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Research Articles

Research Article

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Upper-Extremity Deep Venous Thrombosis: Analysis of 348 Cases

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Abstract

Objectives: In this study, we aimed to determine the clinical features and outcomes of patients with upper-extremity deep venous thrombosis (UEDVT).

Materials and Methods: Three hundered and fourty eight cases diagnosed with UEDVT were included in the study. Risk factors, symptoms, thrombosis localization, and clinical outcomes were examined.

Results: The most common risk factors were central venous catheter (CVC) (82%) and malignancy (45%). Edema (77%) and pain (56%) were the reasons for the admission of patients. The most common site involved was the subclavian vein. Although the frequency of pulmonary embolism was 4%, the rate of 60-day mortality was determined as 9%.

Conclusion: CVC and the presence of malignancy are common risk factors for UEDVT. Despite its low frequency of pulmonary embolism, it has non-low mortality associated with possible underlying diseases.

Keywords: Deep vein thrombosis, upper extremity, low molecular weight heparin, central venous catheter, medical treatment

Introduction

Upper-extremity deep venous thrombosis (UEDVT) is called internal jugular, brachiocephalic, brachial, subclavian, axillary veins thrombosis[1]. It is divided into two etiological classifications, as primary and secondary. Primary UEDVT is defined as spontaneous thrombosis of the subclavian and axillary vein. It includes Paget-Schroetter syndrome, which is defined as venous thoracic outlet syndrome[2]. Thoracic outlet abnormalities may

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occur without an identifiable cause or may be caused by upper extremity movements that create vascular stress causing intimal damage[3]. There is an underlying facilitating reason in secondary UEDVTs. These are central venous catheter (CVC) insertion, malignancy, coagulation abnormalities, and genetic risk factors[4]. In this study, we aimed to determine the risk factors, clinical characteristics, and outcomes of patients diagnosed with UEDVT in our clinic

Materials and Methods

This study was approved by Bozyaka Training and Research Hospital Ethics Review Board in accordance with the Declaration of Helsinki (date: 28.05.2022, protocol no: 2021/92). In our study, we retrospectively examined patients older than 18 years of age, who were diagnosed with UEDVT by Doppler USG, contrast venography, or CTA between November 2001 and August 2019. We recorded the patients' basic clinical characteristics, comorbidity, and risk factors for UEDVT. Symptoms and localization of deep vein thrombosis were determined. Clinical outcomes were determined in terms of pulmonary embolism, mortality, post-thrombotic syndrome (PTS), and recurrence of UEDVT. Considering cancer and other clinical characteristics of the patients, Low-molecular-weight heparin (LMWH) and/or warfarin was administered for three months as a treatment protocol.

Results

During this period, 348 cases were detected. Table 1 shows the clinical characteristics and treatment parameters of these cases. Forty-seven percent of these cases were women with an average age of 57 years. CVC was present in 82% of the cases. The second most common UEDVT risk factor was the presence of malignancy. Twelve percent of the patients had a history of lower-extremity deep venous thrombosis (LEDVT) in the last year. The proportion of patients receiving anticoagulant therapy was 36%.

Symptoms and their distribution are given in Table 2. The most common reason for admission was swelling in the arm and neck (77%). Fifty-six percent of the patients complained of pain at the time of presentation. A lesser of them applied with the complaints of discoloration such as bruising and redness in the extremity. The average admission time to the hospital was 2-6 days and 3.5 days on average.

Table 3 shows the anatomical involvement of UEDVT cases. The most commonly involved venous structure was the subclavian vein. Its continuation, the axillar vein, was the second most frequently affected site. The internal

Table 1. Clinical characteristics and treatment parameters of the cases

Features	n
Age	57
Gender (female)	47
Central venous catheter	82
Malignancy	45
Hemodialysis catheter	14
Surgery and trauma	10
Immobility	8
LEDVT history (in the last year)	12
Anticoagulant therapy	64
Those who do not receive anticoagulant treatment	36

LEDVT: Lower-extremity deep venous thrombosis, n: Number

Table 2. Symptoms and their distribution

Symptoms	n
Edema	77
Pain	56
Color change	19

n: Number

Table 3. Anatomical involvement of UEDVT cases

Involvement	%
Superior vena cava	1
Innominate vein	1
Internal juguler vein	40
Subclavian vein	66
Aksiller vein	51
Brakial vein	19

UEDVT: Upper-extremity deep venous thrombosis, n: Number

jugular vein, which is an important central venous access site, was the third most common UEDVT location with the rate of 40%. Intrathoracic central venous structures including the innominate vein and SVC were the least affected structures with the rate of 1%.

Clinical outcomes are shown in Table 4. Pulmonary embolism was at a low frequency of 4%. We determined the sixty-day mortality as 9%. The rate of PTS with UEDVT morbidity was about 8% (8% of patients with the PTS that causes ongoing swelling, pain, loss of vascular access in the extremity with UEDVT morbidity). In our study, UEDVT had a recurrence rate of 12%.

Discussion

Although UEDVT is uncommon, its frequency is increasing with advanced treatment methods and increased invasive procedures[2,5]. In a study involving 11,564 deep vein thrombosis patients, the incidence of UEDVT was found to be 4.4%[6]. It has historically been considered a benign disease. However, with the demonstration that it causes serious complications such as pulmonary embolism, superior vena cava syndrome, loss of vascular access, post-thrombotic venous insufficiency, its importance has increased Upper extremity deep vein thrombosis is extremely rare compared to lower extremity deep venous thrombosis[7]. The main reasons for this can be counted as the arm veins are less exposed to the effect of gravity and have fewer valves. To reveal the etiological and clinical features of UEDVT disease, which causes morbidity and morbidity, will help to understand the disease and its causes. The most common complaints in patients presenting with a diagnosis of UEDVT are pain and swelling in the arm[8]. In addition, other application complaints are erythema, neck and face swelling. A significant 33-60% of patients with secondary UEDVT do not express symptoms. In our study, patients diagnosed with UEDVT most frequently presented with swelling and arm pain.

Table 4. Clinical outcomes

Clinical outcomes	n
Pulmonary embolism	4
Mortality (60 days)	9
Post thrombotic syndrome	8
UEDVT recurrence	12

UEDVT: Upper-extremity deep venous thrombosis, n: Number

In our study, as in the studies in the literature, the most prominent risk factor was permanent catheter placement. Eighty-two percent of the patients with UEDVT had intravenous instrumentation such as CVC, pacemakers/defibrillators. With increased CVC placement and widespread use of VDU as a diagnostic tool, UEDVT patients are more common[9]. Catheter-induced UEDVT has been associated with catheter size. It has been reported that as the catheter size increases, the frequency of UEDVT increases[10]. In addition, Gonsalves et al.[10] showed that the longer the catheter residence time, the higher the frequency of central vein stenosis and occlusion[11,12]. Thrombosis was detected in 10% of the patients with pacemakers. This thrombosis frequency increases as the number of pacemakers lead increases[13]. Sixty-nine percent of UEDVTs occurring in cancer patients are CVC-related thrombosis[14]. De Cicco et al.[15] identified the first days and left subclavian artery catheterization as risk factors for UEDVT in malignant patients with CVC.

The second important risk factor shown in our study was the presence of cancer[16]. The rate of patients accompanied by cancer was 45%. Although cancer itself creates a prothrombotic process, chemotherapy, hormone replacement therapy, and medical status of cancer patients lay the groundwork for UEDVT[17,18].

In a prospective study involving cancer patients with CVC, symptomatic UEDVT was found at the rate of 6%[15]. In a study conducted with a population that does not have CVC but is known to have cancer.UEDVT in patients with active cancer, 18 times than in those without cancer, those with a history ofcancer were found to have a 7.7-fold increased risk[19]. In another study involving cancer patients, it was reported that UEDVT cases had a high recurrence rate of 18%[20]. Malignancy and catheter-related thrombosis are associated with recurrence[21].

PTS is one of the morbidities of UEDVT and although its frequency is between 6 and 37%, Thiyagarajah et al.[22] associated it more with primary UEDVT, reporting it as 14% for secondary UEDVT. Venous hypertension due to venous thrombus that persists in PTS occurs as a result of damage to the venous valves (especially for the lower extremity) and the deterioration of the microcirculation due to congestion and edema. In our study, the frequency of PTS was found to be 8%.

Recurrence and major bleeding rates in UEDVT patients are similar to those in LEDVT patients[23,24]. This reveals the need and necessity for treatment[25]. However, similar major bleeding frequency requires treatment follow-up as in LEDVT patients.

In a recent study evaluating the treatment approaches of clinicians in UEDVT cases, it was observed that the management of UEDVT patients differed greatly among physicians, only 10% of the physicians adopted the current guidelines and less of them applied the treatment by them. Possibly due to the low quality of data on UEDVT thrombosis, no recommendations were made in the latest American College of Chest Physicians (ACCP) guideline. Considering all these, the lack of research and data on UEDVT prevents the formation and applicability of a treatment consensus.

Its treatment was mainly based on data obtained from LEDVT[25]. The main purpose of the treatment is to alleviate the symptoms of acute UEDVT, to prevent PE and late complications. Medical treatment was applied to our patients, interventional mechanical treatments and surgical treatment were not applied. In our study, we determined the treatment principle of anticoagulation with LMWH or warfarin for three months and/or removal of the catheter in the treatment of our patients. By the ACCP recommendations, patients with cancer were anticoagulated with LMWH and, if not, with warfarin. The rate of patients we followed up with anticoagulation was 64%. During the follow-up, no bleeding problem related to GIS was encountered in our patients, h2 resp antagonist treatment was routinely added to the treatment of the patients and the patients were informed about the hematemesis or melena that may be associated with bleeding. In patients with catheter-associated UEDVT, catheter removal was decided by considering the individual characteristics of the patient, such as the presence of infection, the need for vascular access, the functionality of the catheter, anticoagulant therapy contraindication, response to treatment, and the severity of symptoms. There are different rates of pulmonary embolism in the literature. Although a high incidence of 36% was reported in previous studies, Levy et al.[26] found a low incidence of PE (2%) independent of anticoagulant therapy. In another similar study, in which they used DMAH and warfarin as anticoagulant treatment, although only 40% of 300 patients were anticoagulated, they did not recommend routine anticoagulation to prevent PE due to hemorrhagic complications[27]. Beiswenger et al.[28], found that mortality was higher in the group that did not receive anticoagulant treatment. However, they associated the data with comorbid and demographic characteristics that prevent patients from receiving anticoagulant therapy rather than pulmonary embolism. They found the 30-day and 6-month mortality rates to be 16.55% and 27.5%, respectively[28]. Age, dialysis, central location, ischemic stroke after diagnosis, and cancer at the time of diagnosis have been shown as risk factors for clinical outcomes[28]. In our study, the rate of pulmonary embolism was found to be 4%, and the two-month mortality was 9%. The prognosis of the disease is closely related to the prognosis of cancer, as in our study of the underlying chronic disease.

In the COVID-19 pandemic, we are in, there are case reports diagnosed with UEDVT. Considering the hypercoagulable state caused by SARS-CoV-2 infection, it will not be surprising that it is included in the etiology of UEDVT[29].

Conclusion

UEDVT is a less common disease with a thrombotic process than LEDVT. CVC and malignancy are the most common risk factors for UEDVT. UEDVT should be kept

in mind especially in patients suffering from pain and swelling with the stated risk factors.

Despite its low prevalence of PE, it probably has non-low mortality associated with underlying diseases such as malignancy. Close follow-up should be carried out in terms of UEDVT in patients who received or were diagnosed with a treatment with CVC, and patients should be told that such a complication may occur. In addition, as soon as the symptoms of the disease begin, they should be told to apply to the nearest hospital at an early stage. Anticoagulant treatment should be applied due to the risk of PTS and recurrence.

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Ethics

Ethics Committee Approval: This study was approved by Bozyaka Training and Research Hospital Ethics Review Board in accordance with the Declaration of Helsinki (date: 28.05.2022, protocol no: 2021/92).

Informed Consent: This study does not require patient consent.

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Authorship Contributions

Surgical and Medical Practices: İ.E., M.B.K., Concept: İ.E, M.B.K., Design: İ.E., M.B.K., Data Collection or Processing: İ.E., M.B.K., Analysis or Interpretation: İ.E., M.B.K., Literature Search: İ.E., M.B.K., Writing: İ.E., M.B.K.

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Research Article

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Endovascular Treatment of Subclavian Artery Stenosis: Single-Center Experience

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Abstract

Objectives: The etiology of subclavian artery stenosis can be very different, but typically the most common form is due to atherosclerotic disease. Interventional treatment is generally indicated for upper limb ischemia, vertebrobasilar symptoms, subclavian steal syndrome, and coronary steal syndrome. Endovascular stenting is preferred over surgery because of its high success rate, less invasive nature, and minimal complication rate. In this study, the characteristics of patients treated endovascularly due to subclavian artery stenosis in our center and their procedural details will be examined.

Materials and Methods: We retrospectively analyzed patients with subclavian artery stenosis treated by endovascular techniques in our center between January 2019 and January 2021.

Results: Twelve (80%) of 15 patients with stenotic subclavian arteries were successfully treated with endovascular techniques. In 3 patients, the procedure was terminated with failure. The mean age of the patients was 64.66 years and 3 (20%) of the patients were female. All of the patients were receiving antihypertensive treatment with the diagnosis of hypertension, and 4 (26.66%) patients had diabetes mellitus, 13 (86.66%) patients had hyperlipidemia, and 13 (86.66%) patients had coronary artery disease. In 2 patients, the lesion was diagnosed in the right subclavian artery.

In 7 patients, the procedure was performed with the telescopic method from a transfemoral approach. In 5 patients, the transradial approach was used. In 3 patients, the procedure was performed directly over the transbrachial approach.

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Balloon expandable stent implantation was performed in 10 (66.66%) patients at the end of the procedure, and self-expandable stent implantation was performed in 1 patient.

Conclusion: In conclusion, signs and symptoms can be managed effectively with endovascular techniques in patients with significant subclavian artery disease. In this study, we shared our real-life data on subclavian artery endovascular treatment.

Keywords: Subclavian artery disease, endovascular treatment, patient characteristic

Introduction

The etiology of subclavian artery stenosis may be multifold; however, it is typically due to atherosclerotic disease and is initially suspected when an inter-arm brachial systolic blood pressure (SBP) difference is present in physical examination[1]. However, other causes such as dissection, radiation-induced inflammation fibromuscular disease, and various vasculitides, especially Takayasu arteritis, are not infrequent[2]. Generally, the left subclavian artery is about four times more likely to be affected than the right subclavian artery[3]. The cases are usually over the age of 50 years. It is seen 1.5-2 times more in men than in women. Subclavian artery disease is usually focal and the lesion is mostly in the first 2 cm proximal segment of the vessel from the aortic origin.

Stenosis of the subclavian artery alone is usually asymptomatic due to the rich collateral circulation in the head and neck vessels. Intervention is generally indicated for the upper limb ischemia, vertebrobasilar symptoms, subclavian steal syndrome, and coronary steal syndrome in which stenosis proximal to internal mammary-coronary artery bypass may cause ischemic symptoms. The preferred treatment modality for subclavian stenosis, which can be treated surgically and endovascularly, is endovascular treatment with low complication rates and high success rates. The first reported successful endovascular treatment of subclavian stenosis was done by Bachman and Kim in 1980[4]. Since that time, numerous other reports have been published in the literature with varying degrees of procedural success. We report here our series of 15 patients who underwent intraluminal balloon dilatation and stent implantation of the subclavian stenosis. In this article, it was tried to draw attention to subclavian artery stenosis, which is a rare site of atherosclerosis, it was planned to discuss endovascular treatment methods, feasibility and complications of the procedure were emphasized.

Materials and Methods

The study was planned as a retrospective registry study. Patients who underwent endovascular treatment for subclavian artery stenosis in our center between January 2019 and January 2021 and whose data could be accessed were included in the study. Patients whose data could not be reached were excluded and no additional exclusion criteria were determined.

