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Editorial Office
Address: Trakya Üniversitesi Tıp Fakültesi 22030 Edirne, Turkey
Phone: +90 (284) 235 76 53
E-mail: tmsj@trakya.edu.tr

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CONTENTS

EDITORIAL

Dear readers,

I would like to present to you TMSJ's June issue of 2021. In this issue, you will find 11 articles: consisting of 1 editorial, 3 reviews, 4 original articles, and 3 case reports. Below you can find quick synopses for these articles.

Having discussed its distinct and precise anatomy, it is essential to know the variations that might lead to pathologies. Besides its anatomy, various pathological cases should be kept in mind, essentially for surgical interventions and postoperative follow-ups. Successful surgery without incurring significant neurovascular morbidity in this region depends on the detailed knowledge of its vascular anatomy and pathologies. Therefore, Ersoy et al. describes anterior cerebral circulation in a literature review. Şenödeyici et al. aims to discuss the historical development, working mechanisms, present and future clinical applications of zinc-finger nucleases, transcription activator-like effector nucleases, clustered regularly interspaced short palindromic repeats, and prime editors, while presenting the ethical aspects of using these genomes editing tools. Ersoy et al. illustrates iron metabolism in the human body and its pathophysiology during heart failure and give a comprehensive overview of the studies and trials investigating the role of iron in heart failure.

Akay et al. shares their findings on the opinions of medical students about violence against women, the education they receive during medical school regarding violence against women, and how they would manage a case of violence victims if they were to encounter it in their medical careers. İzzettinoğlu et al. presents a datast including the clinical relationship of visual acuity with optical coherence tomography and perimetry in primary open-angle glaucoma. Akay et al. examines medical student journals from the year 2020 around the world. Özyiğit et al. aims to evaluate the relationship between the length of hospital stay and age, gender, and disease characteristics among glioma patients.

Of our 3 case reports, Koçyiğit et al. presents a case of a constrictive pericarditis which is cause of ascites. Sayın et al. shares a rare case of recurrent signet ring cell carcinoma presenting with thrombocytopenia. Finally, Akay et al. presents a case report with fibrin-associated diffuse large B-cell lymphoma secondary to cardiac myxoma.

I would like to thank all the editorial board who contributed to this issue. As TMSJ editorial board, thank you for hosting us in your home. We hope to meet with science in our next issues.

Hilal Sena Çifcibaşı
Editor-in-Chief, Turkish Medical Student Journal
Trakya University School of Medicine, Edirne, TURKEY

Address for Correspondence: Hilal Sena Çifcibaşı, Trakya University School of Medicine, Edirne, TURKEY
e-mail: hilalsena98@gmail.com
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ORCID iDs of the author: HSÇ: 0000-0002-9507-1092.

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ANTERIOR CEREBRAL CIRCULATION: A LITERATURE REVIEW

Berkin Ersoy[1], Bengisu Gür[2], Kaan Çifcibaşı[3], Hasan Orkun İpsalalı[4]

[1]University of Hamburg School of Medicine, Hamburg, GERMANY
[2]Istanbul University School of Medicine, Istanbul, TURKEY
[3]Technical University of Munich School of Medicine, Munich, GERMANY
[4]Marmara University School of Medicine, Istanbul, TURKEY

