20.
11. Cobo A, Garrido N, Crespo J, Jose R, Pellicer A. Accumulation of oocytes: a new strategy for managing low-responder patients. Reprod BioMed Online. 2012; 24:424–32.
12. Ubaldi FM, Cimadomo D, Vaiarelli A, Fabozzi G, Venturella R, Maggiulli R, et al. Advanced maternal age in IVF: still a challenge? The present and the future of its treatment. Front Endocrinol (Lausanne). 2019; 10:94. doi: 10.3389/fendo.2019.00094
13. Vaiarelli A, Cimadomo D, Argento C, Ubaldi N, Trabucco E, Drakopoulos P, et al. Double stimulation in the same ovarian cycle (DuoStim) is an intriguing strategy to improve oocyte yield and the number of competent embryos in a short timeframe. Minerva Ginecol. 2019; 71:372–6.
14. Ferraretti AP, La Marca A, Fauser,BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum. Reprod. 2011; 26: 1616–24
15. Surrey ES, Schoolcraft WB. Evaluating strategies for improving ovarian response_of the poor responder undergoing assisted reproductive techniques. Fertil Steril. 2000; 73: 667–76.
16. Jin B, Niu Z, Xu B, Chen Q, Zhang A. Comparison of clinical outcomes among dual ovarian stimulation, mild stimulation and luteal phase stimulation protocols in women with poor ovarian response. Gynecol Endocrinol. 2018; 34:694–7.
17. Alsbjerg B, Haahr T, Elbaek HO, Laursen R, Povlsen BB, Humaidan P. Dual stimulation using corifollitropin alfa in 54 Bologna criteria poor ovarian responders- a case series. Reprod BioMed Online. 2019; 38:677–82.
18. Bourdon M, Santulli P, Maignien C, Pocate-Cheriet K, Marcellin L, Chen Y, et al. The ovarian response after follicular versus luteal phase stimulation with a double stimulation strategy. Reprod Sci. 2020; 27:204–10.
19. Kuang Y, Chen Q, Hong Q, Lyu Q, Ai A, Fu Y, et al. Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol). Reprod BioMed Online. 2014;29: 684–91.
20. Liu C, Jiang H, Zhang W, Yin H. Double ovarian stimulation during the follicular and luteal phase in women>/=38 years: a retrospective case- control study. Reprod BioMed Online. 2017; 35:678–84.
21. Rashtian J, Zhang J. Luteal-phase ovarian stimulation increases the number of mature oocytes in older women with severe diminished ovarian reserve. Syst Biol Reprod Med. 2018; 64:216–9.
22. Ubaldi FM, Capalbo A, Vaiarelli A, Cimadomo D, Colamaria S, Alviggi C, et al. Follicular versus luteal phase ovarian stimulation during the same menstrual cycle (DuoStim) in a reduced ovarian reserve population results in a similar euploid blastocyst formation rate: new insight in ovarian reserve exploitation. Fertil Steril. 2016; 105:1488–95.
23. Vaiarelli A, Cimadomo D, Trabucco E, Vallefuoco R, Buffo L, Dusi L, et al. Double stimulation in the same ovarian cycle (DuoStim) to maximize the number of oocytes retrieved from poor prognosis patients: a multicenter experience and SWOT analysis. Front Endocrinol (Lausanne). 2018; 9:317.
24. Vaiarelli A, Cimadomo D, Conforti A, Schimberni M, Giuliani M, D'Alessandro P, et al. Luteal phase after conventional stimulation in the same ovarian cycle might improve the management of poor responder patients fulfilling the Bologna criteria: a case series. Fertil Steril. 2020;113(1):121-30. doi: 10.1016/j.fertnstert.2019.09.012.
25. Vaiarelli A, Cimadomo D, Petriglia C, Conforti A, Alviggi C, Ubaldi N, et al. DuoStim- a reproducible strategy to obtain more oocytes and competent embryos in a short time-frame aimed at fertility preservation and IVF purposes. A systematic review. Ups J Med Sci. 2020;125(2):121-30. doi: 10.1080/03009734.2020.1734694.
26. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum Reprod Update. 2012;18(1):73-91. doi: 10.1093/humupd/dmr039.: 22068695.
27. Vaiarelli A, Venturella R, Vizziello D, Bulletti F, Ubaldi FM. Dual ovarian stimulation and random start in assisted reproductive technologies: from ovarian biology to clinical application. Curr Opin Obstet Gynecol. 2017;29(3):153-9. doi: 10.1097/GCO.0000000000000365.
28. Drakopoulos P, Errazuriz J, Santos-Ribeiro S, Tournaye H, Vaiarelli A, Pluchino N, et al. Cumulative live birth rates in IVF. Minerva Ginecol. 2019; 71:207–10.
29. Bosch E, Bulletti C, Copperman AB, Fanchin R, Yarali H, Petta CA, et al. How time to healthy singleton delivery could affect decision-making during infertility treatment: a Delphi consensus. Reprod Biomed Online. 2019; 38:118–30.
30. Cimadomo D, Vaiarelli A, Colamaria S, Trabucco E, Alviggi C, Venturella R, et al. Luteal phase anovulatory follicles result in the production of competent oocytes: intra-patient paired case-control study comparing follicular versus luteal phase stimulations in the same ovarian cycle. Hum Reprod. 2018;33(8):1442-8. doi: 10.1093/humrep/dey217.
31. Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. Hum Reprod. 2013;28(8):2298-307. doi: 10.1093/humrep/det245.
32. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. Hum Reprod Update. 2014;20(6):808-21. doi: 10.1093/humupd/dmu027.
33. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update. 2018;24(1):35-58. doi: 10.1093/humupd/dmx031.
34. Ke H, Chen X, Liu YD, Ye DS, He YX, Chen SL. Cumulative live birth rate after three ovarian stimulation IVF cycles for poor ovarian responders according to the bologna criteria. J Huazhong Univ Sci Technolog Med Sci. 2013;33(3):418-22. doi: 10.1007/s11596-013-1134-7.
35. Kulkarni G, Mohanty NC, Mohanty IR, Jadhav P, Boricha BG. Survey of reasons for discontinuation from in vitro fertilization treatment among couples attending infertility clinic. J Hum Reprod Sci. 2014;7(4):249-54. doi: 10.4103/0974-1208.147491.
36. Troude P, Guibert J, Bouyer J, de La Rochebrochard E; DAIFI Group. Medical factors associated with early IVF discontinuation. Reprod Biomed Online. 2014;28(3):321-9. doi: 10.1016/j.rbmo.2013.10.018.
37. Cimadomo D, Carmelo R, Parrotta EI, Scalise S, Santamaria G, Alviggi E, et al. Similar miRNomic signatures characterize the follicular fluids collected after follicular and luteal phase stimulations in the same ovarian cycle. J Assist Reprod Genet. 2020;37(1):149-58. doi: 10.1007/s10815-019-01607-6.
38. Chen H, Wang Y, Lyu Q, Ai A, Fu Y, Tian H, et al. Comparison of live-birth defects after luteal-phase ovarian stimulation vs. conventional ovarian stimulation for in vitro fertilization and vitrified embryo transfer cycles. Fertil Steril. 2015;103(5):1194-201.e2. doi: 10.1016/j.fertnstert.2015.02.020.
39. Luo Y, Sun L, Dong M, Zhang X, Huang L, Zhu X, et al. The best execution of the DuoStim strategy (double stimulation in the follicular and luteal phase of the same ovarian cycle) in patients who are poor ovarian responders. Reprod Biol Endocrinol. 2020;18(1):102. doi: 10.1186/s12958-020-00655-3.
40. Yang DZ, Yang W, Li Y, He Z. Progress in understanding human ovarian folliculogenesis and its implications in assisted reproduction. J Assist Reprod Genet. 2013;30(2):213-9. doi: 10.1007/s10815-013-9944-x.
41. Mochtar MH, Danhof NA, Ayeleke RO, Van der Veen F, van Wely M. Recombinant luteinizing hormone (rLH) and recombinant follicle stimulating hormone (rFSH) for ovarian stimulation in IVF/ICSI cycles. Cochrane Database Syst Rev. 2017;5(5):CD005070. doi: 10.1002/14651858.CD005070.
42. Balasch J, Fábregues F, Casamitjana R, Peñarrubia J, Vanrell JA. A pharmacokinetic and endocrine comparison of recombinant follicle-stimulating hormone and human menopausal gonadotrophin in polycystic ovary syndrome. Reprod Biomed Online. 2003;6(3):296-301. doi: 10.1016/s1472-6483(10)61848-2.
43. Alviggi C, Conforti A, Esteves SC, Andersen CY, Bosch E, Bühler K, et al. Recombinant luteinizing hormone supplementation in assisted reproductive technology: a systematic review. Fertil Steril. 2018;109(4):644-64. doi: 10.1016/j.fertnstert.2018.01.003.
44. Mennini FS, Marcellusi A, Viti R, Bini C, Carosso A, Revelli A, et al. Probabilistic cost-effectiveness analysis of controlled ovarian stimulation with recombinant FSH plus recombinant LH vs. human menopausal gonadotropin for women undergoing IVF. Reprod Biol Endocrinol. 2018;16(1):68. doi: 10.1186/s12958-018-0386-2.

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A novel diagnostic tool in determining insulin resistance in obese children: Triglyceride / HDL ratio

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Abstract

Background/Aim: It is known that the frequency of obesity and insulin resistance is increased in children. Diagnostic laboratory criteria of insulin resistance are unclear in children despite the widespread use of the Homeostasis model assessment for insulin resistance (HOMA-IR). Serum triglyceride (TG)/high-density lipoprotein (HDL) ratio is reported to reflect insulin resistance in recent studies. We aimed to investigate the usefulness of the TG/HDL ratio in determining insulin resistance and its correlation with the HOMA-IR index in overweight/obese children.

Methods: Patients who presented to the Pediatric Endocrinology outpatient clinic with excessive weight were examined in this retrospective cohort study. The patients were divided into subgroups according to BMI, sex, pubertal stage, and presence of hepatosteatosis, and the HOMA-IR index and TG/HDL ratios were compared.