The study was approved by the University of Health Sciences Turkey, Gülhane School of Medicine Ethics Committee (date: 20/05/2021, decision no: 2021/231).

Diagnosis and Preprocedural Evaluation

The difference of more than 15 mmHg between systolic blood pressures measured in both arms, weakness or absence of a pulse in one arm suggests subclavian artery occlusive disease. The use of doppler ultrasonography for scanning in subclavian artery stenosis and occlusion is a cost-effective and non-invasive screening method. Monophasic post-stenotic flow and reversal of systolic flow in the ipsilateral vertebral artery suggest the presence of >70% stenosis in the proximal segment of the subclavian artery[5]. In the presence of abnormal or suspicious

ultrasound findings, advanced anatomical imaging should be considered. Although digital subtraction angiography (DSA) is considered the gold standard imaging method, today computed tomography angiography (CTA) and magnetic resonance angiography (MRA) are used with high specificity and sensitivity rates and a success rate equal to conventional angiography[5].

The diagnosis was confirmed by CTA before the procedure in our patient's cohort with suspected subclavian artery stenosis. At the same time, with CTA, lesion characteristics, degree of calcification, collateral circulation, the relationship between vertebral artery, left internal mammary artery (LIMA) and subclavian artery stenosis can be evaluated. This is very important for determining the technique before the process and choosing the material to be used, increasing the success of the process (Figure 1).

Risk factor control is recommended in all patients with subclavian artery occlusive disease to reduce cardiovascular risk[5]. For this purpose, for every patient who did not use antiaggregant and statin therapy, acetylsalicylic acid and high-intensity statin therapy were initiated. Other atherosclerosis risk factors of the patients were controlled. At the same time, symptom-based investigations were conducted for coronary, carotid and lower extremity arterial diseases.

Procedural Details

For all patients, the procedures were performed in the catheter laboratory under local anesthesia. In most cases, the anterograde transfemoral approach is used as the first choice. Once arterial access is established, 5000-8000 IU heparin is administered intravenously.

In our cohort, the procedure was performed with the telescopic method from a transfemoral approach in seven patients. For this purpose, we used 7 or 8 Fr destination sheath and multi-purpose catheter or right Judkins catheter. The schematic view of the transfemoral approach is described in Figure 2. In five patients, the transradial approach was used, first lesion canalization was performed with 0.014 inches 300 cm coronary wire. After the wire was snared in the descending aorta, the procedure was performed with the help of a destination sheath from the transfemoral approach. In three patients, the procedure was performed directly over the transbrachial approach.

In subclavian arteries intervention, the guidewire selection is performed based on the lesion feature and the sheath/guide catheter planned to be used. Conventionally, a 0.028-0.032 inches (at least 260 cm long) guidewire is used to pass moderate stenosis and provide adequate support throughout the procedure. If necessary, for higher grade strictures and complete occlusions, flat-tipped hydrophilic wires and 0.014-inch or 0.018-inch guidewires on the

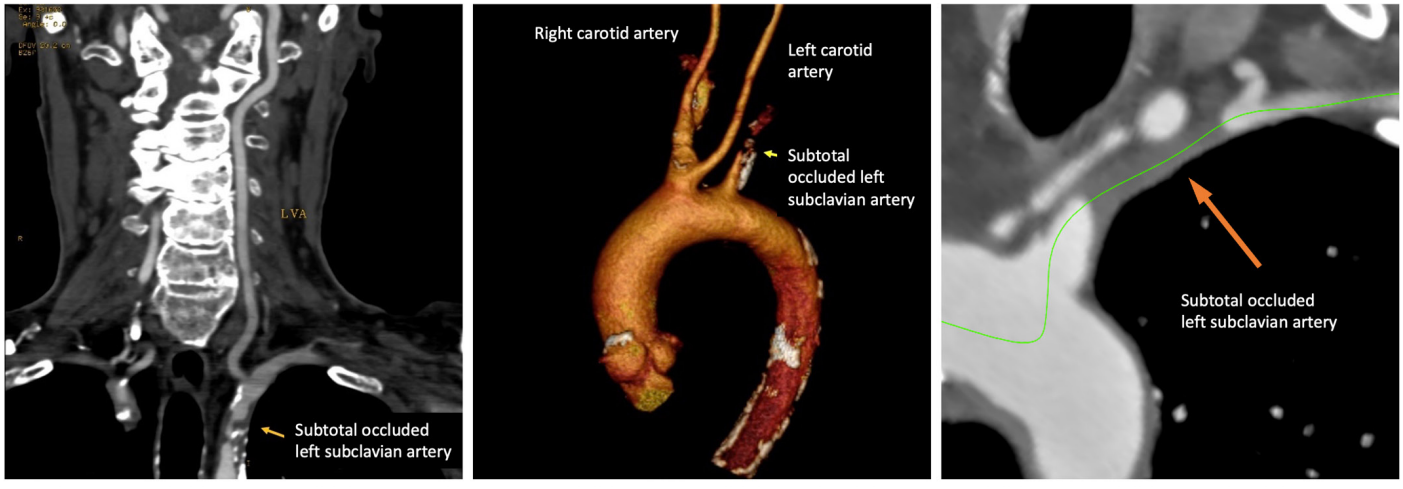

Figure 1. Computed tomographic angiography evaluation before the procedure

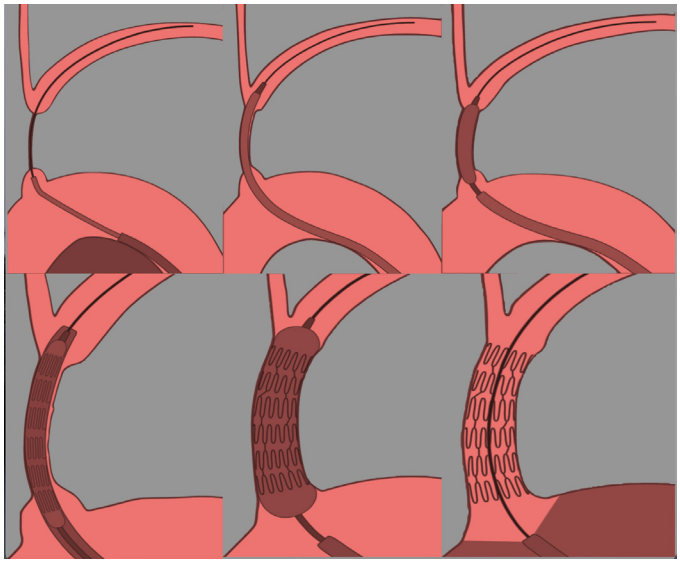

Figure 2. Step by step schematic view of the transfemoral approach

microcatheter support can be used. Stenting, especially balloon-expandable stents, is generally preferred over alone balloon angioplasty in the treatment of subclavian artery stenosis.

Results

The mean age of the patients was 64.66 years and 3 (20%) of the patients were female. The risk factors in this population included hyperlipidemia (13 patients 86.6%), diabetes mellitus (4 patients, 26.6%), hypertension (15 patients, 100%), and smoking (8 patients 53.3%). Coronary artery diseases were present in 13 patients (86.8%); five patients had undergone a coronary artery bypass, of which all had a bypass done using the LIMA (Table 1).

Symptomatology included subclavian steal syndrome and vertebrobasilar insufficiency in nine patients (60%), coronary steal syndrome and protection of a LIMA coronary bypass in five patients (33.3%). Subclavian aneurysm, which is a rare clinic, was our indication in one of our patients (Table 1). Twelve (80%) of 15 patients with subclavian artery stenosis were successfully treated with endovascular methods. In three patients, the procedure was terminated unsuccessfully. The procedure was terminated early because a stroke developed in the subclavian aneurysm patient during the procedure. She recovered without sequelae with anticoagulant therapy in the follow-up. In one patient, the stenosis could not be passed because of a heavily calcified lesion. In the third patient, the procedure was terminated due to heavy calcification and subintimal dissection extending to the brachial artery and thrombus development. These patients, in whom the procedures failed, were started to be followed up under medical treatment because they did not want to undergo surgery, and if they became symptomatic despite medical treatment, it was decided to reschedule endovascular treatment. While the endovascular intervention was performed to a total occluded artery in 8 patients, other patients lesions were not total occluded. Two patients had right subclavian and 13 patients had left subclavian artery disease (Table 1).

Balloon expandable stent implantation was performed in 10 (66.66%) patients at the end of the procedure, and self-expandable stent implantation was performed in one patient. One patient with subclavian stent restenosis was treated only with percutaneous transluminal angioplasty (Table 1).

Discussion

In this study, we shared our real-life experience on subclavian artery endovascular treatment. The basic demographic characteristics of the patients, as well as the technical details of my procedure, were explained. Endovascular therapy has become the primary treatment approach for symptomatic subclavian artery disease. Although surgical revascularization is the preferred treatment option in guidelines and textbooks due to acceptable long-term results, percutaneous endovascular approaches have come to the fore in recent years due to high success rates, long-term patency and low complication rates, shorter hospital-stay and significantly lower cumulative costs compared to surgical treatment[6]. Conventional surgical revascularization can also be performed intrathoracically concurrently with cardiac

Table 1. Basal demographic characteristics and procedure details

	Age	Gender	Comorbidities	Indication	Location	Stenosis degree	Arterial approach location	Stent type and size	Result
Patient 1	77-year-old	Male	HL, HT, CAD	Subclavian artery aneurysm	Right subclavian	-	Right radial	-	Failed due to stroke
Patient 2	78-year-old	Male	HT, CAD, DM, HF	LIMA, coronary steal syndrome	Left subclavian	95 %	Right femoral	10x29 mm balloon expandable stent	Successful
Patient 3	63-year-old	Male	HL, HT, CAD, DM, CKD	LIMA, coronary steal syndrome	Left subclavian	100%	Left radial-snaring-transfemoral	10x30 mm self-expandable stent	Successful
Patient 4	62-year-old	Male	HL, HT, CAD, HF, Stroke, Buerger	LIMA, coronary steal syndrome	Left subclavian	80%	Left brachial	9x37 mm balloon expandable stent	Successful
Patient 5	74-year-old	Male	HL, HT, DM, CAD	Subclavian steal syndrome	Left subclavian	99%	Left brachial	7x40 mm balloon angioplasty	Successful
Patient 6	53-year-old	Female	HL, HT	Subclavian steal syndrome	Left subclavian	90%	Right femoral	8x29 mm balloon expandable stent	Successful
Patient 7	63-year-old	Male	HL, HT, CAD	Subclavian steal syndrome	Right subclavian	90 %	Right brachial	7x29 mm balloon expandable stent	Successful
Patient 8	76-year-old	Male	HT, CKD, HF	Subclavian steal syndrome	Left subclavian	90 %	Right femoral	9x37 mm balloon expandable stent	Successful
Patient 9	65-year-old	Male	HL, HT,	Subclavian steal syndrome	Left subclavian	100%	Right femoral, Left brachial	-	Unsuccessful
Patient 10	56-year-old	Male	HL, HT, DM, CAD	Subclavian steal syndrome	Left subclavian	100%	Left radial- snaring-transfemoral	7x29 mm balloon expandable stent	Successful
Patient 11	65-year-old	Female	HL, HT, CAD	LIMA, coronary steal syndrome	Left subclavian	100 %	Left radial- snaring-transfemoral	9x29 mm balloon expandable	Successful
Patient 12	50-year-old	Male	HL, HT, CAD	Subclavian steal syndrome	Left subclavian	100 %	Left radial- snaring-transfemoral	9x37 mm balloon expandable stent	Successful
Patient 13	69-year-old	Male	HL, HT	Subclavian steal syndrome	Left subclavian	100 %	Right femoral, left brachial	-	Failed due to thrombus
Patient 14	56-year-old	Male	HL, HT, CAD	Subclavian steal syndrome	Left subclavian	100 %	Right femoral	5x24 mm balloon expandable stent	Successful
Patient 15	63-year-old	Female	HL, HT, CAD	LIMA, coronary steal syndrome	Left subclavian	100 %	Right femoral	7x29 mm balloon expandable stent	Successful

HL: Hyperlipidemia, HT: Hypertension, CAD: Coronary artery disease, DM: Diabetes mellitus, CKD: Chronic kidney disease, HF: Heart failure, LIMA: **Left internal mammary artery**

surgery or extra-thoracically as carotid-subclavian, carotid-axillary, subclavian-subclavian and axilla-axillary bypass[7]. Among the surgical revascularization techniques used, carotid-subclavian bypass seems to be more advantageous due to a shorter anastomosis and relatively good patency rates[7].

Depending on the location and severity of the lesion, anterograde (femoral), retrograde (brachial/radial) or combined methods may be preferred in endovascular treatment. The femoral artery approach should be chosen in subtotal lesions with sufficient stump in the proximal subclavian artery. Due to the high fibrocalcific nature of lesion and rich collaterals circulation, it is difficult to pass chronic complete occlusion anterogradely. Therefore, retrograde brachial or radial artery approach may be preferred in total occlusions without an osteal stump, in long segment lesions, in the presence of severe tortuous aorta, in cases where the exit angle of the subclavian artery from the aortic arch is abnormal.

There is no definitive evidence as to whether stenting is more effective than balloon angioplasty alone in subclavian artery occlusive diseases. However, in a systematic review (544 patients) comparing both options, stenting was found to be superior with higher patency rates compared to angioplasty alone[8]. Balloon-expandable stents are preferred rather than self-expandable stents to implant the stent with appropriate sensitivity in lesions in the osteal and proximal regions because there is a possibility of stent dislocation and migration in self-expanding and undersized stents. Moreover, in severe calcified osteal lesions, in addition to easier insertion, balloon-expandable stents provide greater radial force than self-expandable stents. In cases where the lesion is after the vertebral artery, balloon angioplasty should be preferred first, as there is a risk of bending and compression of the stent at the thoracic outlet, especially between the first rib and clavicle. When the lesion does not respond to balloon angioplasty, self-expanding nitinol stents are more appropriate to avoid the possibility of compression by extravascular structures.

While the technical success rate of endovascular treatment in subclavian artery occlusive disease is quite high (97-100%) for stenosis, the success rate for incomplete occlusions decreases because of the length of the occluded segment and the intensity of calcification (46-76%)[6]. De Vries et al.[9], reported in their case series of 110 patients over 10 years that stenting was implanted in 58% of patients with a 93% technical and clinical success and 1% complication rate in the endovascular treatment of symptomatic subclavian artery stenosis. In our case series, twelve (80%) of 15 patients with subclavian artery stenosis were successfully treated with endovascular methods. In three patients, the procedure was terminated unsuccessfully. In a patient who had a subclavian aneurysm, the procedure was terminated early because stroke developed during the procedure, and the other two failed due to total calcific occlusion. In the literature, although long-term follow-up data are insufficient, long-term patency (3-5 years) rate in stenosis is 84%. This rate has been reported as 64% for complete occlusions[10]. In-stent restenosis is a major problem with endovascular procedures. Because the subclavian artery is an elastic and big vessel, the recurrence rate is relatively low and ranges from 5% to 7%[11].

Complication rates ranging from 3% to 11% have been reported with endovascular treatment of subclavian artery stenosis[6]. Reported complications include subclavian artery thrombosis, axillary artery thrombosis, stent migration, stent dislocation, flow-limiting arterial dissection, distal embolization, arterial extravasation, arterial access complications, hematoma requiring transfusion, restenosis, and neurological complications (transient ischemic attack, stroke, hemiplegia, diplopia etc.). In our own case series, complications were observed in two patients (13.33%), the patient with subclavian aneurysm had a stroke complication, and another patient had complications of brachial artery dissection and thrombosis.

Study Limitations

Our study has many limitations. The most important of them is the small number of patients. Another important limitation is that the study was planned retrospectively.

Although the study has very important limitations; we think that it is important that it is a real-life experience and that the etiology, diagnosis, preprocedural evaluation of subclavian artery stenosis and the technical details of the procedure are explained.