ABSTRACT

Anterior cerebral circulation consists of the anterior cerebral artery and middle cerebral artery of the circle of Willis. The anterior cerebral artery's course is classified into two main segment classifications constructed by Fischer and Osburn et al. The perfusion area of the anterior cerebral artery extends medially over the entire frontal and parietal lobes, the septum, and the basal forebrain structures such as the hypothalamus, hypophysis, and optic chiasm. The anterior cerebral artery also provides blood to the rostrum, genu, and body of the corpus callosum. The anterior cerebral artery shows some anatomical variations throughout its course. Aplasia or hypoplasia are the common variants in the A1 segment, whereas fenestration occurs in rare cases. Azygos artery formation is a rare variant of the A2 segment, and recurrent artery of Heubner is an important branch arising from A1 and A2 segments, which is one of the main perforators of structures such as but not limited to the anterior parts of the internal capsule and the lentiform nucleus. Common variations of the middle cerebral artery comprise accessory artery formation or duplication, early branching, and also fenestration. Although cerebral circulation is complex with many collaterals and variations, pathological disturbances in the blood supply can still occur. Anatomical variations, cardiac problems, ethnicity, age, and physical exercise are some of the many risk factors that account for pathological cases such as aneurysms, occlusions, dissections, infarctions, and stroke. Disturbances in the blood supply of such a crucial region may lead to severe disabilities, if not death. Middle cerebral artery syndrome is one of the most important pathologies of the brain, where the outcome is stroke. Whether the underlying etiology is stenosis or obstruction, two main mechanisms can be categorized as atherosclerotic and non-atherosclerotic. Atherosclerotic causes can be thrombus or emboli origin, and non-atherosclerotic causes can be due to hemodynamic compromise, vasculitis, arterial wall dissections, and moyamoya disease. ***Keywords:*** Anterior cerebral artery, middle cerebral artery, aneurysm, infarction

INTRODUCTION

The brain is one of the most complex organs of the human body. Therefore it is highly perfused compared to other organs. Even though the brain is not the heaviest in mass compared to other organs, it still requires the highest energy to carry out its vital functions for the body (1). Despite representing merely 2% of the body mass, the brain uses approximately 50% of the total body glucose (1). The source of glucose, the fuel that the brain runs on, is the blood circulation, hence the reason why two different arterial systems supply it: anterior cerebral circulation and posterior cerebral circulation. Despite serving the same purpose, these two sets of circulation arise from the different branches of the aorta (2).

The aorta is divided into three sections after it courses out of the heart and into the middle mediastinum (2). These parts are the ascending aorta, aortic arch, and descending aorta. Only the aortic arch supplies the brain. The aortic arch has three branches: the brachiocephalic trunk, left common carotid artery, and left subclavian artery, from proximal to distal (2). While the brachiocephalic trunk does not directly contribute to the brain's blood supply and brainstem, the latter further gives off branches to supply the neuraxis.

The left common carotid artery bifurcates into the internal and external carotid arteries at the level of T4 (3). The internal carotid artery (ICA) runs superiorly into the brain to form the anterior circulation of the brain. On the other hand, the subclavian arteries of both sides give off vertebral arteries that run on both sides of the vertebra through the foramen transversarium and enter the brain through the foramen magnum to form the posterior circulation of the brain and the spinal cord (3).

The major suppliers of the anterior cerebral circulation are the anterior cerebral artery (ACA) and middle cerebral artery (MCA) (3). Knowing its distinct and precise anatomy, pathological variations, and possible pathological outcomes of the anterior cerebral circulation is essential for surgical interventions and postoperative follow-ups. Successful surgery without incurring significant neurovascular morbidity in this region depends on the detailed knowledge of vascular anatomy and the relevant pathologies (3).

ANATOMY OF THE ANTERIOR CEREBRAL CIRCULATION

Anterior Cerebral Artery

The knowledge of anatomical variants is crucial for therapeutic success. In their article Sañudo et al. (4) demonstrated that the lack of anatomical knowledge caused around 10% of all medical errors. A certain degree of asymmetry was detected between anterior arteries in 80% of patients in a study conducted by Given et al. (5).

Address for Correspondence: Berkin Ersoy, University of Hamburg School of Medicine, Hamburg, GERMANY
e-mail: berkinersoy@gmail.com
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ORCID iDs of the authors: BE: 0000-0001-7111-648X; BG: 0000-0002-4280-3317; KÇ: 0000-0003-0830-9777; HOİ: 0000-0002-1199-6208.