Results: One hundred and fifty-nine patients aged 5-18 years, with a mean age of 11.4 (3.13) years, were included in the study. Of the patients, 93 (58%) were girls, 99 (62%) were pubertal, and 141 (88.6%) were obese. Seventy-nine (49.6%) patients had insulin resistance. The mean HOMA-IR and TG/HDL were 3.89 (2.29) and 2.71 (1.86), respectively. A positive correlation was found between HOMA-IR value and TG / HDL ratio (r= 0.283, P<0.001). TG/HDL ratio was significantly higher in patients who had insulin resistance and hepatosteatosis than those who did not (P=0.008, P=0.032 respectively). There was no significant difference regarding TG/HDL ratio between female-male, prepubertal-pubertal, overweight/obese patients. There was no correlation between age, body mass index standard deviation score (SDS), and TG/HDL ratio.

Conclusion: TG/HDL ratio is an easy to use, non-invasive, and useful marker of insulin resistance in overweight/obese children regardless of age, gender, pubertal condition, and body mass index standard deviation score.

Keywords: TG / HDL ratio, Insulin resistance, Obese children

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Ethics Committee Approval
The permission was obtained from Gazi Yaşargil Research and Training Hospital ethics committee with 15.1.2021/614 number.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Introduction

The incidence of obesity in children is gradually increasing due to the widespread high-calorie diet and sedentary lifestyle. Obesity, dyslipidemia, hypertension, and glucose metabolism disorder are all components of metabolic syndrome. Insulin resistance and the presence of metabolic syndrome in children are predictors of type 2 diabetes and cardiovascular diseases [1].

The prevalence of dyslipidemia in obese children in Turkey is 21.5% [2]. Many studies showed that high triglyceride (TG) and low high-density lipoprotein (HDL) levels lead to insulin resistance. High TG in serum activates lipoprotein lipase via heparin. Intravascular lipolysis increases and the tissues are exposed to high free fatty acid. High free fatty acids cause insulin resistance through oxidative stress pathways [3]. Insulin resistance and β-cell dysfunction of the pancreas are the two main causes of type 2 diabetes. It has been reported that low HDL and high TG in the circulation can cause cholesterol accumulation in β cells of the pancreas, cellular toxicity, and impairment in insulin secretion, which can directly cause type 2 diabetes [4].

The gold standard diagnostic method in detecting insulin resistance is the hyperinsulinemic-euglycemic clamp method, but this method has little applicability. Instead, "Homeostasis model assessment for insulin resistance (HOMA-IR)" is widely used in determining insulin resistance in children and adults. The HOMA-IR index is affected by many parameters such as age, gender, puberty, and body mass index (BMI) [5]. The fact that HOMA-IR has different threshold values according to gender and puberty confuses clinical practice from time to time. It has recently been reported that the TG / HDL ratio is an easy, non-invasive and useful marker for determining insulin resistance and cardiovascular disease risk [6]. This study aimed to determine the usability of the TG / HDL ratio in determining insulin resistance and its threshold value in overweight/obese children.

Materials and methods

For this study, permission was obtained from the Gazi Yaşargil Research and Training Hospital ethics committee (decision number: 15.1.2021/614). Overweight/obese patients aged 5-18 years who visited the pediatric endocrinology outpatient clinic in Balıkesir Atatürk City Hospital with the complaint of being overweight were included. Patients with syndromic obesity, Cushing's syndrome, chronic disease, or regular medication use, and abnormal thyroid function tests were excluded. Age, gender, anthropometric measurements, pubertal development, the presence of acanthosis nigricans, laboratory tests (glucose, insulin, TG, HDL, total cholesterol, LDL, AST, ALT), and liver ultrasonography findings were recorded retrospectively from the patient files. Height and weight measurements were made with a digital Harpenden stadiometer. Weight, height, BMI (body weight / height^{2,}) and standard deviation scores (SDS) were calculated. Patients with BMI> 2 SDS were considered obese, those with + 1 / + 2 SDS were considered overweight. Testicular volume in males was measured with a Prader orchidometer. Males with a testicular volume of greater than 4 ml, and females with tanner stage≥2 breast development were considered pubertal. HOMA-IR value was calculated with the formula: Fasting insulin level (μU / mL) x fasting glucose (mg / dL) / 405. If the HOMA-IR value was >2.67 in prepubertal males, >2.22 in females, >5.22 in pubertal males, and >3.82 in females, insulin resistance was considered present [7]. The ratio of TG (mg / dL) to HDL (mg / dL) was recorded as TG/HDL value.

Statistical analysis

The sample size was calculated using G*Power version 3 based on the ability to detect a mean difference of TG/HDL ratio between two groups (with insulin resistance, without insulin resistance). The standard deviation used in the calculation was reported in previous trials (Liang et al.) [5]. A confidence level of 95% (P<0.05) and a power of 80% were used. The calculation yielded a minimum sample size of 139. A further increase of 15% was added to allow for attrition, giving an overall sample size of 159 participants. SPSS 24.0 package program was used for statistical analysis. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether the data were normally distributed. Descriptive statistics were used. Mann Whitney U and Kruskal Wallis tests were utilized for the non-normally distributed groups. Spearman correlation analysis was performed to determine the relationship between TG/HDL ratio, age, sex, puberty, HOMA-IR, and other parameters. A P-value of <0.05 was considered statistically significant.

Results

A total of 159 patients aged 5-18 years with a mean age of 11.4 (3.13) years were included in the study. Ninety-three (58%) patients were females, 99 (62%) were pubertal, 18 (11.3%) were overweight, and 141 (88.6%) were obese. The clinical and laboratory characteristics of the patients were shown in Table 1. HOMA-IR was above the threshold value in 79 (49.6%) patients according to gender and pubertal status and insulin resistance were present. Acanthosis nigricans was present in 61 of 79 patients with insulin resistance. In 70 (44%) patients, no fatty liver was detected in ultrasonography. First, second- and third-degree fatty liver were detected in 52 patients, 25 patients, and 2 patients, respectively.

Table 1: The clinical and laboratory finding of patients

	Mean(SD)	(Minimum-Maximum)
Age (year)	11.4(3.13)	(5-18)
Weight (SDS)	2.84(1.05)	(0.13-6.52)
Height (SDS)	0.74(1.27)	(-2.28-6.3)
BMI SDS	2.62(0.63)	(1.1-4.8)
Glucose (mg/dL)	89.71(7.1)	(69-123)
Insulin (μU/mL)	17.71(6.33)	(2-48)
HOMA-IR	3.89(2.29)	(0.44-11.54)
TG/HDL	2.71(1.86)	(0.35-11.28)
Triglyceride (mg/dL)	117.58(61.18)	(28-361)
HDL (mg/dL)	47.68(9.68)	(25-81)
Total Cholesterol (mg/dL)	157.97(32.23)	(42-266)
LDL (mg/dL)	89.15(26.65)	(33-171)
AST (U/L)	23.07(7.31)	(10-62)
ALT (U/L)	22(11.8)	(8-68)

SDS: Standard Deviation Score, BMI: Body Mass Index, HOMA-IR: Homeostasis model assessment for insulin resistance, HDL: High-Density Lipoprotein, TG/HDL: Triglyceride/High-Density Lipoprotein

The mean HOMA-IR value of all patients was 3.89 (2.29), and the mean TG / HDL ratio was 2.71 (1.86) (Table 1). There was no difference in HOMA-IR values between the female-male, overweight/obese patients, and patients with and without fatty liver groups. HOMA-IR values of pubertal patients were significantly higher than those of prepubertal patients

(P<0.001) (Table 2). HOMA-IR was positively correlated with age, weight SDS, BMI SDS, glucose, insulin, TG, and negatively correlated with HDL (Table 3). A positive correlation was found between the HOMA-IR value and the TG/HDL ratio (r=0.283, P<0.001) (Table 3).

Table 2: The comparison of HOMA-IR ve TG/HDL ratio according to groups

	HOMA-IR Median(IQR)	TG/HDL Median(IQR)
male	3.23 (IQR 2.99)	1.92(IQR 1.71)
female	3.24 (IQR 2.81)	2.18(IQR 2.21)
P-value *	0.296	0.268
Prepubertal	2.49(IQR 1.49)	1.89(IQR 1.49)
Pubertal	4.3(IQR 2.85)	2.27(IQR 2.6)
P-value *	<0.001	0.114
No insulin resistance	2.29(IQR 1.29)	1.78(IQR 1.48)
Insulin resistance	5.21(IQR 3.43)	2.63(IQR 2.22)
P-value *	<0.001	0.008
Overweight	2.39(IQR 2.92)	2.07±2.93
Obese	3.32(IQR 2.95)	2.02(IQR2.06)
P-value **	0.118	0.733
No hepatosteatosis	2.92(IQR 2.65)	1.86(IQR 1.48)
Hepatosteatosis	4.15(IQR 3.04)	2.3(IQR 2.61)
P-value *	0.068	0.032

*Mann Whitney U test **Kruskal Wallis test IQR: Interquartile Range, Homa-IR: Homeostasis model assessment for insulin resistance, HDL: High-Density Lipoprotein, TG/HDL: Triglyceride/High-Density Lipoprotein

Table 3: The correlation between HOMA-IR, TG/HDL ratio, and other parameters

	HOMA-IR		TG/HDL	
	r	*P*-value	r	*P*-value
Age (year)	0.306	<0.001	0.14	0.08
Weight SDS	0.296	<0.001	0.079	0.323
Height SDS	0.066	0.415	-0.004	0.957
BMI SDS	0.276	<0.001	0.067	0.403
Glucose (mg/dL)	0.394	<0.001	0.08	0.318
Insulin (µU/mL)	0.986	<0.001	0.281	<0.001
Triglyceride (mg/dL)	0.248	0.002	0.955	<0.001
HDL (mg/dL)	-0.309	<0.001	-0.671	<0.001
Total Cholesterol (mg/dL)	-0.041	0.607	0.199	0.012
LDL (mg/dL)	-0.069	0.388	0.03	0.710
AST (U/L)	-0.211	0.008	0.012	0.886
ALT (U/L)	0.03	0.706	0.09	0.261
TG/HDL	0.283	<0.001		

SDS: Standard Deviation Score, BMI: Body Mass Index, Homa-IR: Homeostasis model assessment for insulin resistance, HDL: High-Density Lipoprotein, TG/HDL: Triglyceride/High-Density Lipoprotein

The TG / HDL ratio was significantly higher in patients with insulin resistance and fatty liver compared to those without (P=0.032) (Table 2). There was no difference in TG/HDL ratio between female-male gender, prepubertal-pubertal, overweight/obese patients (P=0.268 P=0.114 and P=0.733 respectively) (Table 2). There was no correlation between age and TG / HDL ratio, and TG / HDL ratio was positively correlated with insulin, TG, and total cholesterol, and negatively correlated with HDL cholesterol (Table 3).