Conclusion

Depending on the technological developments in endovascular interventions and the increase in operator experience, endovascular treatment in subclavian artery occlusion has become the primary treatment due to low complication and high success rate. In this study, the experience of our center in this field was transferred based on patient and procedural characteristics.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Turkey, Gülhane School of Medicine Ethics Committee (date: 20/05/2021, decision no: 2021/231).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.A., S.E., Design: S.A., M.Ç., S.G., Data Collection or Processing: S.E., M.G., S.G., S.F., S.Y., Analysis or Interpretation: S.A., M.Ç., U.C.Y., Literature Search: S.A., S.Y., S.F., H.T., U.Ç.Y., Writing: S.A., M.Ç.

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

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Effects of COVID-19 Infection on P-Wave Dispersion, P-Wave Peak Time and Atrial Conduction Times

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Abstract

Objectives: The relationship between atrial conduction times and paroxysmal atrial fibrillation has been demonstrated in some studies. There have been case reports showing that coronavirus disease-2019 (COVID-19) infection caused arrhythmic cases including atrial fibrillation. We investigate the effect of different clinical presentations of COVID-19 infection on these parameters in this study.

Materials and Methods: We divided the patients who were infected by COVID-19 into three groups according to computerized tomography and real-time polymerase chain reaction test results. The longest P-wave duration, shortest P-wave durations, P-wave dispersion and P-wave peak duration were calculated in the surface electrocardiography of these patients.

Results: Patients with both real-time polymerase chain reaction test positive and pneumonia had the highest P-wave maximum duration (113.08±9.671 ms vs 102.44±7.412 ms. and 99.18±9.292 ms; p=0.000) and the highest P-wave dispersion (53.34±7.705 ms vs 40.58±4.813 ms. and 35.42±4.116 ms; p=0.000) and the longest P-wave peak time (56.79±7.767 ms vs 51.92 ms ±6.443 ms and 50.55±11.63 ms; p=0.008). P-wave dispersion was found longer in patients with real-time polymerase chain reaction test only compared to patients with only pneumonia (40.58±4.813 ms. vs 35.42±4.116 ms; p=0.000).

Conclusion: Patients with COVID-19 pneumonia with real-time polymerase chain reaction test positivity have longest P-wave dispersion, P-wave maximum duration and P-wave peak duration. It seems that they have a risk of paroxysmal atrial fibrillation. That's why, that group may benefit most from strict electrocardiography follow-up.

Keywords: COVID-19, p-wave dispersion, electrocardiography, atrial fibrillation

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Introduction

Coronavirus disease-2019 (COVID-19) infection has become a pandemic by show up in Wuhan city of China and has been spreading all over the world. More than eight million people were infected by the virus, and it caused more than 400,000 deaths by June 18th, 2020[1]. Although the infectious agent has been previously known, effects of this novel kind of virus on humans have not been clearly demonstrated. The dark parts in the pathogenesis protect its secret. While initially thought to be progressing only with pneumonia, case reports have emerged that it causes myocarditis, arrhythmia problems, coagulopathy, and micro-embolism[2-5]. Drugs with proarrhythmic effects are frequently used in the treatment of COVID-19 infection in our country and in the world[6-8]. In our country, anticoagulant therapy has been added to patients in accordance with the national guidelines[8]. Anticoagulant therapy is thought to reduce the mortality and morbidity of patients[8-10]. This suggests that the possible procoagulant or arrhythmic condition affects patients.

Real-time polymerase chain reaction (rt-PCR) and chest computerized tomography (CT) are used in the diagnosis of COVID-19 disease. The rt-PCR test is seen as the most widely used and most reliable method. However, the sensitivity of rt-PCR test can vary depending on the stage of patients' disease and the appropriateness of the sampling. CT is widely used, too. However, radiological imaging may have difficulty in clearly distinguishing other viral and atypical pneumonias. In our hospital, we apply both CT and rt-PCR tests to the patients in accordance with the national guidelines[8].

Atrial fibrillation is a frequent pathological arrhythmia among elderly people and patients who have comorbid chronic illnesses. It is one of the most common causes of thromboembolism. Some diseases or conditions have been shown to trigger paroxysmal atrial fibrillation. Infectious diseases are the leading causes[11]. It has been shown in some case reports that patients with COVID-19 infection have atrial and ventricular tachyarrhythmias[12,13]. It has shown that COVID-19 infection causes myocarditis[14].

The values of the longest P-wave duration (P-max), P-wave dispersion (PwD) and P-wave peak duration (PwPD) in demonstrating the function of the atrial conduction system and showing the risk of paroxysmal atrial fibrillation have been demonstrated in many studies[15-18].

In this study, we evaluated these parameters by dividing patients into groups with the most common clinical presentations of known COVID-19 infection in patients. As a result, we aimed to show which patients among these groups may have the highest P-max, PwD, and PwPD. Based on this, we wanted to estimate which group of patients might have a higher risk rate of paroxysmal atrial fibrillation. In addition, we wanted to show the effects of COVID-19 infection on the atrial conduction tissue in cases with pneumonia and in cases without pneumonia.

Materials and Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Permission for the research was taken from Republic of Turkey Ministry of Health. Permission number is 2020-05-20T17_56_41. Ethical approval was taken from University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Training and Research Hospital Ethics Committee, with the report number of 509.

Patient selection

Patients who applied to Tatvan State Hospital between the ages of 15 and 90 years were included in the study. Patients who applied to the hospital with COVID-19 infection were divided into three groups according to their diagnostic status. In determining these groups, the decision was made according to the results of CT and rt-PCR tests. Due to the false negative results, patients with two negative PCR results were considered negative. Possible wrong results were tried to be minimized in this

way. In the CT evaluation, the results were based on the reports made by radiologists, compatible with COVID-19 pneumonia according to national and international guidelines. Patients whose CTs were compatible with COVID-19 pneumonia and positive rt-PCR tests were included in group 1. Patients in group 2 were composed of patients who did not suffer from pneumonia but the performed rt-PCR tests were COVID-19 positive. Patients in group 3 were composed of those whose CT results were compatible with COVID-19 pneumonia but had negative rt-PCR tests.

Demographic data of patients, including their age, gender, comorbid vascular, cardiac, renal, pulmonary, and diabetes diseases were noted. The results of the Hemoglobin (Hb), White Blood Cells (WBC), Platelet (Plt), Urea, Creatine, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), C-Reactive Protein (CRP) and Troponin tests of the patients which had been examined prior to any treatment were recorded.

Electrocardiographic Parameters

Twelve lead electrocardiograms (ECGs) were obtained with 10 mm/mV amplitude and 25 mm/s rate with standard lead positions in a supine position. ECGs were manually measured before PCR test results. Heart rate PR interval time and QRS interval duration were calculated and noted. The duration measurements of the P-wave were determined as the duration from the beginning of the P-wave to the end of the P-wave and the duration from the beginning of the P-wave to the end of P-wave on the leads with the longest (P-max) and shortest (P-min) P-wave. PwD was calculated by finding the difference between P-max duration and P-min duration. The time from the start of the P-wave to the peak of the P-wave was calculated and PwPD was found.

Statistical Analysis

Values are expressed as the mean ± standard deviation (SD). Categorical data were compared using χ^2 analysis and Fischer exact test. SPSS-23 Statical analysis software IBM was used for statistical analysis. Normality of the variables was tested with the Kolmogorov-Smirnov method. Comparison among the three groups was performed using a one-way analysis of variance (ANOVA). The Levene's statistic test was performed in the evaluation of variance homogeneity. The Scheffe test was used in variance homogeneous parameters, Tamhane and Games-Howell test in non-homogeneous parameters. The Kruskal-Wallis test was used for variables that did not show normal distribution. The Mann-Whitney U test was used for the parameters in which difference was detected on the Kruskal-Wallis test. Statistically significant p-value was considered as 0.05.

Results

Demographic Information

There were 38 patients in the first group, 36 patients in the second group and 33 patients in the third group. The average age of the patients was 49.3±15.5 years in group 1, 38.5±16.4 years in group 2, and 42.6±18.4 in group 3. While there was no significant difference between group1 and group 3 and between group 2 and group 3, there was a significant difference between group 1 and group 2 ($p<0.05$). There was no significant difference between the groups in terms of coronary artery disease (CAD), chronic renal failure (CRF), diabetes mellitus, hypertension, heart failure and chronic obstructive pulmonary diseases. The demographic characteristics of the study subjects were summarized in Table 1.

Laboratory and Biochemistry Tests Results

There was not significant difference between the groups in terms of Hb, Plt, urea, creatin, ALT, AST and troponin levels. WBC values were significantly higher in group 3 than in group 2 ($p<0.05$) and higher than group 1 ($p<0.05$). However, WBC values were not significant between group 1 and 2. CRP levels were significantly lower in group 2 than in group 1 and group 3 ($p<0.05$) but there was no significant difference between group 1 and 3 in the levels of CRP. The results of laboratory and biochemistry tests are summarized in Table 2.

Electrocardiographic Parameters

There was not significant difference between patients' heart rates, PR interval durations, and QRS interval durations. P-max durations were significantly higher in group 1 than in group 2 and group 3 (113.08±9.671 ms vs 102.44±7.412 ms vs 99.18±9.292 ms; p=0.000), and there was no significant difference between group 2 and group 3 (p=0.120) (Figure 1). The durations of

Table 1. Demographic characteristics of patients

	Group 1 (Both CT and rt-PCR results compatible with COVID-19)	Group 2 (rt-PCR tests compatible with COVID-19, but CT is not compatible)	Group 3 (rt-PCR tests negative, but CT results compatible with COVID-19)	p-value
Number of patients	38	36	33	
Age	49.37±15.573	38.5±16.466	42.61±18.469	0.022*
Male patients	23	20	19	0.910
Female patients	15	16	14	0.910
Hypertension (%)	7 (18.4)	6 (16.7)	6 (18.2)	0.978
Diabetes mellitus (%)	3 (7.9)	4 (11.1)	3 (9.3)	0.893
Coronary artery disease (%)	2 (5.3)	1 (2.8)	1 (3.0)	0.827
Heart failure (%)	3 (7.9)	1 (2.8)	-	0.205
Chronic obstructive pulmonary disease (%)	4 (10.5)	2 (5.6)	4 (12.1)	0.618
Chronic renal failure (%)	1 (2.6)	1 (2.8)	-	0.637

Values are mean ± standard deviation unless stated. p-value <0.05 is significant.
**p-value: 0.005 between group 1 and 2. p=0.103 between group 1 and 3. But analyses between group 2 and 3 is not significant p=0.305.*
CT: Computerized tomography, rt-PCR: Real-time polymerase chain reaction

Table 2. Hematology and biochemistry test results of the patients

	Group 1 (Both CT and rt-PCR results compatible with COVID-19)	Group 2 (rt-PCR tests compatible with COVID-19, but CT is not compatible)	Group 3 (rt-PCR tests negative, but CT results compatible with COVID-19)	p-value
Number of patients	38	36	33	-
Hemoglobin (g/dL)	14.11±1.894	14.42±1.845	14.84±2.05	0.341
Platelets (*1000 u/L)	211±50	214±50	222±62	0.932
WBC (109/L)	6.23±4.375	5.98±2.284	9.05±4.096	0.000*
Urea (16-43 mg/dL)	33.38±13.36	31.93±17.251	30.34±10.281	0.356
Creatin (0.8-13 mg/dL)	0.96±0.245	0.94±0.309	0.95±0.206	0.544
ALT (0-45 U/L)	25.76±15.079	22.78±13.025	25.48±20.569	0.630
AST (0-35 U/L)	25.13±9.749	22.47±7.516	24.21±11.393	0.549
Troponin (0-42.9 pg/mL)	5.05±7.047	2.43±2.515	10.84±43.459	0.109
CRP (0-5 mg/L)	23.79±28.136	13.33±17.317	37.06±37.047	0.004**

*Values are mean ± standard deviation unless stated. Normal laboratoary ranges are indicated in parentheses. A p-value <0.05 is significant, *: p-value: 0.509 between group 1 and 2. p=0.000 between group 1 and 3. p=0.000 between group 2 and 3, **: p-value: 0.038 between group 1 and 2. p=0.099 between group 1 and 3. p=0.002 between group 2 and 3.*
ALT: Alanine aminotransferase, AST: Acetyl aminotransferase, CRP: C-reactive protein, WBC: White blood cells, CT: Computerized tomography, rt-PCR: Real-time polymerase chain reaction

P-mins were significantly lower in group 1 than in group 3 (59.32±7.353 ms vs 64.18±7.868 ms; p=0,009), and there was not significant difference between groups 1 and 2 (59.32±7.353 ms vs 61.86±5.991 ms; p=0.108) and between group 2 and group 3 (61.86±5.991 ms vs 64.18±7.868 ms; p=0.171). PwDs were significantly higher in group 1 compared to group 2 and group 3 (53.34±7.705 ms vs 40.58±4.813 ms and 35.42±4.116 ms; p=0.000), and significantly higher in group 2 than group 3 (p=0.000) (Figure 2). While PwPDs were significantly higher in group 1 than group 2 and group 3 (56.79±7.767 ms vs 51.92±6.443 ms and 50.55±11.63 ms; p=0.008), there was not significant difference between group 2 and group 3 (p=0.626) (Figure 3). The results of electrocardiographic analysis are summarized in Table 3.

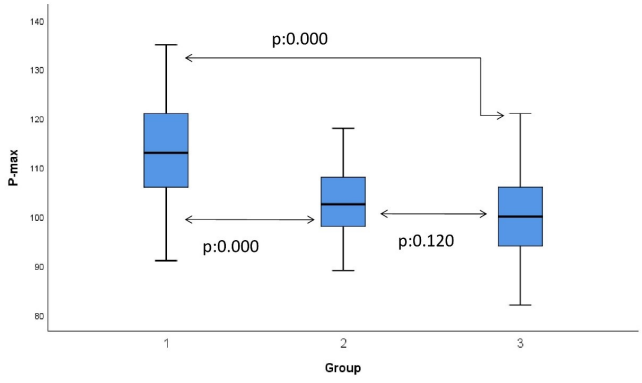

Figure 1. Analysis of patients P-max values between groups

Discussion

In COVID-19 infection, susceptibility to arrhythmia and thrombotic conditions have been demonstrated. The underlying pathophysiology of this condition is unclear. Alteration in P-wave durations has been shown to trigger paroxysmal atrial fibrillation. Paroxysmal atrial fibrillation and atrial arrhythmias can both increase the frequency of thrombotic events and create diastolic dysfunction. In

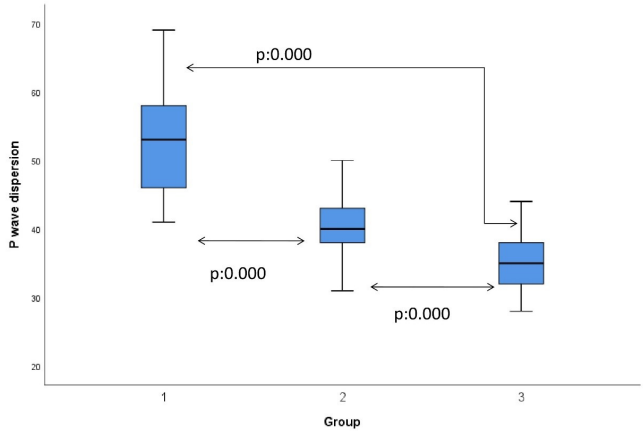

Figure 2. Analysis of patients P-wave dispersion values between groups

Table 3. Electrocardiographic data results of patients

	Group 1 (both BT and rt-PCR results compatible with COVID-19)	Group 2 (rt-PCR tests compatible with COVID-19, but CT is not compatible)	Group 3 (rt-PCR tests negative, but CT results compatible with COVID-19)	p-value
Number of the patients	38	36	33	-
Heart rate (beat/min)	77.5±13.197	81.28±15.978	83.7±17.03	0.234
PR interval duration (ms)	168.89±27.57	160.31±19.76	161.36±27.455	0.533
P-max duration (ms)	113.08±9.671	102.44±7.412	99.18±9.292	0.000[a]
P-min duration (ms)	59.32±7.353	61.86±5.991	64.18±7.868	0.018[b]
P-wave dispersion (ms)	53.34±7.705	40.58±4.813	35.42±4.116	0.000[c]
P-wave peak time (ms)	56.79±7.767	51.92±6.443	50.55±11.63	0.008[d]
QRS interval time (ms)	92.61±17.588	85.83±14.254	86.33±16.547	0.377