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Segmentation and Course of Anterior Cerebral Artery

The ACA arises from the termination point of the ICA and is described as a part of the circle of Willis. There are two main classification schemes for the segments of the ACA. The first, and to our knowledge the oldest classification, Fischer described in 1938 (6). In Fischer's classification, the ACA is divided into five segments: 1 the pre-communicating (A1), 2 below the genu of the corpus callosum (A2), 3 around the genu of the corpus callosum (A3), 4 the terminal branch of the A4, and 5 the terminal branch of the A5 (6). Fischer's classification was further examined and modified by several authors. In the recent modifications to Fischer's classification, the ACA was divided into the pre-communicating segment (A1) and post-communicating segment, which was further divided into infracallosal (A2), precallosal (A3), supracallosal (A4), and posterocallosal (A5) segments (7). In another classification developed by Osborn et al. (8), the ACA was divided into three segments: the segment which runs over the optic chiasm, the segment running vertically and entering into interhemispheric fissure after crossing the anterior communicating artery (ACoA), and a group of terminal segments (8, 9).

The perfusion area of ACA is considered the most variable when compared to other essential blood sources, such as the MCA and posterior cerebral artery (10). The perfusion area extends medially over the entire frontal and parietal lobes, the septum, and the basal forebrain structures such as the hypothalamus, hypophysis, optic chiasm. ACA also provides blood to the rostrum, genu, and body of the corpus callosum. From the perspective of functional centers, ACA thus supplies blood to a large part of the prefrontal and premotor cortex. Through the recurrent artery of Heubner (RAH), blood is perfused to the internal capsule, from the anterior to the beginning of the posterior crus, and the anterior part of the striatum (11).

Anatomical variations and measurements of ACA segments have been studied by magnetic resonance or computed tomography angiography (MRA/CTA) and cadaveric methods. In studies that we reviewed, the number of hemispheres examined was lower in cadaveric studies than in MRA/CTA studies. On the other hand, in MRA/CTA studies, distal branches or low diameter branches could not be examined thoroughly.

Variations of A1 and A2 Segments

Aplasia or hypoplasia of the A1 segment are common anatomical variants. Hypoplasia (in most of the studies defined as a diameter of A1 less than 1.5 mm) of the A1 segment was detected in 10% of cadavers in post-mortem examinations run by Perlmutter et al. (12). In the same study, the rate of aplasia was 2% (12). In cases with hypoplasia or aplasia of the A1 segment, the contralateral ACoA was dilated, allowing the contralateral artery to supply blood to both sides. These variants increase the risk and extent of neural tissue ischemia in the frontal lobe region during intravascular procedures in the area of ACoA or during ischemic episodes (13). Another common variation of the ACA is fenestration. Its prevalence in the A1 region was 0-4% in anatomical studies and 0-2% in MRA/CTA studies (14-17).

A rare variant found in the A2 segment is the azygos-ACA, in which two A1 segments of both hemispheres form a single A2 truck. As a result, an ACoA could not be found in patients with azygos-ACA. This variant was found in around 1.5% of the cases studied by Auguste et al. (18). Azygos-ACA is considered an essential predictor of bilateral frontal strokes, and saccular azygos-ACA aneurysms are relatively common with a prevalence between 13-71% (19).

Variations of Anterior Communicating Artery

A further variation involving the ACA is the accessory anterior cerebral artery. It describes a small, additional artery (so-called median artery of the corpus callosum) branching from the ACoA in addition to two A2 segments of both hemispheres. Its prevalence was around 8% in the studies reviewed by Dimmick et al. (13). In another MRA study conducted by Uchino et al. (14), its frequency was reported as 3%. The description of this variation is relevant before the clipping of ACoA aneurysms.

Due to its short length, fenestration and duplication of ACoA are challenging to differentiate. Thus, they are often grouped in studies. Although it is found frequently in cadaveric studies (20-30%), it is not a common finding in angiographic studies (0-10%) (12, 15, 16, 20). Uchino et al. (20) stated in their case report that improved image quality of MRA can make the accurate diagnosis of tiny variations of ACoA possible, including fenestrations and duplications.

An overview of different variations of A1 and A2 based on several studies can be seen in Table 1 (12, 14-16, 21-23).