Discussion

In 2003, McLaughlin reported for the first time that the TG/HDL ratio in white obese patients reflects insulin resistance, but changes with ethnicity [8]. In recent studies, it has been reported that TG/HDL ratio is strongly associated with insulin resistance in white obese children, especially in Korea and Southeast Asia, but not in Hispanic and black children. [9-11]. In this study, the TG/HDL ratio was significantly higher in overweight/obese children with IR compared to those without IR and correlated with HOMA-IR while it did not change according to age, gender, and pubertal status.

In determining insulin resistance, Behiry et al. [12], Iwani et al. [11], and Yoo et al. [1] reported threshold TG/HDL ratios of 1.36, 1.11, and 2, respectively, among 90, 425, and 769 overweight/obese children, respectively. Pacifico et al. [13] stated a TG/HDL ratio cut-off value of 1.98 to indicate insulin resistance and metabolic syndrome among 541 children, 391 of which were obese. According to Di bonito et al. [14], this value was 2.2 among 5505 children, 4417 of which were obese. We could not determine a TG/HDL cut-off due to the low under the ROC curve area. However, TG / HDL ratio was significantly higher in overweight/obese children with IR compared to those without IR.

It is known that the HOMA-IR value, which is widely used to determine insulin resistance in children, varies according to gender, puberty, and BMI SDS. During puberty, physiological temporary insulin resistance occurs. A decrease in insulin sensitivity in the pubertal period causes an increase in insulin secretion.

It has been shown that insulin resistance increases at the beginning of puberty, peaks at Tanner stage 3, and regresses to prepubertal levels at the end of puberty. The reason for this change in insulin secretion at puberty is not fully known, but it is thought to be related to a mechanism that enhances the anabolic effect of insulin and growth hormone during rapid somatic growth [7]. It has been reported that increased pubertal hormones and changes in fat distribution in girls during adolescence cause higher insulin secretion and resistance compared to boys [15]. We found that HOMA-IR is higher in girls and pubertal children, it increases with increasing age and BMI SDS. The HOMA-IR index is calculated based on serum insulin and glucose levels. Therefore, physiological changes in serum insulin levels cause changes in the HOMA-IR index. The serum TG level used in the TG/HDL ratio, which reflects insulin resistance regardless of the serum insulin level, shows a small change according to age and does not change according to puberty and gender (normal value for TG is 75-99 mg / dL between 0-9 years of age, and 90-129 mg/dL thereafter). HDL remains the same in all age groups (35-45 mg / dL) [16]. For this reason, a single threshold value for TG/HDL ratio can be given independently of gender and puberty in children. Iwani et al. [11] reported that TG / HDL ratio was positively correlated with HOMA-IR and TG / HDL does not change with age, gender, and pubertal status in 271 overweight / obese children. Yoo et al. [1] reported that there was a positive correlation between HOMA-IR and TG / HDL. Çin et al. [17] found a positive correlation between TG / HDL ratio and HOMA-IR in obese adolescents. These results are consistent with our study.

We found no differences between overweight and obese patients in terms of TG/HDL ratio. It was higher in those with fatty liver than those without. Sixty-five percent (n: 52) of the patients with liver steatosis had first-degree steatosis, which suggests that the TG/HDL ratio may be an early marker for detecting insulin resistance and fatty liver.

Metabolic syndrome and impaired glucose tolerance are closely related to insulin resistance. In a study with 122 obese children, Krawcyzk et al. [18] reported that the TG / HDL ratio was 2.09 times higher in children with metabolic syndrome than in those without. In a study conducted in our country, the threshold of TG / HDL ratio to indicate metabolic syndrome was 2.16 in 1171 obese adolescents [17]. Manco et al. [19] reported that a TG/HDL ratio above 2.2 is associated with impaired glucose tolerance. In our study, the patients were evaluated in terms of insulin resistance, but not in terms of metabolic syndrome and glucose intolerance. This was thought to cause a limitation in evaluating the results of our study.

Conclusion

TG/HDL ratio is an easy, non-invasive, and useful indicator of insulin resistance independent of age, gender, pubertal status, and BMI SDS in overweight/obese children. Large-scale studies are needed to determine the optimum TG/HDL ratio threshold value in detecting insulin resistance in our country.

References

1. Yoo DY, Kang YS, Kwon EB, Yoo EG. The triglyceride-to-high density lipoprotein cholesterol ratio in overweight Korean children and adolescents. Ann Pediatr Endocrinol Metab. 2017;22(3):158-63.
2. Elmaoğulları S, Tepe D, Uçaktürk SA, Karaca Kara F, Demirel F. Prevalence of Dyslipidemia and Associated Factors in Obese Children and Adolescents. J Clin Res Pediatr Endocrinol. 2015;7(3):228-34.
3. Zhou M, Zhu L, Cui X, Feng L, Zhao X, He S, et al. The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio as a predictor of insulin resistance but not of β cell function in a Chinese population with different glucose tolerance status. Lipids Health Dis. 2016;15:104.
4. Lim TK, Lee HS, Lee YJ. Triglyceride to HDL-cholesterol ratio and the incidence risk of type 2 diabetes in community dwelling adults: A longitudinal 12-year analysis of the Korean Genome and Epidemiology Study. Diabetes Res Clin Pract. 2020;163:108150.
5. Liang J, Fu J, Jiang Y, Dong G, Wang X, Wu W. TriGlycerides and high-density lipoprotein cholesterol ratio compared with homeostasis model assessment insulin resistance indexes in screening for metabolic syndrome in the chinese obese children: a cross section study. BMC Pediatr. 2015;15:138.
6. Nur Zati Iwani AK, Jalaludin MY, Wan Mohd Zin RM, Fuziah MZ, Hong JYH, Abqariyah Y, et al. TG : HDL-C Ratio Is a Good Marker to Identify Children Affected by Obesity with Increased Cardiometabolic Risk and Insulin Resistance. Int J Endocrinol. 2019;2019:8586167.
7. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu M, Kendirici M, Keskin M, Kondolot M. Insulin resistance in obese children and adolescents: HOMA-IR cut-off levels in the prepubertal and pubertal periods. J Clin Res Pediatr Endocrinol. 2010;2(3):100-6.
8. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. Ann Intern Med. 2003 Nov 18;139(10):802-9. doi: 10.7326/0003-4819-139-10-200311180-00007. PMID: 14623617.
9. Giannini C, Santoro N, Caprio S, Kim G, Lartaud D, Shaw M, et al. The triglyceride-to-HDL cholesterol ratio: association with insulin resistance in obese youths of different ethnic backgrounds. Diabetes Care. 2011;34(8):1869-74.
10. Sumner AE, Finley KB, Genovese DJ, Criqui MH, Boston RC. Fasting triglyceride and the triglyceride-HDL cholesterol ratio are not markers of insulin resistance in African Americans. Arch Intern Med. 2005;165(12):1395-400.
11. Iwani NA, Jalaludin MY, Zin RM, Fuziah MZ, Hong JY, Abqariyah Y, et al. Triglyceride to HDL-C Ratio is Associated with Insulin Resistance in Overweight and Obese Children. Sci Rep. 2017;7:40055.
12. Behiry EG, El Nady NM, AbdEl Haie OM, Mattar MK, Magdy A. Evaluation of TG-HDL Ratio Instead of HOMA Ratio as Insulin Resistance Marker in Overweight and Children with Obesity. Endocr Metab Immune Disord Drug Targets. 2019;19(5):676-82.
13. Pacifico L, Bonci E, Andreoli G, Romaggioli S, Di Miscio R, Lombardo CV, et al. Association of serum triglyceride-to-HDL cholesterol ratio with carotid artery intima-media thickness, insulin resistance and nonalcoholic fatty liver disease in children and adolescents. Nutr Metab Cardiovasc Dis. 2014;24(7):737-43.
14. Di Bonito P, Valerio G, Grugni G, Licenziati MR, Maffeis C, Manco M, et al. Cardiometabolic risk factors in overweight and obese children in ITALY (CARITALY) Study Group. Comparison of non-HDL-cholesterol versus triglycerides-to-HDL-cholesterol ratio in relation to cardiometabolic risk factors and preclinical organ damage in overweight/obese children: the CARITALY study. Nutr Metab Cardiovasc Dis. 2015;25(5):489-94.
15. Moran A, Jacobs DR, Steinberger J, Hong CP, PrineasR, Luepker R, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 1999;48:2039-44.
16. Tümer L, Kasapkara ÇS. Çocuklarda Hiperlipidemi Taraması. Turkiye Klinikleri J Pediatr. 2013;22(4):171-7.
17. Aslan Çin NN, Yardımcı H, Koç N, Uçaktürk SA, Akçil Ok M. Triglycerides/high-density lipoprotein cholesterol is a predictor similar to the triglyceride-glucose index for the diagnosis of metabolic syndrome using International Diabetes Federation criteria of insulin resistance in obese adolescents: a cross-sectional study. J Pediatr Endocrinol Metab. 2020 24;33(6):777-84.
18. Krawczyk M, Rumińska M, Witkowska-Sędek E, Majcher A, Pyrżak B. Usefulness of the Triglycerides to High-Density Lipoprotein Cholesterol ratio (TG/HDL-C) in prediction of metabolic syndrome in Polish obese children and adolescents. Acta Biochim Pol. 2018;65(4):605-11.
19. Manco M, Grugni G, Di Pietro M, Balsamo A, Di Candia S, Morino GS, et al. Triglycerides-to-HDL cholesterol ratio as screening tool for impaired glucose tolerance in obese children and adolescents. Acta Diabetol. 2016;53(3):493-8.

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A case of incidental pulmonary benign metastasizing leiomyoma

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Informed Consent
The authors stated that the written consent was obtained from the parents of the patient presented with images in the study.

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Abstract

Benign metastasizing leiomyoma is exceedingly rare. Pulmonary benign metastasizing leiomyoma is defined as the metastasis of leiomyoma to the lung tissue. It has a benign histological character, but the presence of metastasis is contradictory. First reported in 1937, it is generally seen among women of young reproductive age and premenopausal women who have undergone uterine leiomyoma or hysterectomy, and the most common site of metastasis is the lung. The prognosis is generally very favorable, and it rarely undergoes a malignant transformation. However, various studies suggest that pulmonary and pleural leiomyoma may cause acute respiratory failure. Radiological findings have a wide spectrum ranging from primary malignant neoplasms to metastatic diseases. Benign metastasizing leiomyoma is usually incidentally found on chest X-rays, and it appears as nodules arising from the benign proliferation of smooth muscles. Here, we present a 46-year-old patient who had a hysterectomy 15 years ago due to uterine leiomyoma and was recently diagnosed with benign leiomyoma which metastasized to the lung. It should be kept in mind that benign leiomyomas with pulmonary metastasis may rarely undergo malignant transformation.