Values are mean ± standard deviation unless stated. A p-value <0.05 is significant, [a]: p=0.000 between group 1 and 2, p=0.000 between group 1 and 3, p=0.120 between group 2 and 3, [b]: p=0.108 between group 1 and 2, p=0.009 between group 1 and 3, p=0.171 between group 2 and 3, [c]: p=0,000 between group 1 and 2 , p=0.000 between group 1 and 3, p=0.000 between group 2 and 3 , [d]: p=0.005 between group 1 and 2, p=0.013 between group 1 and 3, p=0.626 between group 2 and 3.
P-max: Maximum P-wave, P -min: Minimum P-wave, ms: miliseconds, CT: Computerized tomography, rt-PCR: Real-time polymerase chain reaction

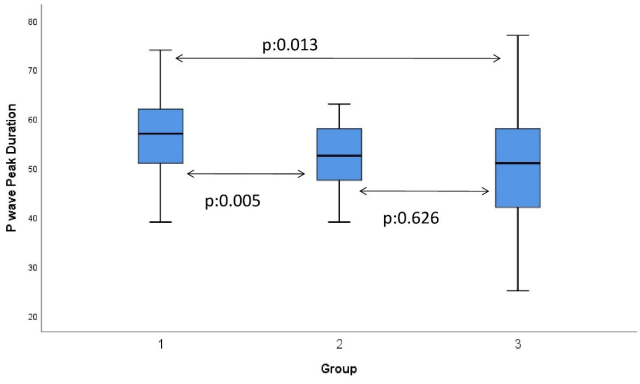

Figure 3. Analysis of patients P-wave peak duration values between groups

this study, P-max, PwD and PwPD values were highest in patients with positive COVID-19 rt-PCR test and in those with CT compatible with COVID-19 pneumonia. PwD was found to be higher among patients with positive COVID-19 rt-PCR test compared to patients with negative rt-PCR test, even if there was no infiltration in the lung. In patients with positive rt-PCR test, patients demonstrating infiltration in CT had higher PwD than patients whose CT was non-compatible with COVID-19 pneumonia.

In the light of these findings, patients with positive rt-PCR test and pneumonia have the longest PwD and PwPD and have highest risk for developing atrial arrhythmias. Rt-PCR positivity increases P-wave times. Based on this finding, rt-PCR positivity is one of the predictable results that may increase susceptibility to atrial arrhythmias in COVID-19 patients. We have seen in the results of this study that even if there is no pneumonic infiltration, there are more changes in the atrial conduction times in patients who have positive rt-PCR test result compared to patients with a negative test result. These results have been given rise to the thought that rt-PCR test positive patients may have an atrial or myocardial involvement with COVID-19 infection.

In addition, even if pneumonic infiltration is not shown in CT, it can be concluded that patients with proven COVID-19 infection by rt-PCR tests have increased susceptibility to atrial arrhythmias compared to patients with negative rt-PCR test results. In the light of this information, patients with pneumonia with rt-PCR positivity seem to be the group that has the highest risk of atrial tachyarrhythmia; that is why, that group may benefit most from strict ECG follow-up. Even if pneumonic infiltration is not observed in CT, it seems that patients with rt-PCR positivity have higher atrial conduction times compared to patients with negative rt-PCR results with COVID-19 compatible infiltration.

Conclusion

In COVID-19 infected patients with rt-PCR positivity, especially in those with COVID-19 pneumonia in CT, P-max, PwD and PwPD were longer than in other patients. This difference persists in patients with CT negative, and as a result, it shows an increased susceptibility to atrial arrhythmias in these patients. Based on this, it is thought that strict ECG monitoring may be beneficial in PCR positive patients.

Ethics

Ethics Committee Approval: Ethical approval was taken from University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Training and Research Hospital Ethics Committee, with the report number of 509.

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.B., E.İ., Concept: B.B., E.İ., Design: B.B., E.İ., Data Collection or Processing: B.B., E.İ., Analysis or Interpretation: B.B., E.İ., Literature Search: B.B., E.İ., Writing: B.B., E.İ.

Conflict of Interest: The authors report no conflicts of interest. The authors are the only responsible of the content which is written in this paper.

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Research Article

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Krill Oil Prevents Atherosclerosis in an Experimental Model

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Abstract

Objectives: The major aim of coronary artery disease management is to reduce the formation and progression of atherosclerotic plaque. Omega-3 fatty acid supplementation that is obtained from fish has been shown to reduce cardiovascular events. Krill (Euphasia superba) are small crustaceans that live in cold seas like the Antarctic Ocean. Recently, due to its high content, and form of the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), krill oil has become popular in researches dealing with the prevention of cardiac disorders and cancer. This study aims to analyze the effect of krill oil supplements on atherosclerosis in rats treated with high-fat diet and streptozotocin-induced diabetes mellitus.

Materials and Methods: Twenty Sprague-Dawley male rats were split into two groups: the control group (C group) and the krill oil group (KO group). Each group was fed with a high-fat diet for six months and streptozotocin was injected subcutaneously to obtain an experimental model of atherosclerosis. The KO group received a 50 mg daily supplement of krill oil orally. Rats were sacrificed after six months for biochemical and histopathological examinations of the aorta and coronary arteries.

Results: The atherosclerosis model was confirmed by elevated levels of low-density lipoprotein (LDL), triglyceride (TG), total cholesterol (TC), and glucose, also decreased insulin and high-density lipoprotein (HDL). The atherosclerosis index (TC/HDL) was lower in the C group compared to the KO group ($p=0.012$). Serum native thiol and total thiol levels were higher; however, the disulfide level was lower in the KO group. This result was statistically significant ($p<0.001$).

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In the KO group, there was a significant decrease in the number of foam cells discovered in the tissue examined from the aorta and coronary arteries with hematoxylin and eosin staining.

Conclusions: The present study indicates that krill oil supplements attenuate the number of foam cells in the aorta and coronary arteries, indicating the preventive effect of krill oil on atherosclerosis.

Keywords: Krill oil, omega-3, antioxidants, atherosclerosis, experimental model

Introduction

In 1904, Felix Marchand became the first person to use the word "atherosclerosis" to include the various arterial lesions and to emphasize the presence of lipid material in the lesions[1]. In addition to lipid accumulation in the vascular intima and wall, atherosclerosis is associated with inflammation[2,3]. These lesions cause luminal occlusion or may result in thrombosis and arterial embolism. Atherosclerosis manifests itself in the arterial vascular system as coronary artery disease (CAD), peripheral artery disease, carotid artery disease, renal artery occlusion, and abdominal aortic aneurysm. CAD is one of the leading causes of morbidity and mortality in the world. Therefore, the basic principle of combating CAD is to reduce atherosclerotic plaque formation and progression[4]. The initial lesion of atherosclerosis is fatty streaking. This lesion occurs as a result of the accumulation of lipid-loaded macrophages in the intima of the arteries[5]. The main factors that lead to the formation of these fatty lines and atherosclerosis are impaired lipid metabolism, vascular cellular activation, and inflammation[6]. In addition to dyslipidemia, hyperglycemia, hypertension, and obesity are also modifiable risk factors of atherosclerosis and eventually CAD. A healthy and balanced diet with regular exercise is the most important recommendation to prevent atherosclerosis[7]. A healthy diet includes a low-fat diet, particularly low in saturated fats, to reduce serum cholesterol levels. Furthermore, in the case of dyslipidemia, serum cholesterol levels can be controlled with drugs such as statins[8]. Statins prevent the formation of atherosclerosis both by preventing the synthesis of cholesterol in the liver and by showing an anti-inflammatory effect on the artery wall[9]. In recent years, long-chain omega-3 polyunsaturated fatty acids such as EPA and DHA, which are mainly found in fish, have been gaining interest and starting to take their place in the prevention of atherosclerosis[10]. Krill (*Euphausia superba*) are small crustaceans that resemble shrimp in appearance and live in cold seas such as the Norwegian Sea and the Antarctic Ocean. Krill is more commonly known as whale food; however, it is also a food source for seals, squid, other fish, seabirds, and to a lesser extent humans. Krill oil is an important source of omega-3 and is obtained from krill. It is purer than fish oil, and it can be used wherever fish oil is used. Recently, due to its high omega-3 content in the form of EPA and DHA, krill oil has become popular in research dealing with the prevention of cardiac disorders and cancer. Omega-3 found in normal fish oil differs from fatty acids, and its bioavailability is much higher. Apart from the fatty acids, krill oil contains triglycerides, vitamin A, vitamin E, tocopherol, astaxanthin, and flavonoid[11].

There are many risk factors for atherosclerosis. One of these is hyperlipidemia and diabetes mellitus. This study aims to analyze the effect of the long-term administration of krill oil supplements on atherosclerosis in rats treated with high-fat diet and streptozotocin-induced diabetes mellitus.

Materials and Methods

The study protocol was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (no: 18/05, date: 31.01.2018). All animals used in the study

were treated following the criteria specified in the Guide for the Care and Use of Laboratory Animals. Providing experimental animals and all experimental stages were carried out at Kırıkkale University Hüseyin Aytemiz Experimental Research and Application Laboratory.

The Animals and Experiment Preparation

This experimental study included a total of 20 male Sprague-Dawley rats weighing 255±25 g and aged three to four months. The animals were kept at a temperature of 21±1 °C and humidity of 50-55%. A maximum of seven or eight rats were placed per cage. Water and food were provided ad libitum and all rats were fasted before the experiment and kept in a 12-hour light and 12-hour dark cycle.

The rats were randomized using a table of random numbers and divided into C and KO Groups, each containing 10 animals[12]. The body weights of the rats were measured and recorded at the beginning and end of the experiment. Since rats are atherogenesis-resistant animals, the atherogenesis model was applied[13]. Each group was fed with a high-fat diet and injected with a single dose of streptozotocin subcutaneously to obtain an experimental model of atherosclerosis[14,15]. The fat diet contained 31.5% animal fat. Streptozotocin 40-80 mg/kg (Glentham Life Sciences, England) was administered subcutaneously to both groups to produce diabetes mellitus. Three days after the injection of streptozotocin, the blood glucose from the tail vein of the rats was measured with a glucose meter (Accu-Chek® Active, Roche Diagnostics, Basel, Switzerland) working with the "glucose-oxidase peroxidase" method and the formation of diabetes mellitus was controlled. While a normal blood glucose level was accepted as 90-110 mg/dL, those with blood glucose levels above 200 mg/dL were considered diabetic[16]. Blood glucose levels were found to be above 200 mg/dL in all animals. Each day 50 mg of krill oil was added to the KO Group in their daily diet by the gavage method (Superba™ Krill Oil, Aker BioMarine ASA, Oslo, Norway). All animals were fed with this diet for six months. The rats who died during the experiment were planned to be excluded from the study. Two animals from each group died before the experiment; therefore, both groups consisted of eight animals each.

Anesthesia

After six months, anesthesia was applied with 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacıbaşı, Turkey) and 10 mg/kg xylazine hydrochloride (Alfazyme®, Alfasan International BV, Woerden, Holland) intraperitoneally.

Surgical Procedure

All of the rats were placed in the supine position. The sternum was opened using surgical scissors to reach the heart. Animals were sacrificed after blood and tissue samples were taken.

Biochemical Analysis of Blood

Blood samples were taken from the right atrium for biochemical tests (Figure 1). Serum samples were centrifuged at 2,000 rpm for eight minutes and then kept at –80 °C. An automatic biochemical analyzer, BS800M (Mindray, P.R. China), was used to measure the levels of HDL, LDL, TG, TC, glucose, and insulin. Then, the atherosclerosis index (TC/HDL) was calculated using these results[17]. Using the serum, native thiol (mmol/L), total thiol (mmol/L), and disulfide levels (mmol/L) were calculated spectrophotometrically (Shimadzu UV-1201 spectrophotometer, Kyoto, Japan).

Histopathological Analysis

Tissue samples were taken from the coronary arteries and ascending aorta for histopathological examination. Rats were sacrificed after biochemical and histopathological examinations. Ascending aorta and coronary artery tissues were kept in a 10% formol solution and sections parallel to the long axis were taken semi-perpendicularly for a routine tissue follow-up. After the tissue follow-up, samples were placed in paraffin, and sections 5-micron thick were taken from the paraffin blocks. Hematoxylin-eosin staining was used to detect foam cells in the walls of the aorta and coronary arteries (Bio-Optica, Milan,

Italy). All images were digitized using a light microscope (Olympus AX80; Olympus Optical, Tokyo, Japan).

Statistical Analysis

The data obtained in the study were evaluated (SPSS Inc., U.S.A.) using Windows 16.0 program for statistical analysis. Descriptive statistics were given as mean ± standard deviation (mean ± SD). Comparison of continuous and ordinal variables among the groups was performed with the Kruskal-Wallis variance analysis. The Mann-Whitney U test was used for group comparisons. For preventing significant inflation, a p-value <0.05 was considered statistically significant.

Results

Induction of atherosclerosis and diabetes in rats after a high-fat diet and streptozotocin injection was confirmed by elevated levels of TC, TG, and LDL and increased glucose and decreased insulin levels. The HDL and insulin levels in the C group were lower than in the KO group. TC, LDL, TG, and glucose levels were found to be lower in the KO group than in the C group. A statistically significant difference was found in the C group atherosclerosis index (TC/HDL) when compared to the KO group. The results obtained are shown in Table 1.

Serum native thiol and total thiol levels measured in the KO group were lower than in the C group and the serum disulfide level in the KO group was lower than the C group. When the two groups were compared, a statistically significant difference was found (p<0.001). The data are shown in Table 2.

There was no statistically significant difference in the pre-experiment body weights between the KO group and the C group. It was determined that the weight gain was less in the K group after the experiment. A statistically significant difference was found between the body weights after the experiment between the KO group and the C group. The results are shown in Table 3.