Recurrent Artery of Heubner

Otto Heubner first described the recurrent artery of Heubner in 1872 (24). RAH is also called the median striatal artery and is one of the main perforators of structures such as the anterior parts of the internal capsule and the lentiform nucleus, and the head of the caudate nucleus (24). Embryologically, it is formed by the fusion of several smaller arteries. It arises from the A1 or A2 segment of the anterior cerebral artery, mainly in the area of the ACoA. In the cadaveric study conducted by Loukas et al. (25), the frequency of the branching of this artery from the ACoA was found to be 62.3%. The branching followed this from the proximal A2 with 23.3%, and the branching from the A1 segment with 14.3%. In the same study, the diameter of the same artery was found to be approximately 0.8 mm (25). Results, including the site of origin, mean diameter, and length of the RAH of several reviewed studies, are illustrated in Table 2 (12, 24-30).

Other Rare Variants

A rare variation considering the course of the proximal part of the ACA is its infraoptic course. The infraoptic course arises from the ICA at the level of the ophthalmic artery and terminates in the proximal segments of the ACA, thereby constitutes an anastomosis between the anterior cerebral circulation and the ICA (31).

Another rare variant with an abnormal anterior cerebral artery course is the persistent primitive olfactory artery (PPOA). Embryologically, the primitive olfactory artery (POA) is considered to be a rostral division of the primitive internal artery. The proper ACA develops from the POA. In the case where POA keeps its embryological course along with the olfactory bulb, it is described as PPOA. The characteristic course of this artery is associated with an increased risk of aneurysms (32).

Middle Cerebral Artery

Common variations of the middle cerebral arteries (MCAs) comprise accessory or duplicated MCA, early branching, and fenestrations. There are different classifications of additional MCAs.

Teal et al. (33) classified the additional MCAs into two major groups in 1973: duplicated and accessory MCAs. The accessory MCA, arising from the ACA in the A1 segment, was documented in 0.4% of the patients in the study conducted by Loukas et al. (25) and 3% of the post-mortem examinations conducted by Jain (34).

Duplicated MCAs, on the other hand, branch from the distal internal carotid arteries and were found to be present in about 2% of patients in the same study. In another study, conducted by Komiyama et al. (35), the prevalence of both arteries was found to be 0.4% and both accessory and duplicated MCAs supplied either the anterior temporal lobe or the anterior frontal lobe.

Another common categorization of the additional MCAs is the Manelfe classification (36). Manelfe grouped accessory MCAs in 3 different sub-categories. Type 1 MCA is the typical duplicated MCA, type 2 branches from the proximal anterior cerebral artery as an accessory MCA, and type 3 arises from the distal anterior cerebral artery as another form of accessory MCA (36).

Table 1: A review of several studies that examined the proximal part of ACA (12, 14-16, 21-23).

Study, year	*Study method*	*Number of hemispheres (n)*	*Mean A1 diameter, length (mm)*	*Anatomical variations of A1 and A2 (%)*	*Mean ACoA diameter, length (mm)*	*Anatomical variations of ACoA (%)*
Perlmutter et al., 1976 (12)	Cadaveric	100	2.6, N/A	Hypoplasia: 10 Aplasia: 1-2 Fenestration: 4	1.5, N/A	Duplication-Fenestration: 30%
Uchino et al., 2006 (14)	MRA	1782	N/A	Unilateral Aplasia: 5-6 Fenestration: 1.2 Azygos ACA: 2.0	N/A	N/A
Shatri et al., 2019 (15)	MRA	1026	2.05, 14-1	Unilateral Agenesis: 5.65 Fenestration: 0.58	1.16, 2-99	Hypoplasia or Aplasia: 15.66% Fenestration: 3.89% Duplication: 0.6%
Şahin et al., 2018 (16)	CTA	1502	N/A	Hypoplasia: 14.6 Aplasia: 2.53 Fenestration: 1.06 Trifurcation: 4.53 Azygos ACA: 1.46	N/A	Fenestration-Duplication: 10.12%
Karatas et al., 2016 (21)	Cadaveric	200	1.87(R)-1.96(L), 14.44(R)-13.72(L)	Hypoplasia: 2 Aplasia: 1	1.43(R)-1.95(L), N/A	Hypoplasia: 20% Aplasia: 1%
Shatri et al., 2017 (22)	MRA	266	2.09, 13.96	N/A	1.5, 2-74	N/A
Yeniceri et al., 2017 (23)	MRA	768	1.58(R)-1.60(L), N/A	N/A	N/A	N/A