Keywords: Leiomyoma, Hysterectomy, Neoplasm, Metastasis

Introduction

Benign metastasizing leiomyoma, a rare condition with different clinical and radiological features, was first reported by Steiner in 1939 [1]. It usually occurs in women of reproductive age and with a history of uterine leiomyoma. Less than 200 cases have been reported in the literature [2]. In the presence of leiomyoma with benign metastasis, a history of uterine leiomyoma should be questioned.

Benign metastasizing leiomyoma can be seen in many parts of the body, including the lungs the most, and the lymph nodes, muscles, heart, bones, and the central nervous system [3]. Clinical symptoms vary depending on the region of involvement. Most patients are asymptomatic, and the lung lesions are detected incidentally [4]. A case of secondary respiratory failure due to cardiac involvement of the cystic leiomyoma and another one due to the involvement of the left lung lower lobe bronchus were reported in the literature [5].

In chest radiographs, benign pulmonary metastatic leiomyoma typically manifests as multiple pulmonary nodules. Unusual appearances, such as a miliary pattern, and a pedicular pulmonary leiomyoma with a giant cystic formation, have also been reported [6]. We reported this case due to its rarity.

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Case presentation

In September 2019, a 46-year-old female patient, from whom informed consent was obtained to publish this case report, visited our outpatient clinic with a severe dry cough complaint for the last 2 weeks. She had undergone a hysterectomy in 2004 due to uterine leiomyoma. Her family's medical history was uneventful. Physical examination including breast examination was normal. Bilateral parenchymal nodules were observed on the patient's chest radiograph (Figure 1). Computed thorax tomography (CT) was requested for a detailed examination. Enlarged lymph nodes were observed in the mediastinum and the bilateral hilar regions in CT. The largest one, located in the lower right paratracheal region had a short diameter of 1 cm. Scattered in both lung parenchyma, the largest lymph node in the left lung was 2x2 cm, located in the lower lobe superior segment. The nodules were compatible with multiple metastases (Figures 2A, 2B, 2C). Positron emission tomography (PET-CT) revealed no pathological hypermetabolic activity in the multiple lymph nodes scattered across both lung parenchyma (Figure 3A, 3B). Wedge resection was performed with the Video-Assisted Thoracoscopic System (VATS). Microscopic examination revealed benign spindle cells without pleomorphism and mitosis, forming bundles around the alveoli (Figure 4). In immunohistochemical staining, estrogen receptor (ER) and progesterone receptor (PR), actin, desmin, vimentin, cytokeratin, and BCL 2 were positive, while CD34, CD10, CD99 were negative (Figures 5, 6). Histopathological examination of the excised specimen revealed a pulmonary metastatic benign leiomyoma.

Our patient was discharged uneventfully 5 days after the operation. There was no change in pulmonary nodules and or mass recurrence in the thorax CT scan 6 months after the operation.

Especially in reproductive and premenopausal women with a history of uterine leiomyoma, a pulmonary metastatic benign leiomyoma should be kept in mind in the presence of nodules in the lung.

Figure 1: Pulmonary nodules on posteroanterior chest radiography

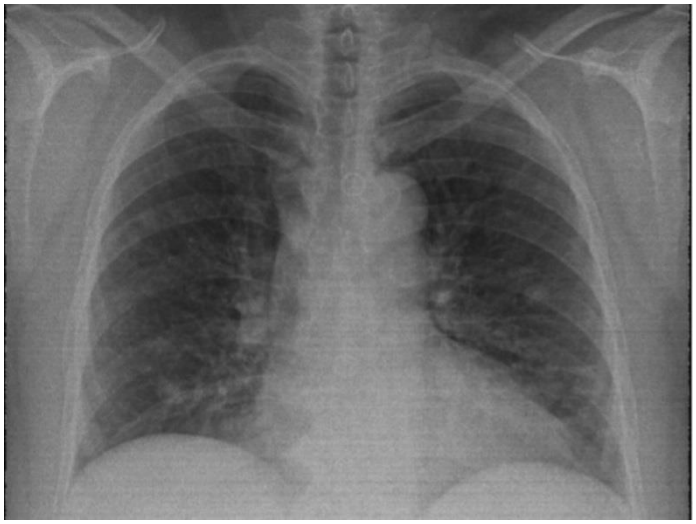


Figure 2: CT image of parenchymal nodules in the lung

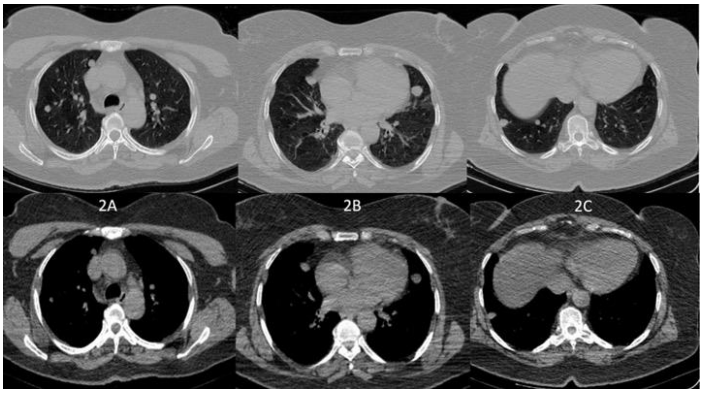


Figure 3: PET-CT view of parenchymal nodules in the lung

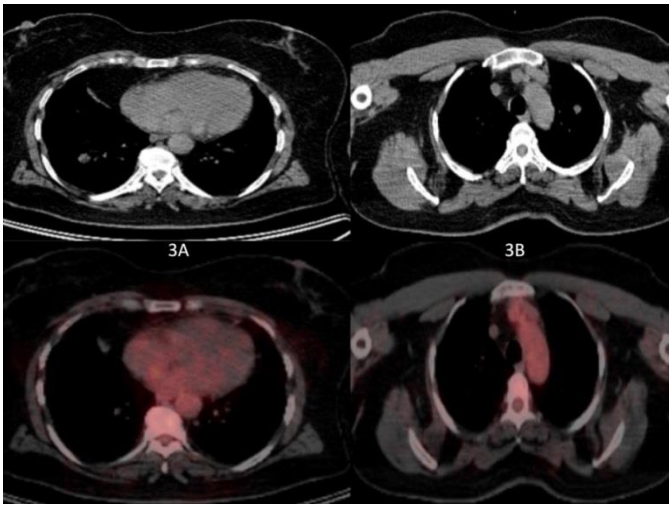


Figure 4: Benign spindle cells forming bundles around the alveoli (HEx200)

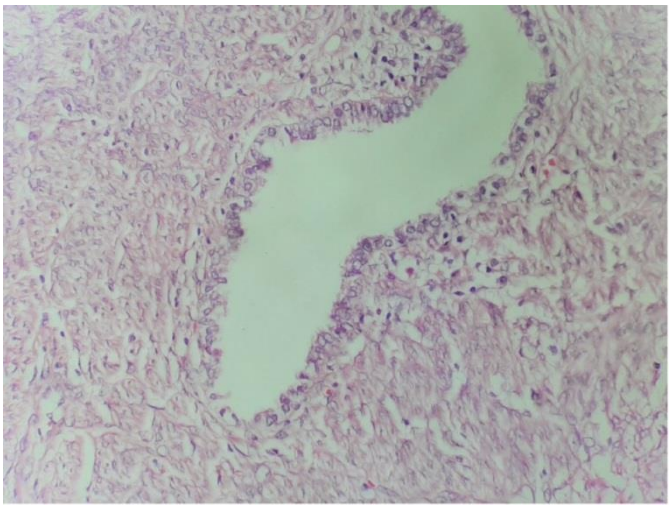


Figure 5: Actin positivity in smooth muscle cells (Actinx400)

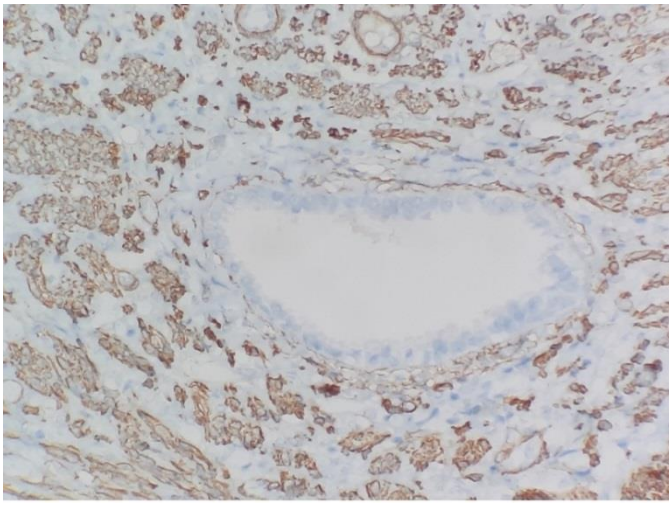
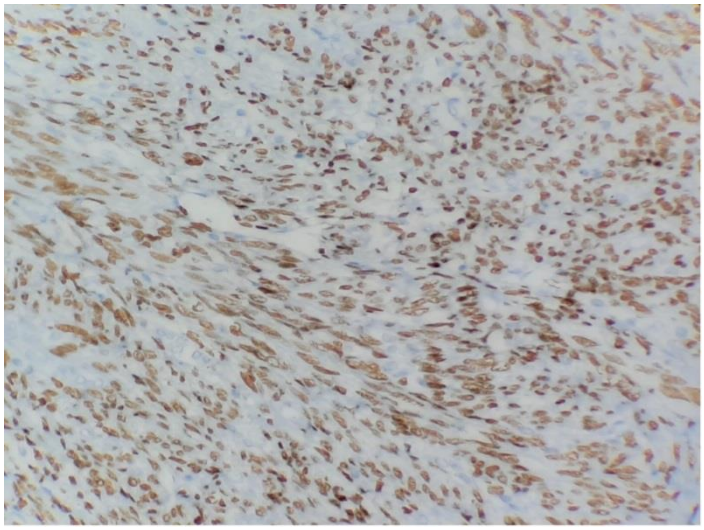


Figure 6: Estrogen receptor positivity in smooth muscle cells (ERx400)



Discussion

Uterine leiomyoma is the most common pelvic tumor in women of reproductive age with an incidence varying between 12-25% [7]. Metastasizing leiomyoma is directly related to uterine leiomyoma and may develop years after myomectomy or hysterectomy. Benign metastasizing leiomyoma is most common among females aged 30-74 years. It may occur in different regions including the lung, liver, heart, breast, and esophagus [8], and rarely undergoes malignant transformation [2].