Table 1. Biochemical data in blood

Parameter	KO group (n=8) Mean ± SD	C group (n=8) Mean ± SD	p-value
HDL (mg/dL)	22.51±2.64	14.60±3.12	**<0.001**
LDL (mg/dL)	29.33±1.41	41.38±1.48	**<0.001**
TG (mg/dL)	180.40±13.42	219.01±42.16	**0.027**
TC (mg/dL)	61.70±4.38	79.05±1.36	**<0.001**
Atherosclerosis index (TC/HDL)	2.74±1.65	5.41±0.43	**0.012**
Glucose (mg/dL)	221.30±10.70	259.55±14.25	**<0.001**
Insulin (µU/mL)	24.12±0.35	27.32±0.38	**<0.001**

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride, TC: Total cholesterol, KO: Krill oil, C: Control, SD: Standard deviation

Table 2. The antioxidant markers of the groups in the study

Parameter	KO group (n=8) Mean ± SD	C group (n=8) Mean ± SD	p-value
Serum disulfide level (mmol/L)	15.96±4.15	20.17±0.65	**0.001**
Serum native thiol level (mmol/L	172.61±22.11	78.31±11.95	**0.001**
Serum total thiol level (mmol/L)	267.15±16.42	110.46±6.32	**0.001**

KO: Krill oil, C: Control, SD: Standard deviation

Table 3. Bodyweight before and after the experiment

Parameter	KO group (n=8) Mean ± SD	C group (n=8) Mean ± SD	p-value
Pre-experiment bodyweight (g)	257.5±14.6	251.4±12.9	**0.061**
Bodyweight (g) after the experiment	512.8± 25.2	540.5±32.6	**<0.001**

KO: Krill oil, C: Control, SD: Standard deviation

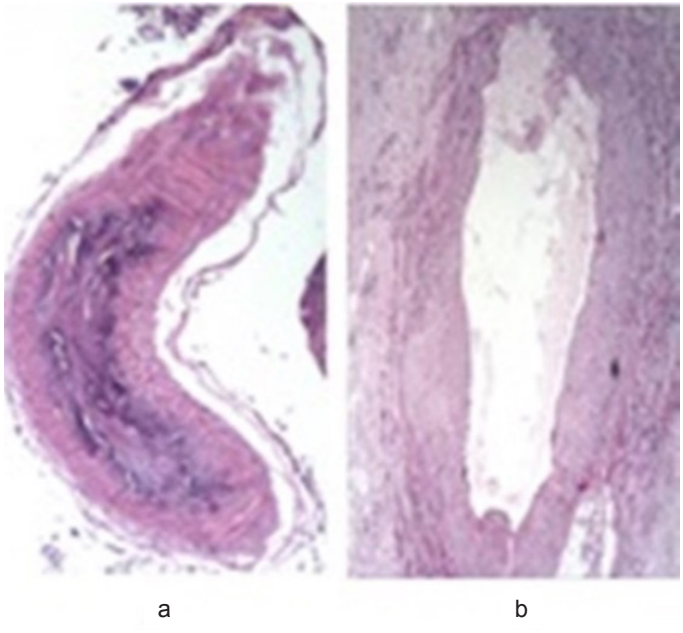

Figure 1. Hematoxylin and eosin staining of the aorta, and coronary artery tissue in the control group. a) shows medial calcific sclerosis, a thickened arterial wall and stenosis of the lumen. b) shows the irregular arterial lumen, inflammatory cells in the arterial wall and lumen

There was a significant increase in the number of foam cells in the aorta in the C group (Figure 1). Figure1a shows medial calcific sclerosis, a thickened arterial wall, and stenosis of the lumen. These are the early signs of atherosclerosis.

Figure 1b shows the irregular arterial lumen, inflammatory cells in the arterial wall and lumen, and migration of smooth muscle cells from media to intima.

The KO group showed a decreased number of foam cells, with a regular structure of the arterial wall and the intima**.** Images are shown in Figure 2.

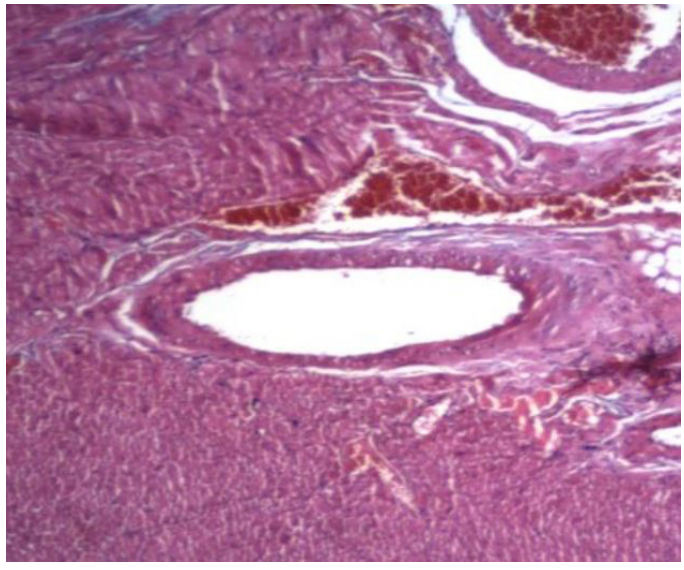

Figure 2. Hematoxylin and eosin staining in the aorta and coronary artery tissue in the krill oil group

Discussion

The main finding from this experimental study is that krill oil is proven to be effective in reducing the development of atherosclerosis in rats treated with high-fat diet and streptozotocin-induced diabetes mellitus. This result was observed in the aorta and coronary arteries.

Atherosclerosis is the most significant cause of heart attacks and strokes, characterized by the thickening and hardening of the arterial walls. It begins in childhood and clinical consequences are visible after a long asymptomatic period[18]. Atherosclerotic lesions are formed during the chronic inflammatory process. In various studies on humans, causes such as dyslipidemia, diabetes mellitus, hypertension, smoking, older age, male gender, heredity, obesity, and lifestyle are among the modifiable and

unchangeable risk factors for atherosclerosis. Lifestyle includes diet and physical activity, which has been shown to play a significant role in the prevention of cardiovascular disease[19]. Believed to protect the cardiovascular system, nutrients such as fish oil and krill oil, which are sources of omega-3, have become very popular and are widely available on the market; reports on their health benefits are driving a steady increase in seafood consumption. Even though human consumption of fish-based food products has increased, the amount of fishing has not risen for a long time and is not expected to grow. A constant increase in fish consumption alongside a decrease in fish resources has created an imbalance between supply and demand. This situation has led to the need for new supplies. Krill, a crustacean living in the Antarctic Ocean, has come to the forefront as such a resource[20].

In epidemiological studies, it has been reported that the risk of CAD increases as the TC and LDL levels increase in humans. It has been stated that the oxidative change of LDL has a significant effect on the pathogen of atherosclerosis[21,22]. Many human and animal studies have shown that fish and krill oils cause significant changes in serum lipid levels[23]. When studies investigating the effect of KO on serum lipids in humans were examined, a meta-analysis was found to report a decrease in LDL and TG[24,25].

Metabolic syndrome is an endocrinopathy in which insulin resistance starts with systemic disorders such as dyslipidemia, hypertension, obesity, diabetes mellitus, and CAD. It does this by causing atherosclerosis in the arteries[26]. In our study, there was no difference in the mean body weight between the groups at the beginning of the experiment; however, a statistically significant difference was found at the end of the study ($p=0.061$). It was determined that the average weight gain was higher in the C group. The more severely impaired lipid profile and reduced glucose and insulin levels seen in the C group may explain the greater weight gain in this group. In addition, low insulin levels and increased glucose levels were detected in both groups with the experimental diabetes mellitus induced by streptozotocin. The high glucose level and low insulin level in the C group were higher than the KO group. This result was statistically significant ($p<0.001$). These results may indicate that krill oil is effective in preventing the development of atherosclerosis.

The atherosclerosis index is used to evaluate cardiovascular risk and is calculated using the ratio of serum TC to HDL; this index is a strong indicator of coronary heart disease. The TG/HDL ratio evaluates the risk of CAD compared to TC or LDL cholesterol levels. In hypercholesterolemic animals, there is an increase in atherosclerosis index, which is a risk factor for atherosclerosis[27]. Our results showed a statistically significant difference between the C Group and KO Group regarding the atherosclerosis index ($p=0.012$). According to their calculated atherosclerotic index value, this result puts the C group in the high cardiovascular risk group.

Krill oil contains omega-3 fatty acids and a variety of antioxidants that differ from those in fish oil[28]. These antioxidant properties are derived from astaxanthin, vitamin A, vitamin E, marine tocopherol, and flavonoid[29,30]. Unlike fish oil, krill oil has a high content of antioxidants, which has been shown in many studies[31,32]. Krill oil also does not have EPA and DHA in the form of TG as in fish oil, instead, omega-3 fatty acids bind to phospholipids[33]. This binding of omega-3 fatty acids, mainly in the form of phosphatidylcholine, is thought to increase both its bioavailability and antioxidant properties[34]. In many studies, it has been reported that chronic inflammation plays a main role in the pathophysiology of atherosclerosis[35]. Antioxidants have been shown in studies to reduce inflammation[36]. Native thiol, total thiol, and disulfide levels examined from serum in our study are antioxidants, which are indicators of oxidative stress[37]. They have critical roles in thiol-disulfide homeostasis, antioxidant protection, detoxification, apoptosis, regulation of enzymatic activity, and cellular signaling mechanisms[38]. In our study, native thiol and total thiol levels were found to be higher and

disulfide levels to be lower in the KO group compared to the C group ($p<0.001$). From these results, due to the antioxidant content of krill oil, we can say that it may be more effective in preventing the development of atherosclerosis.

The first stage of atherosclerosis that can be seen under a microscope is the formation of fatty streaks from foam cells[39]. Parolini et al[40]. created an atherosclerosis experimental model on mice with apoE-deficiency. Throughout their studies in which they wanted to show the effect of krill oil on lipid levels and histopathological examination, they demonstrated that as a result of histopathological examination of the ascending aorta, abdominal aorta, and iliac arteries, the development of atherosclerotic plaque in krill oil decreased. In our study, as a result of histopathological examination of the ascending aorta and coronary artery tissues stained with hematoxylin-eosin, an important increase in the number of foam cells, fatty streak, medial calcific sclerosis, and a thickened arterial wall, which are the early signs of atherosclerosis in the aorta, was found in the control group. In addition to these findings, it showed the irregular arterial lumen, inflammatory cells in the artery wall and lumen, and the migration of smooth muscle cells from the environment to the intima. There was a decreased number of foam cells with a regular structure of the arterial wall and the intima in the KO Group. These histopathological results obtained in the KO Group show that krill oil has a protective effect from atherosclerosis. This result is similar to the results of Parolini et al. [40] We also demonstrated the antioxidant effect of krill oil by its change in oxidative stress markers in the blood.

Study Limitations

The limitation of this study is that pro-inflammatory oxidant markers secreted by foam cells were not examined.

Conclusion

Krill oil, as a new source of EPA and DHA, is suggested to attenuate atherosclerosis in the present study. Prospective randomized trials using krill oil as a supplement for humans are warranted to confirm these results.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (no: 18/05, date: 31.01.2018).

Informed Consent: Not obtained since the study is an animal experiment study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.Y., A.B., A.T.K., Concept: G.Y., A.B., Design: G.Y., A.B., A.T.K.., Data Collection or Processing: G.Y., A.B., Analysis or Interpretation: G.Y., A.T.K., Literature Search: G.Y., A.T.G., Writing: G.Y., A.B., A.T.K.

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Research Article

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Patients with Vitamin D Deficiency Are at Higher Risk of Developing Calcified and Mixed Plaques

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Abstract

Objectives: Vitamin D plays a role in the cardiovascular system through its pleomorphic effects. In some studies, it has been reported that the relationship between vitamin D deficiency and coronary artery calcification is inconsistent. In this study, it was aimed to evaluate the relationship between the vitamin D level and coronary artery calcium score (CACS), plaque presence, and plaque type.

Materials and Methods: Included in this retrospective cohort study were 719 patients who had no previously known coronary artery disease (CVD), and for whom coronary computed tomography angiography (CCTA) was performed between 2015 and 2019. Patients were classified as normal, inadequate, or deficient according to their levels of vitamin D deficiency. They were evaluated according to the presence of plaque on their CCTA or CACS >0 atherosclerosis. Moreover, patients were separated into four groups, comprising zero-plaque (those that were not plaque according to the plaque type), mere fatty plaque (CACS=0), mere calcified plaque, and mixed plaque. Age, sex, smoking status, diabetes mellitus, hypertension, and hyperlipidemia were evaluated as traditional risk factors.

Results: In 18.4% of the patients, the vitamin D levels were normal, whereas they were inadequate in 65% and deficient in 16.7%. The median CACS of the patients was 0 (range: 0-3759), and mere fatty plaque was found in 13.5% of patients,

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whereas 13.4% had mere calcified plaque, and 27.5% had mixed plaque. A negative correlation was detected between the vitamin D levels and CACSs (r=0.345; p<0.001). The median CACS in those with vitamin D deficiency was higher when compared to those with inadequate and normal levels (normal: 0 vs inadequate: 0 vs deficient: 7; p<0.001). Regardless of the traditional risk factors, vitamin D deficiency was found to be an independent predictor of atherosclerosis [odds ratio (OR): 6.9; 95% confidence interval (CI): 3.53-13.52; p<0.001], fatty plaque (OR: 3.04; 95% CI: 1.34-6.87; p=0.008), mere calcified plaque (OR: 13.11; 95% CI: 3.53-13.52; p<0.001), and mixed plaque (OR: 14.27; 95% CI: 5.58-36.50; p<0.001). Moreover, regardless of the traditional risk factors, the vitamin D deficiency increased the risk of fatty plaque development by 2.37 times in patients with CACS: 0 (OR: 2.37; 95% CI: 1.01-5.62; p=0.045).

Conclusion: A decrease in vitamin D level is associated with an increase in the CACS, and the development of calcified and mixed plaque is more likely when there is vitamin D deficiency. Depending on the incidence of CVDs and vitamin D deficiency in asymptomatic patients, vitamin D supplements can be beneficial.

Keywords: Vitamin D, coronary artery calcium score, atherosclerosis, coronary artery disease

Introduction

Vitamin D deficiency, which is a major public health problem worldwide, has been shown to be an important risk factor in cardiovascular diseases[1,2]. In cardiovascular diseases, coronary artery disease (CAD) is one of the leading causes of morbidity and mortality. Growing evidence has suggested that many factors, including vitamin D, play a role in coronary plaque formation[2-4]. Vitamin D deficiency affects a large number of cells involved in atherogenesis (such as immune cells, endothelial cells, smooth muscle cells, and cardiomyocytes)[5-8].

Coronary artery calcification (CAC) is the pathognomonic finding of atherosclerosis, as well as a good marker of atherosclerotic plaque load[9,10]. Moreover, coronary calcium measurements have been associated with histological measurements of atheromatous plaque[11,12]. The growing evidence supports the role that vitamin D plays in the development of cardiovascular impacts and CAC; however, there have also been studies that have shown no relationship between them[13-18]. Different geography and patient groups form the basis of this contrast. However, no studies that have assessed the relationship between the vitamin D level and the coronary artery calcium score (CACS), presence of plaque, and plaque-type in a wide cohort could be found.

In this study, it was aimed to examine the role of vitamin D deficiency by evaluating the relationship between vitamin D levels and the CACS, presence of plaque, and plaque type.

Materials and Methods

Study Population

This retrospective cohort study was designed and undertaken at the Cardiology and Thoracic Surgery Clinics of the İstanbul Yedikule Training and Research Hospital. All aspects of the research were carefully designed to comply with the 2013 Declaration of Helsinki, as well as the principles of good clinical practices. The relevant ethics committee granted approval of the study [İstanbul Training and Research Hospital, University of Health Sciences, Clinical Research Ethics Committee (decision date/no: 24.07.2020/2481)]. Furthermore, the consent of all participants was also obtained in both verbal and written form before the study began.

Included in the study were 719 patients who had no previously known CAD, and for whom coronary computed tomography angiography (CCTA) was performed between 2015 and 2019. The exclusion criteria of the study included the presence of cardiac failure, congenital heart

disease, history of asthma, and history of CVD, history of pulmonary embolism, chronic obstructive lung disease, and history of kidney disease.

The demographic data (gender, age, hypertension, diabetes mellitus (DM), hyperlipidemia, and smoking status), the laboratory data, and the CCTA data of all participants were obtained from their patient files using the electronic information system of the hospital.

Laboratory Testing

Results of the blood sample tests were obtained from the patient files as described above. Platelets were measured using the impedance method, and other hemogram parameters were measured using a Sysmex XE 2100 hematology analyzer (Roche Diagnostic, Corp. IN, USA), and hemoglobin was measured photometrically. C-reactive protein was measured using the immunoturbidimetric method, albumin was measured using the bromine cresol green method, triglycerides and total cholesterol were measured using the enzymatic colorimetric method, and high-density lipoprotein (HDL) cholesterol was measured using the homogeneous enzymatic colorimetric method in a Beckman Coulter DX1800 Analyzer (Indianapolis, USA). Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula.