A1 and A2: *Proximal segments of anterior cerebral artery,* ***ACA:*** *Anterior cerebral artery,* ***ACoA:*** *Anterior communicating artery,* ***CTA:*** *Computed tomography angiography,* ***MRA:*** *Magnetic resonance angiography,* ***R:*** *Right,* ***L:*** *Left,* ***N/A:*** *Not available*

Table 2: A Review of several studies that examined the recurrent artery of Huebner (12, 24-30).

Study, year	*Study method*	*Number of hemispheres (n)*	*Site of origin*			*Mean RAH diameter, length (mm)*
			A1 (%)	*A2 (%)*	*J* (%)*	
Perlmutter et al., 1976 (12)	Cadaveric	100	14	78	8	1.0, N/A
Maga et al., 2013 (24)	Cadaveric	140	26.2	33.8	40	1.0, 25.2
Loukas et al., 2006 (25)	Cadaveric	69	14.3	23.3	62.3	0.8, 24
Gomes et al., 1984 (26)	Cadaveric	60	8	57	35	0.8, 23.4
Avci et al., 2003 (27)	Cadaveric	62	8	64	29	N/A
Uzun et al., 2009 (28)	Cadaveric	108	6.2	14.6	79.2	0.67, N/A
Zunon-Kipré et al., 2012 (29)	Cadaveric	40	30	58	12	0.7, 24
Najera et al., 2019 (30)	Cadaveric	50	41	31.5	27.3	0.4, 13

RAH: *Recurrent artery of Huebner,* ***A1 and A2:*** *Proximal segments of the anterior cerebral artery,* ***J:*** *Junction,* ***N/A:*** *Not available*
*At the level of the anterior cerebral artery and anterior communicating artery junction.

PATHOLOGIES OF ANTERIOR CEREBRAL CIRCULATION

Anterior Cerebral Artery

Knowing how crucial the cerebral blood supply is, it undoubtedly concerns if any of the blood vessels do not function properly. From cerebral aneurysms to cerebral occlusions and dissections, any change in the cerebral circulation may lead to a stroke. Although ACA infarction is known to account for only 0.5-3% of ischemic strokes, its severe clinical outcomes such as death, should not be overlooked (37, 38).

Anterior cerebral artery infarcts occur predominantly on the left hemisphere, while bilateral ACA infarcts only account for 0-9% of ACA infarcts (39). Studies on Western populations suggest that ACA infarction rarely stems from ACA atherosclerosis (40). However, embolism from either ICA or the heart is considered the primary reason, given the approximate rate of 63% among ACA infarction patients (40). Studies on Asian populations, on the other hand, suggest different percentages for the listed possible reasons for ACA infarction. In Asian patients, the primary reason is ACA internal atherosclerosis with a large portion of 59%, with the atherosclerotic change mostly occurring in the A2 region of the ACA (40). Large atherosclerotic cerebral infarction occurs later in life, whereas cardioembolic cerebral infarction can occur at any age (41). Although intracranial dissections are less common than extracranial dissections in Western populations, ACA is not the affected region in most cases (38). However, in Asian populations, ACA dissection accounts for 8% of intracranial dissections (38). Cerebral infarction originating from atherothrombosis mainly affects the right hemisphere, and it is not bilateral unless the reasons are chronic atherosclerosis and acute cardioembolism (41).

Major risk factors for ACA infarction are age, heart disease, high blood pressure, and a history of strokes (38). In patients with ACA infarction stemming specifically from dissection, these factors were different: younger age, lower blood pressure, and no underlying heart diseases (38). In dissection cases, stroke develops more suddenly and more commonly after physical exertion, such as defecation, singing, or heavy exercise (38).