Approximately 100 cases of malignant transformation of the metastasizing leiomyoma were reported. The pathogenesis and propagation path of PBML is not clear. It is hypothesized that leiomyoma spreads hematogenously during uterine surgery [8].

However, the progress in primary pulmonary leiomyosarcoma is slow. The incidence of sarcomatous transformation of benign uterine leiomyomas is only 0.1-0.8% and is most common among women in their 50s. [9]. Early diagnosis is important and achieving negative surgical margins and wide resection are the standard treatments. The 5-year survival rate is above 50% in patients with complete resection and recurrence is rare. Since lymph node involvement of primary pulmonary leiomyosarcoma is also uncommon, lymph node dissection is unnecessary. Chemotherapy, radiation therapy or both can be planned as adjuvant therapy for advanced lesions [10].

Lung involvement may present with varying images in lung imaging, ranging from unilateral or bilateral nodules to cystic or cavitary lesions of different sizes [11].

PBML has slow growth potential and a limited effect on lung function. However, it can mimic lung cancers or rare benign diseases of the lung, such as tuberculoma, pneumoconiosis, or sarcoidosis [11].

Approximately 200 BML cases were reported [2]. Its pathogenesis is unknown, but the most common theory is the hematogenous spread of leiomyoma during uterine surgery. Excisional biopsy is used for standard diagnosis. SMA, desmin, actin, ER, and PR are positive in immunohistochemical staining of the pathological specimen [12].

Most cases of PBML do not require treatment, but patients with metastasis, increased nodule size and respiratory failure, and hemoptysis should be treated. Surgical treatment should be preferred in these cases. Depending on the size of the tumor and the state of malignancy, radiotherapy, chemotherapy, or both can be administered. In some PBML cases that are positive for ER or PR, reducing hormone release through a hysterectomy, bilateral adnexectomy, and/or hormone therapy can reduce tumor size [13].

Radiologically, it is difficult to distinguish between the malignant transformation of PBML and benign PBML. The only effective diagnostic imaging tool is PET-CT [14].

In our case, no malignant transformation was observed. Microscopic examination of the piece excised from the superior segment of the left lower lobe revealed spindle cell tumor cells without pleomorphism or nuclear atypia and the pathological report was consistent with PBML.

The standard diagnostic procedure for PBML is excisional lung biopsy. Pathologically, the current criteria used to differentiate a malignant smooth muscle tumor from a benign mass are necrosis, high mitotic index (5 mitoses at 50 HPF), and nuclear atypia [15].

Conclusions

In this disease, malignant transformation is very rare. Early diagnosis and total mass resection are of great importance in the case of malign transformation of PBML. If there is an increase in nodule size in CTs during follow-ups, new lesions are detected or there are findings suggesting malignancy in PET-CT, diagnostic procedures should be performed, and surgery should be planned if necessary.

References

1. Kayser K, Zink S, Schneider T, Dienemann H, André S, Kaltner H, et. al. Benign metastasizing leiomyoma of the uterus: Documentation of clinical, immunohistochemical and lectin-histochemical data of ten cases. Virchows Arch. 2000;37:284-92.
2. Chen S, Liu R-M, and Li T. Pulmonary benign metastasizing leiomyoma: a case report and literature review. J Thorac Dis. 2014;6(6):E92–E98.
3. Kang MW, Kang SK, Yu JH, Lim SP, Suh KS, Ahn JS, et al. Benign metastasizing leiomyoma: metastasis to rib and vertebra. Ann. Thorac Surg. 2011;91(3):924–926.
4. Parenti DJ, Morley TF, Giudice JC. Benign metastasizing leiomyoma: a case report and review of literature. Respiration. 1992;56:347350.
5. Takemura G, Takatsu Y, Kaitani K, Ono M, Ando F, Tanada S, et al. Metastasizing uterine leiomyoma: a case with cardiac and pulmonary metastasis. Pathol Res Pract. 1996;192:622-629.
6. Uyama T, Monden Y, Harada K, Sumitomo M, Kimura S. Pulmonary leiomyomatosis showing endobronchiab extension and giant cyst formation. Chest. 1988;94:644-646.
7. Cramer SF, Patel A. The frequency of uterine leiomyomas. Am J Clin Pathol. 1990;94:435-8.
8. Chen S, Liu RM, Li T. Pulmonary benign metastasizing leiomyoma: a case report and literature review. J Thorac Dis. 2014;6:E92-8.
9. Bharambe BM, Deshpande KA, Surase SG, Ajmera AP. Malignant transformation of leiomyoma of uterus to leiomyosarcoma with metastasis to ovary. J Obstet Gynaecol India. 2014;64:68-9.
10. Arnold LM, Burman SD, O-Yurvati AH. Diagnosis and management of primary pulmonary leiomyosarcoma. J Am Osteopath Assoc. 2010;110:244-6.
11. Pastré J, Juvin K, Grand B, Gibault L, Valcke J, Israël-Biet D. Pulmonary benign metastasizing leiomyoma presented as acute respiratory distress. Respirology. 2017;5(2):00216. doi: 10.1002/rcr2.216.
12. Egberts JH, Schafmayer C, Bauerschlag DO, Jänig U, Tepel J. Benign abdominal and pulmonary metastasizing leiomyoma of the uterus. Arch Gynecol Obstet. 2006;274:319-22.
13. Esteban JM, Allen WM, Schaerf RH. Benign metastasizing leiomyoma of the uterus: histologic and immunohistochemical characterization of primary and metastatic lesions. Arch Pathol Lab Med. 1999;123:960-2.
14. Ogawa M, Hara M, Ozawa Y, Moriyama S, Yano M, Schimizu S, et al. Benign metastasizing leiomyoma of the lung with malignant transformation mimicking mediastinal tumor. Clin Imaging. 2011;35:401-4.
15. Mahadevia PS, Tanaka K, Fineberg S. Rosai and Ackerman's surgical pathology, 9th edition author: Juan Rosai Mosby, Edinburgh, 2004. Diagn Cytopathol. 2006;34:382-3.

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Journal of Surgery and Medicine

Chronic osteomyelitis of the zygomatic bone: Back to Benzathine penicillin

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Informed Consent
The authors stated that the written consent was obtained from the patients and the parents of patients presented with images in the study.

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Abstract

Osteomyelitis (OM) of zygomatic bone (zyb) is a rare disease, characterized by relapses. We present two patients presenting with chronic discharging sinus over zyb, who had been treated in previous centers, with multiple culture–directed antibiotics, over many months, without cure. We administered intramuscular Benzathine Penicillin 1.2 million IU with oral Vitamin C 1000 mg/day, for a total of six months and achieved successful healing. In chronic OM (cOM), most bacteria are sessile, embedded in biofilm. Sessile bacteria are not picked by a swab; therefore, the bone must be biopsied. Sessile bacteria also do not grow well in culture media. Thus, the conventional cultures do not reflect the true organisms causing cOM. The Minimum Biofilm Eradication Concentration of antibiotics cause toxicity. To eradicate the biofilm bacteria in patients whose computed tomography showed absence of a sequestrum, we administer this regimen. Judicious case selection is necessary. This regimen adds to the clinician's armamentarium.

Keywords: Osteomyelitis, Zygomatic bone, Benzathine penicillin, Chronic osteomyelitis, Facial aesthetics

Introduction

Osteomyelitis (OM) is the infection of the bone and bone marrow. OM of the facial bones is a rare disease, but associated with high morbidity. OM of zygomatic bone (zyb) has a low incidence (1.42%). Chronic OM (cOM) is characterized by alternating periods of quiescence and sinus tract drainage [1].

We present two patients of cOM of zyb, who had been treated with various culture-directed antibiotics, over many months, without cure. We administered intramuscular (im) Benzathine Penicillin (BPn) with oral Vitamin C (oVitC) and achieved successful healing of the sinus.

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Case presentation

Case 1

A 12-year-old male presented with history of mild fever and a spontaneous onset diffuse swelling in the left malar region 9 months ago. The lesion was slowly growing in size, painless, and not associated with any dental complaints or swellings in body. Suspecting it to be an abscess, a physician in a previous center had incised the swelling and sent the drained fluid for culture, which revealed a growth of Streptococci. Guided by the sensitivity reports, he was prescribed Amoxicillin–Clavulanic acid 625 mg thrice daily. There was a reduction in the discharge, followed by an increase of foul-smelling discharge. The patient consulted another doctor, and was prescribed intravenous Vancomycin, and over next six months was treated with Linezolid, Clindamycin, Levofloxacin and Metronidazole, in appropriate doses, with no sustained relief. He came to us with a discharging sinus over left malar eminence (Figure 1). A consent was obtained for scientific presentation. Total and differential leucocyte counts were normal, C-reactive protein was elevated, and biochemical tests were normal. We ordered a computed tomography (CT) of the face and did a bone biopsy. The CT revealed a cavitation of the left zyb (Figure 2). Bone culture was negative.

We diagnosed cOM of the zyb and proceeded with BPn 1.2 million IU im, fortnightly and oVitC 500mg BD. The discharge reduced within a month and ceased in two months. The sinus healed in three months.