Vitamin D levels were measured using the 25 (OH) 2D3 radioimmunoassay method (Beckman Coulter, Indianapolis, USA) in an auto analyzer. Vitamin D was classified as normal (vitamin D >30 ng/mL), insufficient (10-30 ng/mL), or deficient (<10 ng/mL)[19].

Coronary Artery Calcification Assessment

All imaging was performed with a 64 multi-slice computed tomography (Toshiba Aquillon, Japan). During the examination, the heart was scanned in the craniocaudal direction, from the carina to the apex. During the process, imaging was performed using the parameters 120 Kvp, 300 mA, 75 mAs, and 3-mm section thickness. Next, all of the images were transferred to the workstation for calcium scoring and evaluated using a Toshiba Aqua 4.1 device (Otawara, Japan). The CACS was calculated considering a threshold of 130 HU, as described by Agatston et al.[20]. A CACS: 0 was evaluated as the absence of CAC. The CACS was categorized into the following five classes: 0, 1-99, 100-399, 400-999 and ≥1000. In all of the coronary segments, the coronary plaque was defined as 1) zero-plaque, 2) calcified (a more intense computed tomography (CT) density than the coronary lumen filled by contrast), 3) non-calcified (less density than the coronary lumen filled by contrast, but more CT density than the connective tissue around it), or 4) mixed (plaque containing both calcified and non-calcified components). One coronary plaque was assigned per coronary segment.

Statistical Analysis

IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA) was used for all of the statistical analyses. To determine whether or not the data were normally distributed, the Kolmogorov-Smirnov test was applied. While the numerical variables were given as the mean ± standard deviation or median (minimum-maximum), the categorical values were given as numbers and percentages. The chi-square test and Fisher exact test were applied to compare the categorical data. The student t-test or Mann-Whitney U test was used in the comparison of numerical variables in two groups according to the normality of the distribution. The ANOVA test (post hoc: Bonferroni test) or Kruskal-Wallis H test (post hoc: Dunn test) was used to compare the numerical variables between the plaque types according to the normality of the distribution. The Spearman correlation analysis was used for the relationship between the CACS and the vitamin D level. Age, gender, smoking status, DM, hypertension, and hyperlipidemia were considered as traditional risk factors. The association of vitamin D deficiency and the presence of plaque was used in the logistic regression analysis and adjusted traditional risk factors. $P<0.05$ was considered as statistically significant.

Results

The mean age of patients was 51.9±6.9 (range: 35-65) years, 63.3% were male, and the median vitamin D level was 17.2 (range: 2.2-57.6). The ratio of patients who had normal vitamin D levels was 18.4% (n=132), whereas it was 65% for those who had inadequate levels, (n=467), and it was 16.7% for those who had vitamin D deficiency (n=120). The median CACS score of the patients was 0 (range: 0-3759), and 59.1% had normal calcium scores, whereas 11.3% had minimal, 16.7% had mild, 9.5% had moderate, and 3.5% had severe calcium scores (Table 1). Demographic and laboratory findings of the patients are shown in detail in Table 1.

Table 1. Demographic and laboratory findings

Variables	All population (n=719)	Vitamin D			
		Normal (n=132)	Insufficient (n=467)	Deficient (n=120)	p-value
Age, years	51.9±6.9	52.4±6.4	51.7±7.1	51.7±7.0	0.558
Gender, n (%)					
Female	264 (36.7)	29 (22.0)	193 (41.3)	42 (35.0)	**<0.001***
Male	455 (63.3)	103 (78.0)	274 (58.7)	78 (65.0)	
Diabetes mellitus, n (%)	211 (29.3)	33 (25.0)	142 (30.4)	36 (30.0)	0.477
Hypertension, n (%)	323 (44.9)	57 (43.2)	206 (44.1)	60 (50.0)	0.464
Hyperlipidemia, n (%)	263 (36.6)	34 (25.8)	173 (37.0)	56 (46.7)	**0.003***
Cigarette smoking, n (%)	301 (41.9)	53 (40.2)	196 (42.0)	52 (43.3)	0.880
CACS	0 (0-3759)	0 (0-587)	0 (0-3759)	7 (0-1100)	**<0.001***
Normal, n (%)	425 (59.1)	115 (87.1)	263 (56.3)	47 (39.2)	**<0.001***
Minimal, n (%)	81 (11.3)	2 (1.5)	60 (12.8)	19 (15.8)	
Mild, n (%)	120 (16.7)	9 (6.8)	80 (17.1)	31 (25.8)	
Moderate, n (%)	68 (9.5)	4 (3.0)	50 (10.7)	14 (11.7)	
Severe, n (%)	25 (3.5)	2 (1.5)	14 (3.0)	9 (7.5)	
Plaque, n (%)					
No plaque	328 (45.6)	99 (75.0)	197 (42.2)	32 (26.7)	**<0.001***
Yes	391 (54.4)	33 (25.0)	270 (57.8)	88 (73.3)	
Only fatty plaque	97 (13.5)	16 (12.1)	66 (14.1)	15 (12.5)	**<0.001***
Only calcific plaque	96 (13.4)	6 (4.5)	67 (14.3)	23 (19.2)	
Mixt plaque	198 (27.5)	11 (8.3)	137 (29.3)	50 (41.7)	
Hemoglobin, g/dL	13.6±1.5	13.6±1.2	13.6±1.6	**13.3±1.5**	**0.008***
Platelet, x10^3 µL	256.9±61.3	251.8±58.5	257.1±60.1	261.5±68.5	0.455
HDL, mg/dL	50.2±12.8	**55.7±13.6**	49.5±11.7	47.1±14.2	**<0.001***
LDL, mg/dL	136 (35-410)	144 (37-275)	136 (35-410)	127.5 (48-353)	0.068
Triglyceride, mg/dL	132 (36-992)	**118 (36-491)**	133 (40-992)	145 (57-857)	**0.033***
Albumin, g/dL	4.4±0.3	4.4±0.3	4.4±0.3	**4.2±0.3**	**<0.001***
Creatinine, mg/dL	0.7±0.2	0.7±0.2	0.7±0.2	0.7±0.2	0.505
hs-CRP, mg/L	0.5 (0-5.5)	**0.2 (0-1.5)**	0.6 (0-5.5)	0.5 (0-3.9)	**<0.001***
Vitamin D, ng/mL	17.2 (2.2-57.6)	31.9 (9.3-57.6)	17.2 (10-29.9)	7.5 (2.2-9.8)	**<0.001***

Numerical variables were shown as mean ± standard deviation or median (min-max).
Categorical variables were shown as number (%).
**p<0.05 shows statistical significance.*
Bold characters differ between groups (posthoc: Bonferroni or Dunn's test)
Vitamin D was classified as normal (vitamin D >30 ng/mL), insufficient (10-30 ng/mL), or deficient (<10 ng/mL)[19].
CACS: Coronary artery calcium score, HDL: High density lipoprotein, LDL: Low density lipoprotein, hs-CRP: High sensitivity C-reactive protein, n: Number

A negative correlation was detected between the vitamin D levels and CACS scores (r=-0.345; p<0.001) (Figure 1a). The median CACS in those with vitamin D deficiency was higher when compared to those with inadequate and normal levels (normal: 0 vs inadequate: 0 vs deficient 7; p<0.001). In terms of the plaque distribution, the ratio of those who had calcified plaque among those with vitamin D deficiency (normal: 4.5% vs inadequate: 14.3% vs deficient 19.2%; p<0.001) and the ratio of those who had mixed plaque (normal: 8.3% vs inadequate: 29.3% vs deficient 41.7%; p<0.001) were detected higher (Figure 1b) (Table 1). In the CACS: 0 patients, it was determined that the ratio of those with a mere fatty plaque was higher in those who had vitamin D deficiency when compared to those who had inadequate or normal samples. In those who had inadequate vitamin D, the ratio of those with

Table 2. Demographic and laboratory findings according to plaque types

Variables	No plaque (n=328)	Plaque type			p-value
		Only fatty plaque (n=97)	Only calcific plaque (n=96)	Mixed plaque (n=198)	
Age, years	51.2±6.2	50.5±7.1	51.0±7.9	54.0±6.9	**<0.001***
Gender, n (%)					
Female	82 (25.0)	44 (45.4)	38 (39.6)	100 (50.5)	**<0.001***
Male	246 (75.0)	53 (54.6)	58 (60.4)	98 (49.5)	
Diabetes mellitus, n (%)	73 (22.3)	30 (30.9)	34 (35.4)	74 (37.4)	**<0.001***
Hypertension, n (%)	125 (38.1)	45 (46.4)	38 (39.6)	115 (58.1)	**<0.001***
Hyperlipidemia, n (%)	52 (15.9)	38 (39.2)	57 (59.4)	116 (58.6)	**<0.001***
Cigarette smoking, n (%)	125 (38.1)	44 (45.4)	47 (49.0)	85 (42.9)	0.215
CACS	0 (0-0)	0 (0-0)	**11 (1-992)**	**60.5 (1-3759)**	**<0.001***
Normal, n (%)	328 (100.0)	97 (100.0)	-	-	**<0.001***
Minimal, n (%)	-	-	47 (49.0)	34 (17.2)	
Mild, n (%)	-	-	41 (42.7)	79 (39.9)	
Moderate, n (%)	-	-	5 (5.2)	63 (31.8)	
Severe, n (%)	-	-	3 (3.1)	22 (11.1)	
Hemoglobin, g/dL	13.5±1.4	13.8±1.6	13.5±1.5	13.8±1.6	0.069
Platelet, x10³ µL	257.7±60.3	254.5±61	250.6±57.5	259.7±65	0.653
HDL, mg/dL	**54.0±12.7**	47.3±10.5	49.0±14.9	46.0±10.9	**<0.001***
LDL, mg/dL	**143 (37-410)**	131 (49-250)	128.5 (35-299)	131.5 (48-257)	**0.001***
Triglyceride, mg/dL	**124.5 (36-992)**	140 (47-673)	138.5 (46-691)	142.5 (54-857)	**0.002***
Albumin, g/dL	**4.4±0.3**	**4.3±0.3**	4.2±0.3	4.2±0.3	**<0.001***
Creatinine, mg/dL	0.7±0.1	0.7±0.2	0.7±0.2	**0.8±0.2**	**<0.001***
hs-CRP, mg/L	**0.3 (0-1.5)**	0.5 (0-2.6)	0.5 (0-3.9)	**0.7 (0.1-5.5)**	**<0.001***
Vitamin D, ng/mL	20.3 (4-43.6)	20.4 (5.1-39)	14.2 (4-57.6)	14.2 (2.2-44.2)	**<0.001***
Normal, n (%)	99 (30.2)	16 (16.5)	6 (6.3)	11 (5.6)	**<0.001***
Insufficient, n (%)	197 (60.1)	66 (68.0)	67 (69.8)	137 (69.2)	
Deficient, n (%)	32 (9.8)	15 (15.5)	23 (24.0)	50 (25.3)	

Numerical variables were shown as mean ± standard deviation or median (min-max).
Categorical variables were shown as number (%).
**p<0.05 shows statistical significance.*
Bold characters differ between groups (posthoc: Bonferroni or Dun's tests)
CACS: Coronary artery calcium score, HDL: High density lipoprotein, LDL: Low density lipoprotein, hs-CRP: High sensitivity C-reactive protein, n: Number

fatty plaque was found to be higher when compared to the normal ones (normal: 13.9% vs inadequate: 25.1% vs deficient: 31.9%; p=0.013) (Figure 1c).

The median vitamin D level was similar in those with mixed plaque and mere calcified plaque, and the vitamin D levels were lower when compared to those with mere fatty plaque and those with zero-plaque. The median vitamin D levels did not differ significantly in those with and without fatty plaque (Figure 1d and Table 2).

Compared to those with no atherosclerosis, the median CACS level and vitamin D efficiency of those with atherosclerosis (0 vs. 15; p<0.001) were higher (9.8% vs 22.5%; p<0.001). The findings associated with atherosclerosis are detailed in Table 3.

Regardless of the traditional risk factors, vitamin D deficiency was found as an independent predictor for atherosclerosis (OR: 6.9; 95% CI: 3.53-13.52; p<0.001), fatty plaque (OR: 3.04; 95% CI: 1.34-6.87; p=0.008), mere calcified plaque (OR: 13.11; 95% CI: 3.53-13.52; p<0.001), and mixed plaque (OR: 14.27; 95% CI: 5.58-36.50; p<0.001 (Table 4). Moreover, regardless of the traditional risk factors, vitamin D deficiency increased the risk of fatty plaque development by 2.37 times in patients with CAC: 0 (OR: 2.37; 95% CI: 1.01-5.62; p=0.045).

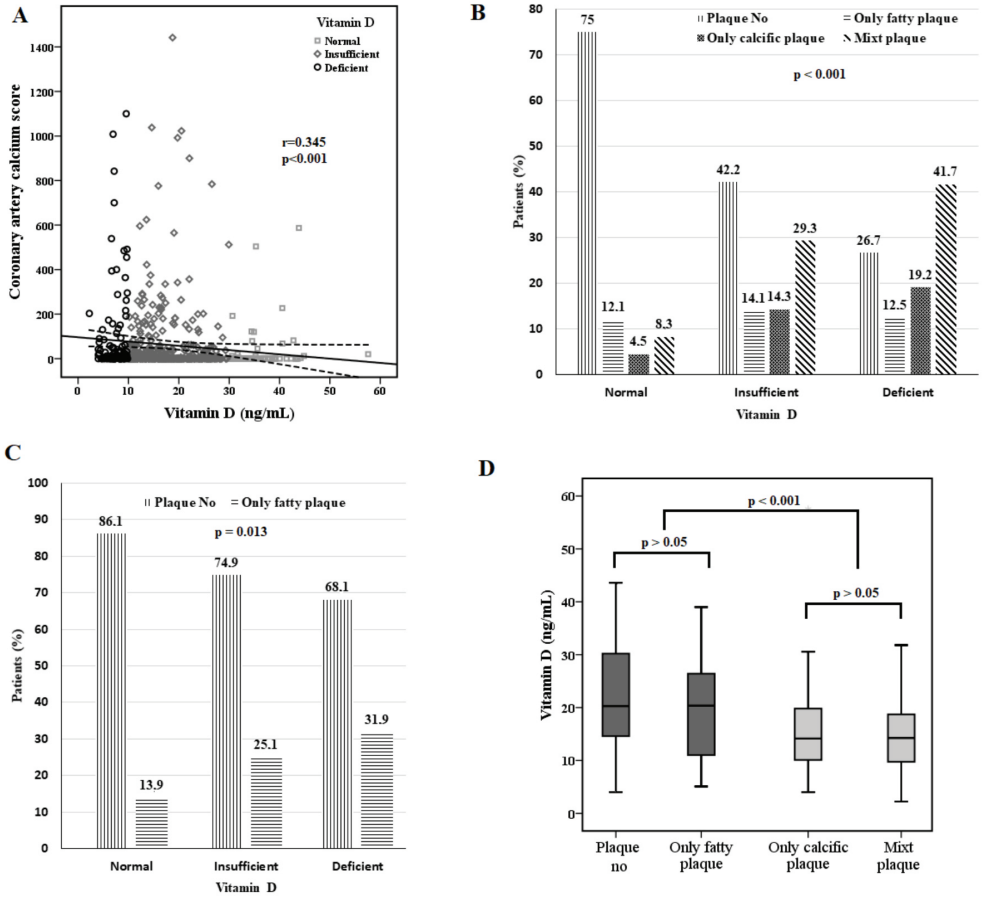

Figure 1. Vitamin D distributions: **a)** Relationship between coronary artery calcium score and vitamin D, **b)** Plaque distributions according to vitamin D sufficiency, **c)** vitamin D levels according to plaque distributions, **d)** Presence of plaque according to vitamin D adequacy in patients with coronary artery calcium score 0