Anterior cerebral artery infarction causes clinical symptoms such as neurological deficits, accompanied by headache and physical exertion at the onset (38). It causes weakness on the limbs, contralateral hemiparesis, or monoparesis that generally affect the legs, sometimes accompanied by weakness on the shoulders and arms (39). The arm and face weakness is mainly associated with Heubner's artery and the medial striate arteries (39). In the affected limbs, sensory dysfunction may also appear (37). Headache is also an essential sign of this condition. Although headache is not considered a typical symptom, approximately 30% of the patients experience it and is defined as a "non-throbbing" headache (39). Patients with dissection have an earlier onset of a headache than patients without dissection (38). Along with these major symptoms, some patients may also present speech disturbances such as aphasia and dysarthria, decreased level of consciousness, grasp reflex, and urinary incontinence (39, 42, 43).

Stroke led by ACA infarction results in 7.8% in-hospital mortality (42). Even if the patients survive, only 9.8% of patients are symptom-free at hospital discharge. The remaining majority is unfortunately left with different levels of disability (42).

Another pathology that needs to be elucidated is ACA aneurysms. The vast majority of ACA aneurysms are located at or adjacent to the ACoA (44). ACA aneurysms that occur distal to the ACoA (A2:vertical or post-communicating portion) are called distal ACA aneurysms, and they are not as common (44). Proximal ACA (A1) is considered a rare location for aneurysms to develop, as they only account for 0.59-4% of intracranial aneurysms (44, 45). For an A1 aneurysm to develop, vascular abnormalities such as hypoplasia, aplasia, or fenestration of the ACA need to be present (45). Sixty percent of A1 aneurysms remain unruptured (45). Distal anterior cerebral artery (DACA) aneurysms, which develop on the A2-A5 segments, account for 2-9% of intracranial aneurysms (46). DACA aneurysms stem from various ACA abnormalities that increase the blood flow and shear stress in DACA and cause aneurysms at the bifurcations (47).

Although rare and mostly asymptomatic, ACA aneurysms might cause symptoms if they are ruptured (48). Ninety percent of ACA aneurysms are asymptomatic and are discovered incidentally (48). Unruptured ACA aneurysms might present with vague or non-specific symptoms such as headache, dizziness, or loss of consciousness depending on the severeness of the situation (47, 48). The risk of rupture depends on several risk factors such as aneurysm size, specific location, female gender, older age (mostly over 60 years), hypertension, and smoking (48). With the present risk factors, physical exercise that increases intracranial pressure may cause the aneurysm to rupture (48). In addition to physical exercise, sexual intercourse, defecation, micturition, excessive caffeine intake, and excessive anger are among the risk factors that may increase intracranial pressure (48). Ruptured aneurysms account for 85% of subarachnoid hemorrhages, which lead to death in 35-39% of the cases (48). Subarachnoid hemorrhages from an aneurysm rupture present with a sudden and severe headache that reaches its maximum intensity within seconds (48). Apart from the particular headache defined as "the worst headache of my life" by many patients, vomiting is another sign that presents approximately half of the patients (48).