Figure 1: Left oblique view of patient 1 showing pus discharge from the sinus over the left zygomatic bone

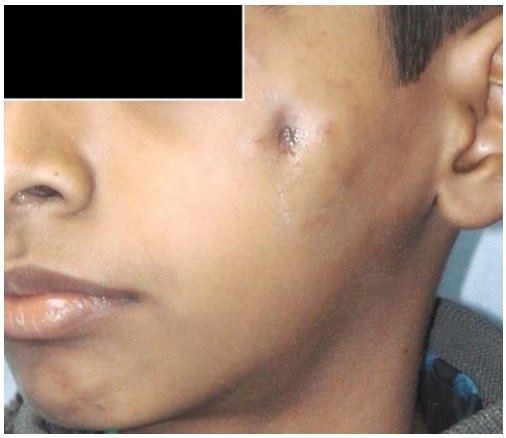
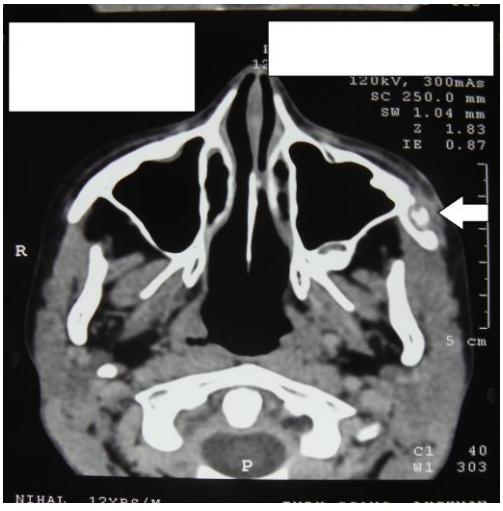


Figure 2: Transverse section of CT scan of the face of patient 1, showing a cavitation of left zygomatic bone (arrow)



Case 2

A 24-year-old female was referred to us with a persistently discharging sinus over the right zyb. Four months ago, the patient had spontaneously developed a diffuse, painful swelling over right zyb, which had not resolved with oral Cefpodoxime and Metronidazole. The swelling spontaneously ruptured. She consulted a surgeon, who curetted the sinus and sent the tissue for culture, which revealed Staphylococcus sensitive to Vancomycin. Over three months, she was treated with multiple antibiotics, but relief was temporary. We diagnosed cOM of the zyb based on the CT of the face (Figure 3) and bone biopsy and started her on BPn 1.2million IU im, fortnightly and oVitC 500 mg BD. Photographic documentation was performed. The discharge ceased in two months and the sinus healed in four months. We continued the treatment for six months.

The patient has been followed up for two years now, without recurrence. There is a depressed scar over the zyb, for which fat grafting is planned (Figure 4).

Figure 3: Coronal section of CT scan of the face of patient 2, showing a cavitation of right zygomatic bone (arrow)

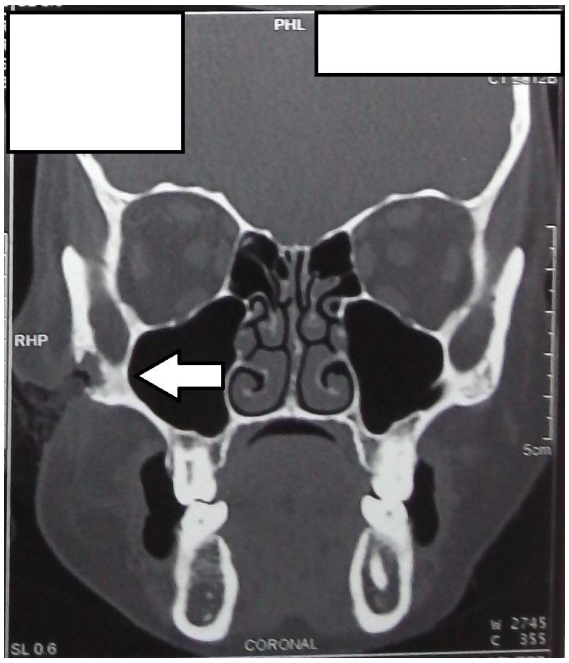
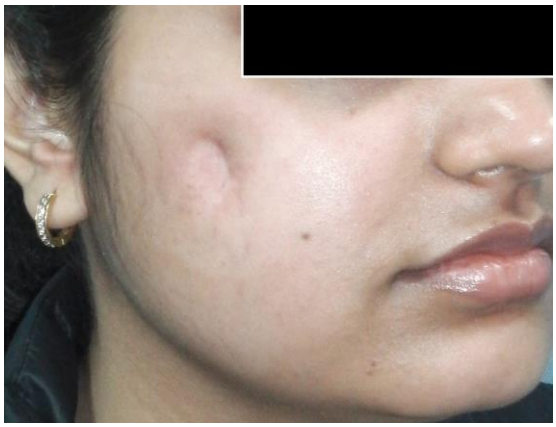


Figure 4: Healed sinus of patient 2, forming a depressed scar over the right zygomatic bone



Discussion

The underlying pathology, rather than the timescale, differentiates cOM from acute OM. Acute OM is a suppurative infection, with edema and small vessel thrombosis, while cOM is biofilm and sessile bacterial infection. In cOM, a small fraction of the microorganisms is free-floating (planktonic) and the rest are embedded within a hydrated polysaccharide matrix with nucleic acid and protein (biofilm) [2].

Culture is the best method to detect the viable bacteria [3]. The sessile bacteria within the glycocalyx-enclosed microcolonies are adherent to the bone and are not picked by swabs. The planktonic bacteria, however, are [2, 4]. The affected bone must be cultured. Also, the sessile bacteria are adapted to grow very slowly. The media used to culture bacteria in laboratory, thus, selects the planktonic bacteria [4].

The conventional method to determine the sensitivity towards antibiotics (Minimum Inhibitory Concentration, MIC) is measured against the planktonic flora. The Minimum Biofilm Eradication Concentration of various drugs is 50 to 1000 times higher than MIC. Such high plasma level of antibiotics cannot be achieved because of toxicity [2, 4].

Staphylococcus aureus is the most common organism causing OM, followed by coagulase-negative Staphylococci, Streptococcus, gram-negative bacilli, and anaerobic organisms [2, 5]. Other bacterial species that are unculturable are also present [2].

Penicillin is effective against Staphylococcus, Streptococcus and other gram-positive bacteria [6]. Penicillin with a biofilm dispersing agent, like oVitC, administered for long enough, has been used to treat psoriasis, rheumatic fever, and Alzheimer-like disease, which are notorious for relapses [6]. Both biofilm and planktonic forms of *S. dysgalactiae* and *S. suis* are shown to be sensitive to penicillin [4].

oVitC inhibits both preformed and new biofilm production. This effect might be due to the anti-quorum sensing activity [7].

Our management is supported by Banham, who treated OM of the maxilla with Penicillin. [8] Merkesteyn treated cOM of the mandible with Penicillin [9].

BPn (depot form of Penicillin) is hydrolyzed to Penicillin G over 14 to 28 days from the intramuscular injection sites [10]. Hydrolysis and slow absorption results in prolonged serum levels [5]. Adults have detectable drug levels for 14 days [10]. Since short duration of treatment is associated with relapse [9], we recommend a treatment of at least six months.

Penicillin is a relatively less toxic antibiotic. Hypersensitivity reactions are the most serious concern, which may be averted by injecting a test dose [10]. Hyperbaric oxygen is another alternative to surgical reperfusion.

Intracellular persistence of S.aureus maybe another cause of cOM. In vivo studies show this to be insignificant [5].

Presence of sequestrum and necrotic tissue necessitate surgical debridement [2, 9]. In selected cases of cOM, without apparent necrotic tissue, we administer this regimen. Debridement of zyb obliterates an important aesthetic landmark of face. Reconstruction, too, has its pitfalls. A careful case selection is emphasized.

Conclusion

In judiciously selected cases of cOM of zyb, BPn 1.2 million IU administered intramuscularly, fortnightly with oVitC 1000 mg, daily, for at least six months, is effective. This regimen adds to the armamentarium of surgeons and is worthy of wider use. The need to sensitize surgeons to the role of BPn with oVitC in eradicating cOM led us to report these cases.

Acknowledgements

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References

1. Mejia B, Bordoy A, Mendez B. Osteomyelitis of the zygomatic bone: a case report and literature review. Internat J Oral maxillofac Surg. 2017;46:308.
2. Calhoun JH, Manring MM, Shirtliff M. Osteomyelitis of the long bones. Semin Plast Surg. 2009;23(2):59–72. doi: 10.1055/s-0029-1214158.
3. Barer MR, Harwood CR. Bacterial viability and culturability. Adv Microb Physiol. 1999;41:93–137. doi: 10.1016/s0065-2911(08)60166-6.
4. Olson ME, Ceri H, Morck DW, Buret AG, Read RR. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res. 2002;66(2):86-92.
5. Masters EA, Trombetta RP, Bentley KLM, Boyce BF, Gill AL, Gill SR, et al. Evolving concepts in bone infection: redefining "biofilm", "acute vs. chronic osteomyelitis", "the immune proteome" and "local antibiotic therapy". Bone Res. 2019;7:20. doi: 10.1038/s41413-019-0061-z. eCollection 2019.
6. Allen HB, Hossain C, Abidi N, Larijani M, Joshi SG. Penicillin: The old/new wonder drug. Adv Tech Biol Med. 2017;5:17.
7. El-Gebaly E, Essam T, Hashem S, El-Baky RA. Effect of Levofloxacin and Vitamin C on bacterial adherence and preformed biofilm on urethral catheter surfaces. J Microb Biochem Technol. 2012;4:131-6.
8. Banham TM. A case of Osteomyelitis of the Superior Maxilla treated with penicillin. J Laryngol Otol. 1949;63(4):233. doi: 10.1017/s0022215100046387.
9. Merkesteyn JPRV, Groot RH, Akker VD, Bakker DJ, Borgmeijer-Hoelen AMMJ. Treatment of chronic suppurative osteomyelitis of the mandible. Int J Oral Maxillofac Surg. 1997;26:450-4. doi: 10.1016/s0901-5027(97)80012-4.
10. Ball AP, Gray JA, Murdoch JMcC. The Natural Penicillins. In: Ball AP, Gray JA, Murdoch JMcC, eds. Antibacterial Drugs Today, 2nd ed. Lancester: MTP Press Ltd; 1978. pp. 8-11.

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Advanced vasospasm in carotid stenting using the distal filter-type embolic protection device: A case report

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Abstract

There are difficulties in the use of embolic protection devices (EPD) for carotid artery stenting (CAS), and various side effects can be observed. We presented a patient who underwent CAS with a distal filter-type (DF) EPD for symptomatic right internal carotid artery (ICA) stenosis and showed advanced vasospasm. A 68-year-old male patient was hospitalized with a pre-diagnosis of transient ischemic attack (TIA) in the form of left-sided weakness. Computed tomography angiography revealed 90% stenosis in the right ICA. On the 5th day, he underwent an angiography, which showed a plaque causing 90% stenosis. A 5F Spider FX (Medtronic Corp.; Minneapolis, MN, USA) was placed in the cervical ICA. At this time, mild vasospasm was observed in the cervical ICA, but the flow was normal. After pre-dilatation, a Protégé 8-6x40 stent was placed in the stenotic segment. Due to the 50% residual stenosis, a post-dilatation was performed. After post-dilatation, advanced vasospasm developed distal to the stent and the flow was completely obstructed. Vasospasm significantly regressed within a few minutes with intra-arterial nitrate administration. There was no change in the neurological examination of the patient during and after the procedure. Although vasospasm is usually a self-limiting complication that resolves without clinical symptoms, it may cause a TIA or an infarct. In CAS procedures, the possibility of a vasospasm should be kept in mind and if it occurs, the irritant should be removed. If there is no improvement, an intra-arterial vasodilator should be administered.