Table 3. Factors associated with atherosclerosis

Variables	Atherosclerosis		p-value
	No (n=328)	Yes (n=391)	
Age, years	51.2±6.2	52.4±7.5	0.022*
Gender, n (%)			
Female	82 (25.0)	182 (46.5)	<0.001*
Male	246 (75.0)	209 (53.5)	
Diabetes mellitus, n (%)	73 (22.3)	138 (35.3)	<0.001*
Hypertension, n (%)	125 (38.1)	198 (50.6)	0.001*
Hyperlipidemia, n (%)	52 (15.9)	211 (54.0)	<0.001*
Cigarette smoking, n (%)	125 (38.1)	176 (45.0)	0.062
CACS	0 (0-0)	15 (0-3759)	<0.001*
Hemoglobin, g/dL	13.5±1.4	13.7±1.5	0.037*
Platelet, x103 µL	257.7±60.3	256.2±62.2	0.732
HDL, mg/dL	54.0±12.7	47.1±12.0	<0.001*
LDL, mg/dL	143 (37-410)	131 (35-299)	<0.001*
Triglyceride, mg/dL	124.5 (36-992)	140 (46-857)	0.001*
Albumin, g/dL	4.4±0.3	4.2±0.3	<0.001*
Creatinine, mg/dL	0.7±0.1	0.8±0.2	<0.001*
hs-CRP, mg/L	0.3 (0-1.5)	0.6 (0-5.5)	<0.001*
Vitamin D, ng/mL	20.3 (4.0-43.6)	15.2 (2.2-57.6)	<0.001*
Normal, n (%)	99 (30.2)	33 (8.4)	<0.001*
Insufficient, n (%)	197 (60.1)	270 (69.1)	
Deficient, n (%)	32 (9.8)	88 (22.5)	

Numerical variables were shown as mean ± standard deviation or median (min-max).
Categorical variables were shown as number (%).
**p<0.05 shows statistical significance.*
CACS: Coronary artery calcium score, HDL: High density lipoprotein, LDL: Low density lipoprotein, hs-CRP: High sensitivity C-reactive protein, n: Number

Discussion

In this study, a negative relationship was found between the CACSs and vitamin D levels, which are indicators of subclinical atherosclerosis in patients without previously known CAD. While a higher percentage of calcified and mixed plaque was detected in patients with vitamin D deficiency, vitamin D inadequacy and deficiency were identified as independent predictors of atherosclerosis. In patients with CACS: 0, the ratio of those with a fatty plaque in vitamin D deficiency was higher and was found as the predictor of fatty plaque, regardless of traditional risk factors. These findings suggested that there may be a higher risk of CAD in asymptomatic patients with inadequate or insufficient vitamin D levels.

CAC, which is found in coronary before the development of clinically significant narrowness, is an important predictor of subclinical atherosclerosis[21]. However, vitamin D deficiency is also considered to be a potential risk factor for CAD due to its contributions to atherosclerosis[22,23]. Although vitamin D plays a role in the cardiovascular system through its pleomorphic impacts, the pathophysiology of its relationship with CAC is not fully understood; however, a number of mechanisms have been put forward. Vitamin D deficiency causes impaired calcium

Table 4. Association of vitamin D deficiency and presence of plaque

Dependent variables	Vitamin D		Nagelkerke R^2
	Insufficient	Deficient	
Atherosclerosis (ref: No plaque or CACS=0)			
OR	3.24	6.9	0.385
95% CI	1.94-5.42	3.53-13.52	
p	**<0.001***	**<0.001***	**<0.001***
Only fatty plaque (ref: No plaque)			
OR	2.08	3.04	0.235
95% CI	1.14-3.80	1.34--6.87	
p	**0.017***	**0.008***	0.001
Only calcific plaque (ref: No plaque)			
OR	5.81	13.11	0.415
95% CI	2.11-16.08	4.04-42.50	
p	**0.001***	**<0.001***	**<0.001***
Mixed plaque (ref: No plaque)			
OR	5.43	14.27	0.494
95% CI	2.46-11.98	5.58-36.50	
p	**<0.001***	**<0.001***	**<0.001***
Only calcific plaque (ref: Only fatty plaque)			
OR	4.61	8.62	0.272
95% CI	1.48-14.31	2.29-32.45	
p	**0.008***	**0.001***	**<0.001***
Mixed plaque (ref: Only fatty plaque)			
OR	4.91	6.72	0.258
95% CI	1.92-12.54	2.27-19.89	
p	**0.001***	**0.001***	**<0.001***
Mixed plaque (ref: Only calcific plaque)			
OR	1.12	1.18	0.064
95% CI	0.40-3.15	0.39-3.60	
p	0.837	0.764	**0.002***

Age, gender, smoking, diabetes mellitus, hypertension and hyperlipidemia were adjusted in all analysis.
Those with normal vitamin D levels were considered as reference.
**p<0.05 shows statistical significance.*
Vitamin D was classified as normal (vitamin D >30 ng/mL), insufficient (10-30 ng/mL), or deficient (<10 ng/mL)[19].
OR: odds ratio, CI: confidence interval, ref: Reference

balance and secondary hyperparathyroidism. Differences in calcium and parathyroid hormone homeostasis are a predisposing factor for vascular calcification[24]. A study on pigs found that vitamin D deficiency increased the karyopherin α4 expression and NF-λB activation[25]. As a result, it was suggested that increased chronic inflammation of epicardial adipose tissue accelerates the progression of CAD[26]. It was suggested that vitamin D plays a role in

the coronary calcification process by acting on antigen-presenting cells, such as dendritic cells and macrophages, by suppressing cholesterol intake[27]. However, vitamin D has an impact on all stages of atherosclerotic plaque formation, destabilization, and rupture[28].

In the current study, a negative correlation between vitamin D and CACS was detected, but lower Vitamin D levels were determined only in patients with the calcified plaque and mixed plaque. Conflicting results have been reported in studies that researched the relationship between vitamin D and CAC in the literature[15-18]. This may have depended on research being conducted in different geographical regions. Moreover, this may have been associated with the fact that the impacts of traditional cardiovascular risk factors (age, sex, smoking status, DM, hypertension, hyperlipidemia) were not eliminated[29]. These risk factors may affect the relationship between vitamin D and CAC. In the current study, it was observed that a significant relationship continued, even in the regression model, in which the effects of these risk factors were eliminated. Furthermore, in the case of vitamin D deficiency or inadequacy, it was found that the probability of the presence of calcified and mixed plaque increased when compared to the patients with fatty plaque.

Mere non-calcified plaque was observed in 4-38% of the asymptomatic patients[30-32]. A meta-analysis showed that only 1% of patients with CACS: 0 were diagnosed with acute coronary syndrome after presenting with acute chest pain, normal troponin level, and suspected electrocardiography[33]. Moreover, it was determined in this study that CACS >0 had a 99% sensitivity value, 57% specificity value, 24% positive predictive value, and 99% negative predictive value for acute coronary syndrome[33]. In this research, it was observed that while the fatty plaque ratio in the whole population was 13.5%, this rate increased to 25.1% in vitamin D inadequacy and 31.9% in vitamin D deficiency in patients with CACS: 0. Furthermore, it was found that vitamin D deficiency, independent of traditional risk factors, increased the likelihood of fatty plaque by about 2.4 times in this patient group. This can speed up the atherosclerotic process due to the increased presence of calcium from simple fatty plaque to mixed plaque, which is the very early stage of CAD[11,12].

Vitamin D supplement reportedly does not change coronary artery plaque load in patients with calcified plaque[12], but it has been associated with improvement in cardiac results[23,34]. As far as observed in the current research, no studies that have assessed the effect of vitamin D supplements in patients with a fatty plaque in their coronary could be found. Therefore, the atherosclerotic process can be slowed down or prevented with vitamin D supplements, especially in patients with vitamin D deficiency or inadequacy. To this end, randomized controlled studies are needed.

The strengths of this study were the wide number of samples and the consideration of mixing factors. However, there were some significant restrictions. Due to fact that the study was a retrospective research, the $1.25(OH)_2D$ levels of the patients could not be measured. Circulating vitamin D levels are transmitted by the VDR signal. Therefore, the measured vitamin D levels did not reflect the circulating active form of the $1.25(OH)_2D$ levels. Given the complex nature of its metabolism and signal, referring to systemic levels of vitamin D alone may be insufficient to fully understand its physiological effect, especially in disease conditions. Moreover, CAC can develop over time, and the development of current calcium lesions in patients was not investigated.

Conclusion

It was found that there was a negative relationship between the vitamin D levels and the CACS, and there is a higher risk of atherosclerosis and developing calcified and mixed plaque in vitamin D deficiency. Moreover, considering the increase in the rates of fatty plaque in vitamin D deficiency in patients with a CACS of 0, it is thought that the atherosclerotic process begins and CAD may speed up. Depending on the incidence of CVDs and vitamin D deficiency in asymptomatic patients, vitamin D supplements can be beneficial.

Ethics

Ethics Committee Approval: İstanbul Training and Research Hospital, Clinical Research Ethics Committee (decision date/no: 24.07.2020/2481).

Informed Consent: All of the participants' consents were obtained in both verbal and written form before the study began.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Do Cardiological Characteristics Explain the Mortality Rate Disparity Between Genders in COVID-19?

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Abstract

Objectives: The coronavirus disease-2019 (COVID-19) mortality risk in males is twice as high compared to females. The literature lacks data regarding how the cardiological parameters influence the mortality rate imbalance between genders in the setting of COVID-19. This study aims to investigate how cardiological parameters vary between genders in COVID-19 disease—a potential explanation for the increased mortality rate in males in the setting of COVID-19.

Materials and Methods: We included 458 adult patients with a confirmed diagnosis of COVID-19 disease. Demographics, comorbidities, laboratory findings, and electrocardiogram parameters were compared between males and females.

Results: Of 458, a total of 63 (14.2%) patients died, and 82 (17.9%) were followed up in the intensive care unit during the hospitalization. Although the median age between males and females looked like to be similar, the mortality rate was significantly higher among males (44% vs 9.5%, p=0.006). High-sensitive troponin T, presentative of myocardial injury, was considerably higher in dead patients than survivors ($p<0.05$); however, it did not present a significant difference between genders. Electrocardiogram features did not show substantial differences, as well.

Conclusion: This study could provide important insights into the currently mostly enigmatic issue, gender-based death disparity in COVID-19, that cardiological characteristics do not impact the death imbalance.

Keywords: COVID-19, cardiology, ECG, electrocardiogram, gender, mortality

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Introduction

The coronavirus disease-2019 (COVID-19) pandemic is continuing to present a grave threat to humanity. A vast number of people, particularly elders and those with comorbidities, died due to the disease, and the numbers are still increasing dramatically[1]. One of the most prominent comorbidities to increase the disease's severity and mortality is cardiovascular disease (CVD) [2]. The association between CVD and COVID-19 is mutual; COVID-19 may lead to various CVD pathologies, including myocarditis, arrhythmias, and heart failure (HF), while preexisting CVD magnifies the possibility of unfavorable outcomes in patients with COVID-19[2-4].

A wealth of clinical and epidemiological data demonstrated the enhanced male-gender role in mortality[5,6]. The COVID-19 mortality risk in men was twice as high compared to that in women[5]. Various possible issues that may be responsible for the imbalance of COVID-19 mortality between genders, ranging from lifestyles to differences in chromosomal structure, were discussed[7]. However, the exact cause has remained unrevealed. Moreover, the literature lacks data regarding how the cardiological parameters influence the mortality rate imbalance between genders in the setting of COVID-19. This study aims to investigate how cardiological parameters vary between genders in COVID-19 disease—a potential explanation for the increased mortality rate in males in the setting of COVID-19.

Materials and Methods

In this single-center retrospective study, we reviewed the records of COVID-19 patients from April 28th, 2020 to October 30th, 2020. We analyzed demographics, comorbidities, laboratory findings, and electrocardiographic parameters. We included a total of 458 adult COVID-19 patients who presented to our center. The outcome of the study population was death or discharge with a cure. All patients were tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We medicated patients with 5-day favipiravir oral tablets.

The Ministry of Health of Turkey and Institutional Review Board of Kafkas University approved the study protocol (reference number: 80576354-050-99/277, approval date: 25.11.2020). Demographics, clinical characteristics, and laboratory findings at the admission time were collected from the medical record. High-sensitive troponin T (hs-TnT) value at admission was also collected from the medical record. All 12-lead electrocardiograms (ECG) performed on hospital admission were analyzed by a blinded cardiologist (M.K) using a standardized comprehensive electrocardiogram (ECG) reading protocol[8]. ECG analysis included intervals, rate, rhythm, axis, QRS morphology (including bundle branch block), and ST or T-wave abnormalities. Corrected QT interval (QTc) was calculated using the Hodge formula. Demographics, comorbidities, laboratory findings, and ECGs parameters were compared between males and females.

The inclusion criteria were: 1) age >18 years; 2) A definite diagnosis of COVID-19. Exclusion criteria was the presence of ST-segment elevated myocardial infarction (STEMI) and the presence of grade 2 or 3 conduction block. These patient groups were referred to another center due to our center's lack of angiography laboratory.

Chronic heart failure was defined as a left ventricle ejection fraction <50% according to previous records, and patients with an estimated glomerular filtration rate (eGFR) <60 (mL/minute) were determined as chronic kidney disease.

Statistical Analysis

Data obtained from this study were evaluated by using the SPSS 20 program. Continuous data were expressed as mean ± standard deviation; categorical data were expressed as percent (%). Data were evaluated with the Kolmogorov-Smirnov test in terms of normal distribution. The use of an independent t-test to analyze continuous data showing normal distribution and a Mann-Whitney

U-test for the analysis of variables not showing normal distribution was planned. Crosstabs (chi-square test) were used for the comparison of categorical data. The statistical significance level was accepted as $p<0.05$.

Results

Of 458, a total of 65 (14.2%) patients died, and 82 (17.9%) were followed up in the intensive care unit during the hospitalization. Although the median age between males and females looked like to be similar, the mortality rate was significantly higher among males (44% vs 9.5%, p=0.006). Demographic, clinical and laboratory characteristics are summarized in Table 1. For patients followed up in the intensive care unit, males showed a significantly higher proportion than females (21.4% vs 14.1%, p=0.041). In 408 (89.1%) patients, at least one COVID-19 related symptom, was observed. The most common presented symptom was cough (35.6%), followed by dyspnea (32.3%) and fatigue (28.6%). It is worthy of note that overall symptoms did not demonstrate a significant difference between genders.