A1 segment of ACA is the principal source of the blood supply for the circle of Willis (49). Not only is the ACA a principal blood supply, but it also branches to the striate arteries that supply blood to a broad range of crucial areas: anterior hypothalamus, septum pellucidum, and the anterior and inferior portions of corpus striatum (49). The hypoplastic A1 segment is an uncommon congenital variation with a frequency of 1-13% (49). A1 segment is considered hypoplastic if its width is less than 50% of the contralateral A1 segment or if its diameter is less than 1 mm (45, 49). In some cases, if the A1 segment is hypoplastic or aplastic, the ACoA supplies the territory (39). However, the absence of such a large blood supply can still be an issue. Hypoplasia or aplasia contributes to an increase in local hemodynamic forces on the contralateral side, and this increases wall shear stress (32, 45). A1 segment hypoplasia is classified as a predisposing factor for occlusion pathologies (49). Therefore, disturbances of the supply stemming from hypoplasia or aplasia of the ACA lead to ischemic strokes of the anterior cerebral area (49). Around 83.3% of A1 hypoplasia-related ischemic strokes were thought to stem from occlusions, especially within the striate arteries (49). In 76.2% of ipsilateral hemispheric ischemia, A1 segment hypoplasia, mostly on the right side, was associated (49). A1 hypoplasia was also associated with 19.2% of hemispheric infarction cases and 4.8% of brainstem or cerebellar ischemic stroke cases (49). 41.9% of patients with A1 segment aneurysms had vascular abnormalities such as fenestrations, hypoplasia, and aplasia on the contralateral side (45). Although many agree that A1 segment hypoplasia has the potential to cause important clinical presentations, there is evidence that it might even be asymptomatic if its collaterals compensate for the disturbances (49).

Middle Cerebral Artery

The MCA has an extensive vascular territory with an elaborate course contributing to the arterial supply of the anterior circulation of the cortex. However, due to its long course, structure, and effects of systemic diseases, many pathologies associated with various clinical presentations have been documented in the literature. For a more focused look, this part will include the MCA syndrome only.

Middle Cerebral Artery Syndrome (Disease)

Restriction of blood supply to the MCA is defined as MCA syndrome or MCA disease. The outcome of this blood flow restriction is stroke (50). Bogousslavsky et al. (51) state that stroke results from either stenosis or occlusion of the MCA. Factors resulting in cerebral infarction are categorized by Wong et al. (52) categorised factors resulting in cerebral infarction as embolism, thrombosis, hemodynamic compromise, local occlusion of branches, and a mixture of these etiologies. In terms of conditions contributing to the development of stroke, the aetiologies can be grouped into two main categories: atherosclerotic MCA syndrome and non-atherosclerotic MCA syndrome.

According to the study conducted by Ahn et al. (53), the origin of MCA disease in 72.6% of their patients was found to be atherosclerosis. Another study found that only in 12.5% of the patients, the MCA obstruction was due to local thrombosis, whereas in 40% of the patients it was due to embolism of the heart and the ICA, and in 47.5% of the patients its origin was undetermined (54). Other studies found out that one-third is due to embolism of all cases of MCA syndrome (51, 55). In their study, Lee et al. (56) found that 76 out of 107 patients had atherosclerosis and 31 patients suffered from cardiac embolism. Whether atherosclerotic or non-atherosclerotic, the infarcts are categorized as cortical infarcts, border zone infarcts, and penetrating artery infarcts. In their study, Kim et al. (40) found that the non-atherosclerosis group was younger when compared to the atherosclerosis group (40.1 ± 10.5 years, 45.7 ± 8.9 years respectively), and the women were more affected than men (58.3% to 44.4%).

Cortical Infarcts

In their study, Wong et al. (52) defined cortical infarcts as smaller than 5 mm in diameter. They also found no cortical infarct was isolated and suggested that cortical infarcts usually are asymptomatic and co-exist with other types of infarcts (border zone and penetrating artery).

Border Zone Infarcts

Wong et al. (52) found that border zone infarcts can exist as both single and multiple infarcts, where the latter is more common (73.3% multiple, 33.3% single). They describe the underlying pathology as cholesterol crystals (52, 55). However, cholesterol crystals are not the sole pathology in border zone infarcts, since Torvik (57) and Beal et al. (58), in their studies, reported platelet-emboli-occluded leptomeningeal arteries where border zone infarcts were seen.

Penetrating Artery Infarcts

Penetrating artery infarcts (PAI) can be seen as both single and multiple. In their study, Wong et al. (52) found no PAI larger than 15 mm. They found that the lesions were identical to that of lacunar infarcts where the lesion is due to lipohyalinosis. The underlying mechanism in this type of infarct is the occlusion of MCA in the origin of a penetrating artery that results in lacuna-like infarcts (59). Occasionally, small embolisms may also cause the block leading to PAI.