Keywords: Carotid stenosis, Carotid stenting, Vasospasm, Distal filter, Embolic protection device

Introduction

Carotid artery stenting (CAS) is recommended as an alternative treatment to carotid endarterectomy operation (CEA) in the centers where the complication rate for symptomatic and asymptomatic patients is below 6% and 3%, respectively. The frequency of minor stroke was higher in CAS compared to CEA operations [1, 2]. Embolic protection devices were developed to reduce the risk of distal embolism during the CAS procedure. These can be classified as distal occlusion balloons, distal filters (DF), and proximal occlusion devices. Nowadays, combined systems are also used, the most common ones in daily practice being the DFr group EPDs. While there are publications reporting that the perioperative stroke risk is lower in EPD use, there are also studies which report that there is no significant difference in neurological complications between the patients in whom an EPD was or was not used [3-5]. There are difficulties in the use of EPDs, and side effects may occur. We here present a patient who underwent CAS with a DF type EPD due to symptomatic right internal carotid artery (ICA) stenosis and showed advanced vasospasm.

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Informed Consent
The authors stated that the written consent was obtained from the patient presented with images in the study.

Conflict of Interest
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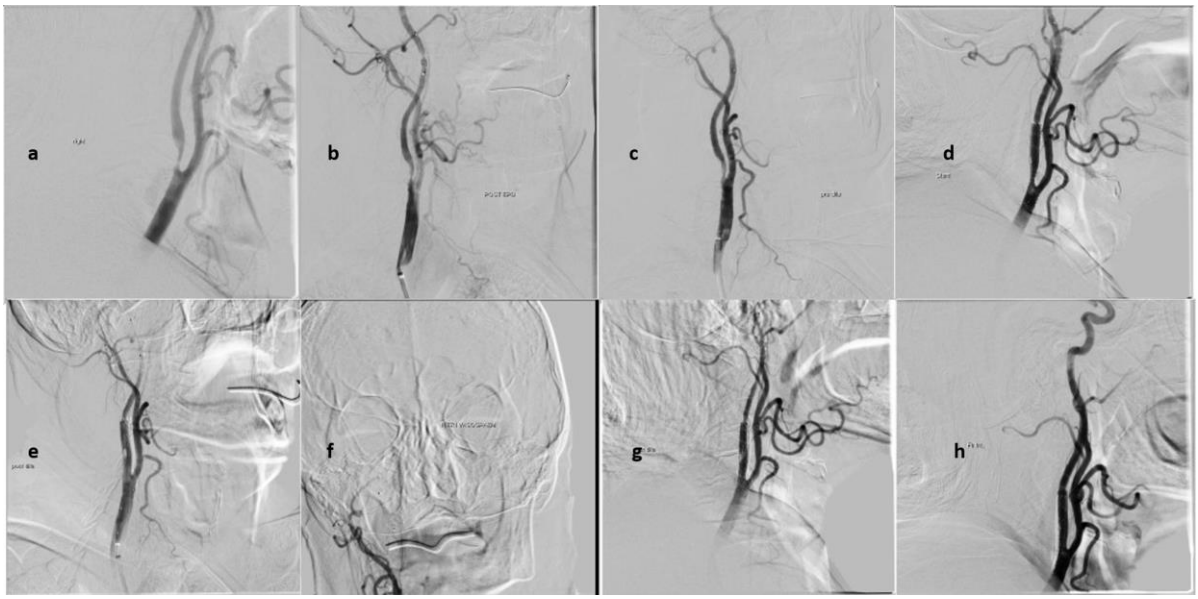
Figure 1: (a) Diagnostic examination revealed a 90% stenosis. (b) Mild vasospasm was observed in the cervical ICA after the distal filter was placed. (c) After pre-dilatation. (d) There was approximately 50% residual stenosis after stenting. (e) After post-dilatation (f) After post-dilatation, there was no flow in the ICA. (g) Immediately after intra-arterial nitrate administration. (h) Five minutes after intra-arterial nitrate administration.



Case presentation

In September 2020, a 68-year-old male patient with known hypertension, diabetes mellitus and a history of coronary artery bypass surgery eight years ago was admitted to the emergency department of our hospital due to left-sided weakness that started five hours ago. On admission, the neurological examination showed that the left nasolabial fold was flattened and the muscle strength for left upper and lower extremities were 3/5 and 4/5, respectively. His National Institutes of Health Stroke Scale (NIHSS) score was 5. No acute pathology was detected in diffusion-weighted magnetic resonance imaging (MRI). Computed tomography angiography (CTA) revealed a 90% stenosis in the right ICA. The patient was hospitalized and acetylsalicylic acid and ticagrelor were started. Within six hours of follow-up, the neurological examination returned to normal; transient ischemic attack (TIA) was considered in the etiology of carotid stenosis and right ICA stenting was planned. On the 5th day, the patient's neurological examination was normal, and he was taken to angiography. A 6F long sheath was placed in the right ICA over the 8F femoral access sheath. Diagnostic examination revealed an ulcer extending from the right CCA to the ICA and a plaque forming a 90% stenosis at its narrowest point. After the stenotic segment was passed with a micro-guidewire, a 5F Spider FX (Medtronic Corp.; Minneapolis, MN, USA) EPD was placed in the distal cervical ICA. Vasospasm developed in the segment where the DF was opened, but the flow was normal. Then, pre-dilatation was performed with a 2.75x20 mm balloon catheter, and a Protege 8-6x40 (Medtronic Corp.; Minneapolis, MN, USA) stent was placed in the stenotic segment. Due to 50% residual stenosis, a post-dilatation was performed with a 5x15 mm balloon catheter. After post-dilatation, advanced vasospasm developed distal to the stent and the flow was completely obstructed in the cervical ICA. Meanwhile, the neurological examination did not deteriorate. After intra-arterial nitrate administration, vasospasm significantly regressed within a few minutes.

The 5F Spider FX EPD was properly collected. In the control injection, the ICA and intracranial circulation were normal and there was no residual stenosis (Figure 1). The patient, whose neurological examination was normal, was discharged after 24 hours of follow-up. Informed consent was obtained from the patient before discharge. At the 3rd month control, the neurological examination was normal, and no residual stenosis was detected in Doppler USG.

Discussion

The DF group forms the most extensively used EPDs in daily practice to reduce the risk of distal embolism in CAS. The advantage of distal filter group EPDs over proximal protective devices and distal balloon devices is that cerebral perfusion can continue during the procedure. However, there are also several disadvantages: The stenotic segment must be passed without cerebral protection; if pre-dilatation is needed, it must be performed without cerebral embolic protection; in patients with tortuous vascular structures, the implantation can sometimes be arduous or even impossible. Also, they may cause vasospasm or dissection, thrombus may occur directly in the filter, and there is a possibility of thrombus formation within the EPD, which may embolize distally during retrieval or filter malposition. In a randomized controlled study comparing the use of a proximal occlusion device and a DF, vasospasm was observed in 23% of the DF group and 2% of the proximal occlusion group. There was no significant difference between the two groups in terms of clinical events [6]. In a retrospective study evaluating CAS cases in whom DFs were used, vasospasm causing more than 50% stenosis developed in 9.1% of the patients, which spontaneously regressed within a few minutes in all patients [7].

A study comparing first-, and second- generation DF and no DF use reported that vasospasm developed in 67% of those in the first-generation DF group, 17% of those in the second-generation DF group, and 14% of those in the non-DF group [8].

Another study on 640 cases in which DF was used reported that 4.29% of the patients developed a vasospasm large

enough to disrupt the flow, which regressed after intra-arterial nitrate administration without giving out clinical signs [9], as was the case in our patient. Vasospasm is more common among females, in those with high tortuosity index, and during long procedures [10].

Even in the most experienced hands, DFs move a little during the process. This can trigger a vasospasm due to endothelial irritation or damage done by the micro-guidewire or DF. Although it usually resolves spontaneously with withdrawal of the irritant, intra-arterial nitrate, nimodipine or milrinone administration may be required. A vasospasm can also make it quite difficult to recover the DF. Although vasospasm is usually a self-limiting complication that resolves without clinical symptoms, it may cause a TIA or an infarct. The risk is especially high if it is overlooked or noticed late [11-13]. Advanced vasospasm can cause flow interruption in the ICA, which may be confused with an ICA occlusion.

Conclusion

In CAS procedures, the possibility of a vasospasm should be kept in mind. If it occurs, first, the irritant should be removed, and if there is no improvement, an intra-arterial vasodilator should be administered. The key is keeping the risk of vasospasm in mind and detecting it early.