Although most laboratory results showed a significant difference between genders, the medians were in normal ranges. Hs-TnT was significantly higher in dead patients

Table 1. Demographic, clinical and laboratory characteristics

	Overall (n=458)	Male (n=238)	Female (n=220)	p-value
Gender, n (%)		238 (52)	220 (48)	0.4
Age (years), median [IQR]	58 (36-71)	55 (34-71)	58 (39-71)	0.556
Death, n (%)	65 (14.2)	44 (18.5)	21 (9.5)	**0.006**
Intensive care unit, n (%)	82 (17.9)	51 (21.4)	31 (14.1)	**0.041**
Initial vital signs				
SBP (mmHg), median [IQR]	120 (110-120)	120 (110-120)	120 (110-130)	0.054
DBP (mmHg), median [IQR]	70 (60-80)	70 (60-80)	70 (60-80)	0.698
Heart rate, median [IQR]	84 (78-96)	85.5 (78-99)	84 (78-92)	0.144
Saturation (%), median [IQR]	93.5 (90-95.2)	93 (90-96)	94 (90-95)	0.323
RR/minute, median [IQR]	20 (20-22)	20 (20-22)	20 (20-22)	0.088
Symptoms at arrival				
Symptomatic, n (%)	408 (89.1)	210 (88.2)	198 (90)	0.545
Fever, n (%)	88 (19.2)	51 (21.4)	37 (16.8)	0.211
Cough, n (%)	163 (35.6)	94 (39.5)	69 (31.4)	0.069
Dyspnea, n (%)	148 (32.3)	79 (33.2)	69 (31.4)	0.676
Fatigue, n (%)	131 (28.6)	65 (27.3)	66 (30)	0.525
Nausea, n (%)	40 (8.8)	18 (7.6)	22 (10)	0.348
Diarrhoea, n (%)	10 (2.2)	3 (1.3)	7 (3.2)	0.16
Anosmia, n (%)	11 (2.4)	3 (1.3)	8 (3.6)	0.099
Anorexia, n (%)	25 (5.5)	8 (3.4)	17 (7.8)	**0.038**
Ageusia, n (%)	12 (2.6)	3 (1.3)	9 (4.1)	0.058
Sore throat, n (%)	59 (12.9)	28 (11.8)	31 (14.1)	0.458
Chest pain, n (%)	55 (12)	32 (13.4)	23 (10.5)	0.334
Abdominal pain, n (%)	19 (4.1)	7 (2.9)	12 (5.5)	0.178
Headache, n (%)	73 (16)	35 (14.8)	38 (17.3)	0.465
arthralgia/myalgia, n (%)	79 (17.2)	38 (16)	41 (18.6)	0.45
Laboratory findings at admission				

Table 1. continued

Hgb (g/dL), median [IQR]	14.2 (12.7-15.7)	15.3 (13.5-16.4)	13.4 (12.3-14.5)	**<0.001**
WBC (×10³/μL), median [IQR]	7.68 (5.58-7.68)	8.47 (6.05-12.39)	6.96 (5.3-10.71)	**0.001**
Lymphocyte (×10³/μL), median [IQR]	1.5 (0.92-2.06)	1.45 (0.83-2.01)	1.57 (1.06-2.14)	**0.042**
Neutrophil (×10³/μL), median [IQR]	5.22 (3.35-9.18)	5.83 (3.96-10.23)	4.58 (3.07-7.81)	**<0.001**
PLT (×10³/μL), median [IQR]	209 (168-267)	199 (162-253)	226 (179-280)	**0.003**
Hs-TnT(ng/L), median [IQR]	4.97 (3-12.47)	6.86 (3-14.54)	4.06 (3-9.63)	**0.009**
ProBNP (pg/mL), median [IQR]	58.7 (18.2-301.9)	47.1 (14.4-305.3)	69.3 (24.2-296.9)	0.343
CRP (mg/L), median [IQR]	13.5 (3.3-53.3)	19.8 (4.2-71.6)	10.5 (2.6-33.3)	**0.002**
Procalcitonin (ug/L), median [IQR]	0.059 (0.04-0.128)	0.071 (0.044-0.141)	0.52 (0.038-0.108)	**0.003**
Creatinine (mg/dL), median [IQR]	0.87 (0.72-1.13)	1.01 (0.85-1.19)	0.74 (.064-0.92)	**<0.001**
D-Dimer (μg/mL), median [IQR]	436 (224-1086)	463 (217-953)	421 (227-1119)	0.619
Comorbidities				
Hypertension, n (%)	157 (34.3)	75 (31.5)	82 (37.3)	0.194
Diabetes, n (%)	51 (11.1)	25 (10.5)	26 (11.8)	0.655
Cigarette smoking, n (%)	119 (26)	88 (37)	31 (14.1)	**<0.001**
Coronary artery disease, n (%)	42 (9.2)	27 (11.3)	15 (6.8)	0.094
chronic heart failure, n (%)	21 (4.6)	14 (5.9)	7 (3.2)	0.167
COPD, n (%)	123 (26.9)	70 (29.4)	53 (24.1)	0.199
CKD (eGFR <60 mL/min/m²), n (%)	22 (4.8)	13 (5.5)	9 (4.1)	0.493
Cancer, n (%)	10 (2.2)	6 (2.5)	4 (1.8)	0.607
Previous hypothyroidism, n (%)	12 (2.6)	3 (1.3)	9 (4.1)	0.058
Cerebrovascular disease, n (%)	15 (3.3)	6 (2.5)	9 (4.1)	0.346
Previous atrial fibrillation, n (%)	13 (2.8)	3 (1.3)	10 (4.5)	**0.034**

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, RR: Respiratory rate, Hgb: Hemoglobin, WBC: White blood count, PLT: Platelet, hs-TnT: High-sensitivity troponin T, BNP: B type natriuretic peptide, CRP: C-reactive protein, COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, IQR: Interquartile range, n: Number

than survivors (p<0.05); however, it did not present a significant difference between genders. Procalcitonin (normal rage <0.5 ug/L) was significantly higher in females than males [median (interquartile range), 0.071 (0.044-0.141) vs 0.52 (0.038-0.108)] (Table 1). The most common comorbidity presented in our data was hypertension in 157 patients (34.3%), followed by COPD in 123 patients (26.9%). Frequencies of comorbidities were similar between genders except for previous atrial fibrillation (1.3% in males vs 4.5% in females, p=0.034). The smoking ratio was significantly higher among males than females (37% vs 14.1%, p<0.001).

ECG of 313 participants was available. The electrocardiographical features are summarized in Table 2. For electrocardiographic (ECG) parameters, dead patients had longer QRS intervals and more frequent ST-segment/T-wave changes than surviving patients (both p-value, <0.05). Bundle branch block and early repolarization showed higher proportions in males, while the frequency of ST-segment/T-wave change was higher for females. QRS interval was higher, and QTc was lower among males compared to females. It is noteworthy that QTc and QRS medians were in normal ranges in both genders. All remaining ECG features were similar between genders. Farther, when a comparison was made covering only deceased patients, ECG features and hs-TnT level did not show significant difference between males and females (Table 3).

Table 2. Electrocardiographic characteristics

	Overall (n=313)	Male (n=164)	Female (n=149)	p-value
Sinus, n (%)	301 (96.8)	161 (98.2)	142 (95.3)	0.15
Atrial fibrillation/flutter, n (%)	10 (3.2)	3 (1.8)	7 (4.7)	0.34
Axis change, n (%)	73 (23.3)	45 (27.4)	28 (18.8)	0.071
Heart rate (b.p.m), median [IQR]	82 (72-91)	80 (72-90)	83 (72-92)	0.286
PR interval (ms), median [IQR]	150 (130-150)	150 (130-166)	148 (130-160)	0.962
P-wave dispersion (ms), median [IQR]	40 (30-55)	40 (30-55)	40 (30-53)	0.081
QRS interval (ms), median [IQR]	90 (84-98)	90 (85-100)	88 (80-95)	**<0.001**
QTc (ms), mean [SD]	429 (37)	422 (38)	436 (35)	**0.001**
ST-segment/T-wave change, n (%)	119 (38)	52 (31.7)	67 (45)	**0.016**
fragmented QRS, n (%)	98 (31.3)	54 (32.9)	44 (29.5)	0.518
Isolated S1Q3T3 pattern, n (%)	22 (7.1)	13 (8)	9 (6)	0.505
Bandle branch block, n (%)	55 (17.6)	36 (22)	19 (12.8)	**0.033**
Premature atrial/ventricular contraction, n (%)	38 (12.1)	19 (11.6)	19 (12.8)	0.752
Early repolarization, n (%)	46 (14.7)	39 (23.8)	7 (4.7)	**<0.001**

QTc: Corrected QT, IQR: Interquartile range, n: Number

Table 3. Comparison of electrocardiographic characteristics and hs-TnT level in deceased patients.

	Overall (n=43)	Male (n=28)	Female (n=15)	p-value
Atrial fibrillation/flutter, n (%)	6 (9.2)	2 (4.5)	4 (19)	0.08
Axis change (n=43), n (%)	15 (34.9)	8 (28.6)	7 (46.7)	0.318
PR interval (ms), median [IQR]	160 (130-178)	155 (129-176)	165 (137-176)	0.320
P-wave dispersion (ms), median [IQR]	40 (35-60)	40 (37-60)	50 (32-60)	0.949
QRS interval (ms), median [IQR]	95 (86-100)	96 (87-108)	95 (87-98)	0.277
QTc (ms), mean [SD]	431 (56)	420 (54)	451 (54)	0.089
ST-segment/T-wave change, n (%)	26 (60.5)	15 (53.6)	11 (73.3)	0.326
fragmented QRS, n (%)	14 (32.6)	11 (39.3)	3 (20)	0.308
Isolated S1Q3T3 pattern, n (%)	6 (14)	6 (21.4)	0 (0)	0.076
Bandle branch block, n (%)	11 (25.6)	9 (32.1)	2 (13.3)	0.276
Premature atrial/ventricular contraction, n (%)	8 (18.6)	4 (14.3)	4 (26.7)	0.419
Early repolarization, n (%)	2 (4.7)	1 (3.6)	1 (6.7)	0.999
hs-TnT (ng/L), median [IQR] (n=14)	45 (31-83)	41 (38-70)	50 (20-83)	0.999

QTc: Corrected QT, hs-TnT: High-sensitivity troponin T, SD: Standard deviation, IQR: Interquartile range, n: Number

Discussion

Here, to our knowledge, we presented the first study addressing the role of cardiological characteristics in sex-dependent death-disparity in COVID-19. Our study revealed that the mortality rate was twice higher among males than females in a similar line with previous reports[5,9]. The majority of the patients who were followed up in the intensive care unit (ICU), which indicated disease severity, were males. When comparing males and females, initial vital signs, symptoms, laboratory results, and comorbidities showed similar features. Additionally, when the cardiological characteristics, including hs-TnT, the indicator of cardiac injury, were compared, we could not find a significant difference between genders. However, the smoking rate was higher among males.

A vast number of reports showed the striking dominance of male sex, around twice as high, in the overall mortality in patients with COVID-19[5,10]. The most extensive study to date, OpenSAFELY[5], has assessed data from over 17 million patients in the United Kingdom and identified that males have over twice the as high increased risk of death as females. Another data from five European countries (France, Italy, Spain, Switzerland, and Germany) presented a similar outcome[6]. These critical reports confirmed an imbalance of COVID-19 severity, hospitalization, and mortality between men and women.

A range of hypotheses, from lifestyles to differences in chromosomal structure, from underlying comorbidities to variation in sex hormones, have been argued regarding gender-based death disparity in the context of COVID-19.

According to prior reports, for cardiological characteristics, there were various ECG abnormalities associated with poor outcomes in patients with COVID-19[11,12]. In a study, ventricular arrhythmia and sinus tachycardia were associated with higher mortality in COVID-19 patients[13]. Another study highlighted that prolonged QTc was an independent risk factor of mortality in COVID-19[14]. Romero et al.[15] found out that T wave inversion was also associated with mortality in the setting of COVID-19 disease. Furthermore, according to Abrams et al.[16], longer QTc and left bundle branch block on admission showed an increased risk of death. For ECG changes in the current study, dead patients had longer QRS intervals, more frequent ST-segment/T-wave changes than surviving patients. However, when a comparison was made, these ECG features did not show a significant difference between males and females. Bundle branch block and early repolarization showed higher proportions in males. However, it is hard to link just the two ECG changes to the increased mortality; while, critical ECG abnormalities such as fragmented QRS, QTC, ST-segment/T-wave changes showed no difference between genders.

According to previous reports, myocardial injury, which was defined as troponin value above the 99th percentile upper reference limit, was directly correlated with increased mortality in patients with COVID-9[17]. Similarly, in our study, the median values of hs-TnT at admission were markedly higher in the dead patients than in the survivors. Nevertheless, it showed similar medians between genders.

COVID-19 patients with underlying comorbidities in both young and older individuals show more severe symptoms and higher mortality irrespective of gender[18,19]. A field that has pioneered this dialogue is underlying cardiovascular disease (CVD)[2,20]. Past evidence presented that patients with a history of CVD met with unfavorable outcomes of COVID-19 more frequently[21]. The current study showed similar proportions of CVD in both genders. It can be suggested that CVD did not influence male-based increased mortality in patients with COVID-19.

We also assessed the impact of CVD risk factors such as hypertension, advanced age, and smoking. Arterial hypertension, which is clearly a male-biased disease, is an excellent example of a sex-based death imbalance in COVID-19[22]. A retrospective observational study by Gao et al.[23] has observed a two-fold increase of COVID-19 mortality in hypertensive individuals, highlighting a clear relationship between hypertension and COVID-19. However, the proportion of hypertension was similar in both genders in our data.

Older age, another classical CVD risk factor, is most profoundly implicated in COVID-19-associated mortality. The OpenSAFELY study has identified that hazard ratios increase with age, increasing from 2.40 in those over 60 years old to 20.61 in individuals over 80 years old[5]. Similarly, in our study, the mortality rate was positively correlated with advanced age. However, notably, the median age in both genders showed no difference.

Ahmed et al.[9] argued the role of smoking in COVID-19 in a commentary. They suggested that men could potentially have poorer respiratory outcomes in SARS-Cov-2 infection because they were more likely to smoke. They also highlighted that this remained speculative as gender-stratified smoking rates were not specifically reported in the published COVID-19 literature. When we looked at our data, the percentage of smoking was higher among males in comparison to females. Also, smoking was significantly higher in dead patients than in those who survived. Additionally, when a comparison was made among only dead patients, the smoking ratio was higher in males than in females. Herein, could smoking, as a cardiovascular disease risk factor, explain the male-based mortality rise in COVID-19? Possible, but more evidence is needed in this field.

As for the other arguments that potentially help explain the high mortality of males in the context of COVID-19, many genes that play crucial roles in immune responses are present on the X chromosome. Current evidence demonstrates that the differential expression and regulation of X-linked genes between males and females play significant roles in sexual dimorphic responses to infection[24]. Furthermore, the sex-related difference in the immune system, sex hormone milieu, and other unknown causes may contribute to the high mortality of males in stressful conditions, including COVID-19[25]. According to some reports, sex and sex hormones affect many components of the circulating and tissue-based renin-angiotensin-aldosterone system (RAAS), including ACE2[26]. Nevertheless, reports from several preclinical studies agree that ACE2 is frequently higher in males than in females, mainly under pathological conditions[27,28]. It is a point of question whether this influences death disparity between genders.

Ultimately, numerous reports, with various hypotheses, tried to explain the higher mortality rate in males than in females in the setting of COVID-19. However, the exact cause has remained unclear.

In this study, we focused on the potential cardiological influence on the sex-based disparity for COVID-19 deaths. However, we did not meet any cardiological characteristics including ECG parameters and hs-TnT and comorbidities such as hypertension, diabetes, chronic heart failure, chronic kidney disease (CKD), cancer, hypothyroidism, COPS, CVD, cerebrovascular disease, and age, which contribute to the increased mortality rate among males. Future studies should explore the effects of additional factors on COVID-19 susceptibility/mortality disparity within the context of sex groups to disentangle these issues.

Study Limitations

This study has several significant limitations that must be acknowledged: 1) we did not have previous ECGs of patients and, thus, we could not distinguish between new-onset vs. chronic ECG abnormalities, 2) we only assessed ECGs which were available on admission; herewith, we cannot exclude that a later cardiac involvement with subsequent ECG changes might have resulted in some differences between the groups, 3) This study lacks echocardiographic imaging; echocardiographic work-up in patients with COVID-19 was often challenging due to efforts to mitigate exposure risk of health care workers, 4) Since we were lack of detailed data about pharmacological therapy for comorbidities, we could not calculate how treatment affected survival, in-depth. Long-term observations and prospective studies are needed.

Conclusion

This study could provide important insights into the currently mostly enigmatic issue, gender-based death

disparity in COVID-19, that cardiological characteristics do not impact the death imbalance. More comprehensive studies are needed in this area.

Ethics

Ethics Committee Approval: Institutional Review Board of Kafkas University approved the study protocol (reference number: 80576354-050-99/277, approval date: 25.11.2020).

Informed Consent: Not required (retrospective study).

Peer-review: Externally peer-reviewed.

Authorship Contributions

Conception and/or Design: T.O., Acquisition of Data: M.K., G.P., Analysis and/or Interpretation of Data: T.O., Drafting of the Manuscript: T.O., Critical Revision: M.K., Statistical Analysis: T.O., Supervision: M.K.

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