References

1. Ederle J, Dobson J, Featherstone RL, Bonati LH, van der Worp HB, de Borst GJ, et al. Carotid artery stenting compared with endarterectomy in patients with symptomatic carotid stenosis (International Carotid Stenting Study): an interim analysis of a randomised controlled trial. Lancet (London, England). 2010;375(9719):985-97.
2. Kernan WN, Ovbiagele B, Black HR, Bravata DM, Chimowitz MI, Ezekowitz MD, et al. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2014;45(7):2160-236.
3. Barbato JE, Dillavou E, Horowitz MB, Jovin TG, Kanal E, David S, et al. A randomized trial of carotid artery stenting with and without cerebral protection. J Vasc Surg. 2008;47(4):760-5.
4. Vos JA. Evidence overview: benefit of cerebral protection devices during carotid artery stenting. The Journal of cardiovascular surgery. 2017;58(2):170-7.
5. Doig D, Turner EL, Dobson J, Featherstone RL, Lo RT, Gaines PA, et al. Predictors of Stroke, Myocardial Infarction or Death within 30 Days of Carotid Artery Stenting: Results from the International Carotid Stenting Study. European journal of vascular and endovascular surgery the official journal of the European Society for Vascular Surgery. 2016;51(3):327-34.
6. Tatli E, Buturak A, Grunduz Y, Dogan E, Alkan M, Sayin M, et al. Comparison of anti-embolic protection with proximal balloon occlusion and filter devices during carotid artery stenting: clinical and procedural outcomes. Postepy w kardiologii interwencyjnej = Advances in interventional cardiology. 2013;9(3):221-7.
7. Kwon BJ, Han MH, Kang H-S, Jung C. Protection Filter-Related Events in Extracranial Carotid Artery Stenting: A Single-Center Experience. Journal of Endovascular Therapy. 2006;13(6):711-22.
8. Clemens MS, Daniel AH, Adel MM. Iatrogenic vasospasm in carotid artery stent angioplasty with distal protection devices. Neurosurgical Focus FOC. 2008;24(2):E12.
9. Reimers B, Schlüter M, Castriota F, Tübler T, Corvaja N, Cernetti C, et al. Routine use of cerebral protection during carotid artery stenting: results of a multicenter registry of 753 patients. The American Journal of Medicine. 2004;116(4):217-22.
10. Wang Q, Liu C, Yan B, Fan X, Zhang M, Li Y, et al. Correlation of Extracranial Internal Carotid Artery Tortuosity Index and Intraprocedural Complications during Carotid Artery Stenting. European Neurology. 2012;68(2):65-72.
11. Ishihara H, Ishihara S, Niimi J, Neki H, Kakehi Y, Uemiya N, et al. Risk factors and prevention of guiding catheter-induced vasospasm in neuroendovascular treatment. Neurologia medico-chirurgica. 2015;55(3):261-5.
12. Vijayvergiya R, Otaal PS, Bagga S, Modi M. Symptomatic carotid vasospasm caused by a distal-protection device during stent angioplasty of the right internal carotid artery. Tex Heart Inst J. 2010;37(2):226-9.
13. Cardaioli P, Giordan M, Panfili M, Chioin R. Complication with an embolic protection device during carotid angioplasty. Catheterization and cardiovascular interventions: official journal of the Society for Cardiac Angiography & Interventions. 2004;62(2):234-6.

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Journal of Surgery and Medicine

Watershed infarction after honey intoxication

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Informed Consent
The authors stated that the written consent was obtained from the patient presented with images in the study.

Conflict of Interest
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Abstract

Watershed infarction indicates ischemic lesions at the junction of two central artery regions. These lesions are the cause of 10% of all brain infarctions. In watershed infarctions, disrupted hemodynamics constitute the pathophysiology. A specific type of honey in the Black Sea region of Turkey, “mad honey,” contains grayanotoxin and may cause intoxication. We herein report a case with watershed infarction after honey intoxication. The patient presented with dizziness and paresthesia in the left arm after eating “mad honey.” Imaging revealed carotid stenosis and watershed infarction.

Keywords: Watershed infarct, Mad honey, Stroke

Introduction

Watershed infarction indicates ischemic lesions at the junction of two central artery regions [1]. These lesions are the cause of 10% of all brain infarctions [2] and are divided into subcortical and cortical infarctions [3]. Subcortical watershed infarctions are usually small lesions in the centrum semiovale or corona radiata parallel to the lateral ventricle. In contrast, cortical watershed infarctions are cortical lesions between the anterior, middle, and posterior brain arteries [4]. The general principle in the pathophysiology of Watershed infarctions is a hemodynamic disruption [5].

A specific type of honey in the Black Sea region of Turkey, also called “mad honey,” contains grayanotoxin and sometimes causes intoxication [6]. "Mad honey" poisoning is potentially fatal if left untreated, but fatal cases have not been reported in the literature [7]. Symptoms of grayanotoxin intoxication are dose-related. In mild cases, dizziness, weakness, excessive sweating, hypersalivation, nausea, vomiting, hypotension, bradycardia, and paresthesia are observed. Severe cases may present with life-threatening cardiac complications such as an atrioventricular block [8].

In our case report, we aimed to draw attention to the watershed infarction in a patient who presented to the emergency department with honey intoxication.

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Case presentation

An 88-year-old female patient was admitted to the emergency room with dizziness and paresthesia in the left arm. She was well-oriented and cooperative. Her Glasgow Coma Score (GCS) was 15, blood pressure was 70/30 mmHg, heart rate was 45/minute, and respiratory rate was 14 /minute. Her complaints had begun after eating honey and were present for 5 hours. She had a history of hypertension and chronic kidney disease. There was no right-left motor impairment; however, she had hypoesthesia in the left upper extremity and sinus bradycardia in the ECG. Blood hemogram and biochemical values were normal. While no acute pathology was observed in the brain computerized tomography (CT) images, watershed infarction was seen in diffusion MRI (Figures 1 and 2). Echocardiography revealed no cardiac pathologies. In the carotid Doppler ultrasound, 50% and 70% stenoses were detected in the right and left internal carotid arteries (ICA), respectively. The patient was admitted to the neurology service for follow-up and treatment. Approval was obtained from the patient for the publication of this case report.

Figure 1: Diffusion-Weighted Imaging (DWI) - MRI

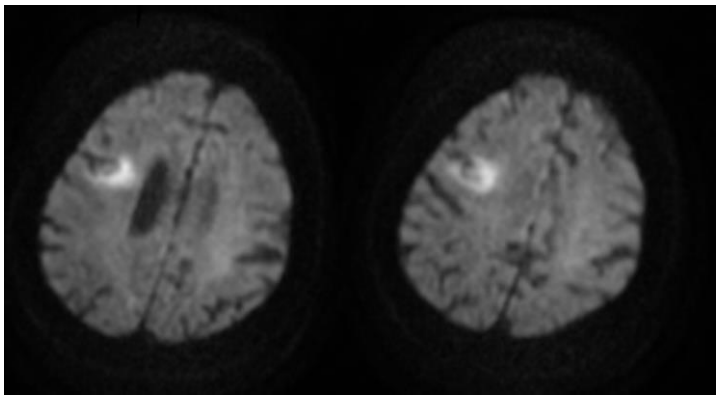
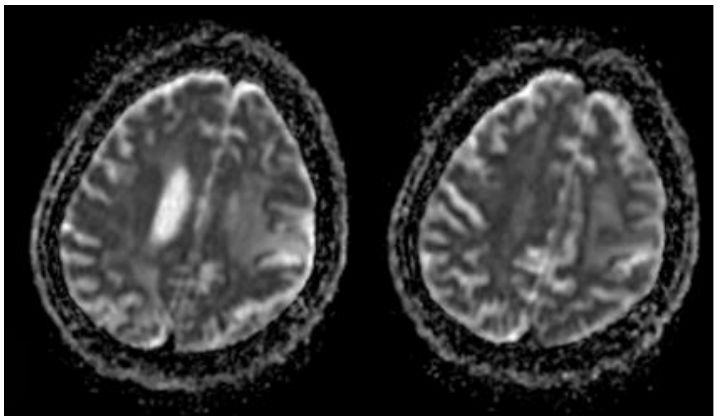


Figure 2: (A) – Apparent Diffusion Coefficient (ADC) - MRI



Discussion

Watershed infarctions occur because of severe stenosis of the craniocervical arteries due to impaired hypoperfusion in case of cardiac arrest or severe hypotension [9]. Studies showed the relationship between carotid stenosis and watershed infarction [10]. Caplan and Hennerici reported the coexistence of severe carotid stenosis and watershed infarction in a patient with a transient neurological disorder [11].

Beekeeping is an everyday activity among the local people in the Eastern Black Sea region. The honey sometimes contains grayanotoxin and causes poisoning [12]. The toxic effects are rarely fatal and usually last no more than 24 hours. After eating toxic honey, dizziness, weakness, sweating, nausea and vomiting, salivation, blurred vision, fainting, tremors, numbness in the extremities, and loss of consciousness may be observed. Other probable complaints include low blood pressure, bradycardia, complete atrioventricular block, and shock [13].

Conclusions

As in our case, watershed infarction can be observed in patients admitted to the emergency department after consuming honey. Physicians should keep in mind a possible infarction in patients with neurological complaints after consuming honey, and the necessary tests should be performed for etiological research and diagnosis.

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References

1. Schneider M. Durchblutung und sauerstoffversorgungdes gehirns. Verh Dtsch Tes Kreislaufforsch. 1953;19:3-12.
2. Torvik A. The pathogenesis of watershed infarcts in the brain. Stroke. 1984; 15(2):221-3.
3. Derdeyn CP, Khosla A, Videen TO, Fritsch SM, Carpenter DL, Grubb RL Jr, et al. Severe hemodynamic impairment and border zone--region infarction. Radiology. 2001;220(1):195-201.
4. Moriwaki H, Matsumoto M, Hashikawa K, Oku N, Ishida M, Seike Y, et al. Hemodynamic aspect of cerebral watershed infarction: assessment of perfusion reserve using iodine-123-iodoamphetamine SPECT. J Nucl Med. 1997;38(10):1556-62.
5. Yong SW, Bang OY, Lee PH, Li WY. Internal and cortical border-zone infarction: clinical and diffusion-weighted imaging features. Stroke. 2006;37(3):841-6.
6. Gunduz A, Turedi S, Uzun H, Topbas M. Mad honey poisoning. Am J Emerg Med. 2006;24(5):595-8.
7. Von Malottki K, Wiechmann HW. Acute life-threatening bradycardia: food poisoning by Turkish wild honey. Dtsch Med Wochenschr. 1996;121(30):936-8.
8. Ergun K, Tufekcioglu O, Aras D. A rare cause of atrioventricular block: mad honey intoxication. Int. J.Cardiol. 2005;99:347–348.
9. Moustafa RR, Izquierdo-Garcia D, Jones PS, Graves MJ, Fryer TD, Gillard JH, et al. Watershed infarcts in transient ischemic attack/minor stroke with 50% carotid stenosis: hemodynamic or embolic? Stroke. 2010;41(7):1410-6.
10. Del Sette M, Eliasziw M, Streifler JY, Hachinski VC, Fox AJ, Barnett HJ. Internal borderzone infarction: a marker for severe stenosis in patients with symptomatic internal carotid artery disease. For the North American Symptomatic Carotid Endarterectomy (NASCET) Group. Stroke. 2000;31(3):631-6.
11. Caplan LR, Hennerici M. Impaired clearance of emboli (washout) is an important link between hypoperfusion, embolism, and ischemic stroke. Arch Neurol. 1998; 55(11):1475-82.
12. Onat FY, Yegen BC, Lawrence R, Oktay A, Oktay S. Mad honey poisoning in man and rat. Rev Environ Health. 1991;9(1):3-9.
13. Ozhan H, Akdemir R, Yazici M, Gündüz H, Duran S, Uyan C. Cardiac emergencies caused by honey ingestion: a single centre experience. Emerg Med J. 2004;21(6):742-4.

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