Atherosclerotic Causes

Atherosclerotic MCA syndromes result from either intracranial atherosclerosis or extracranial atherosclerosis (37). Intracranial atherosclerosis arise within the MCA, and extracranial atherosclerotic causes of infarctions are due to embolism that arises in the proximal ICA, heart chambers, and aorta (52, 54). Lee et al. (56) stated although rare in Caucasians, atherosclerosis of MCA is more common in Asians, and therefore should be included in the differential diagnosis of ischemic strokes (51). In a study conducted by Kim et al. (37), ischemic strokes due to atherosclerosis were found to be in MCA in 34% of the cases. They also found that most cases were due to intracranial atherosclerosis.

One other cause of MCA syndrome is artery-to-artery embolism. When small embolisms are released, there exist two possible results: total clearance and ischemia. The latter is, as presumed by Wong et al. (52), the low risk regarding the ability of cerebral circulation in terms of arterial anastomoses and collateral supply, which prevents the formation of infarct and doles out adequate blood flow that prevents the formation of large ischemic area. These infarcts caused by small emboli usually result in cortical infarcts that appear to be less harmful, as they are more benign and affect a smaller area. Wong et al. (52) state that this is due to anastomoses formed during a rather long process of MCA stenosis caused by atherosclerosis. As mentioned above, the common pathology underlying artery-to-artery embolism is cholesterol crystals (52). Atheroma in MCA can cause occlusion and lead to PAI when the block is formed in the origin of a penetrating artery (59).

Non-atherosclerotic Causes

Although atherosclerotic lesions are the most common causes of MCA strokes, many non-atherosclerotic causes also play a role in the development of infarctions. Kim et al. (60) state that moyamoya disease (MMD), arterial wall dissections, and vasculitis are some of these etiologies and these etiologies are the underlying lesions for younger individuals.

In their study, Kim et al. (60) found that 28 patients had concentric stenosis with a smaller diameter. MMD is typically symptomatic from the midst of the 3rd decade to the 4th decade of life (60). Scott et al. (61) state that 2/3 of the affected population are female. Kim et al. (60) state that hemodynamic compromise may exist along MMD and when affected unilaterally, MMD on its own may not cause any symptoms by hemodynamic stability and collateral perfusion.

CONCLUSION

With the brain being one of the most complex organs in the human body, it undoubtedly has crucial tasks. In order to maintain its vital functions, the brain must be sufficiently perfused. Accordingly, it is supplied by two distinct arterial systems: the anterior cerebral circulation and the posterior cerebral circulation. The major suppliers of the anterior cerebral circulation are ACA and MCA. Having discussed its distinct and precise anatomy, it is essential to know the variations that might lead to pathologies. Besides its anatomy, various pathological cases should be kept in mind, essentially for surgical interventions and postoperative follow-ups. Successful surgery without incurring significant neurovascular morbidity in this region depends on the detailed knowledge of its vascular anatomy and pathologies.

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MAIN GENOME EDITING TOOLS: AN OVERVIEW OF THE LITERATURE, FUTURE APPLICATIONS AND ETHICAL QUESTIONS

Eylül Şenödeyici[1], Dengiz Koray Şahintürk[1], Bilge Rana Akbolat[1], Arzu Dindar[1], Selin Sefer[1], Gül Feride Anğay[1], Selma Demir[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Medical Genetics, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

The popularity of genome editing technologies in the scientific community has been on the rise for several years. These technologies are slowly becoming a ray of hope for many patients with genetic diseases, thanks to their immense potential for clinical application. New genome editing tools are being rapidly developed and introduced, while pre-existing ones are being perfected. In the process beginning with the completion of the Human Genome Project to the first clinical trials focusing on cancer immunotherapy and treating blindness, studies on genome editing have increased exponentially. The clustered regularly interspaced short palindromic repeats system is a Nobel Prize-winning genome editing tool celebrated by many researchers and is often praised due to its ease of use, low cost, and efficiency compared to other acknowledged genome editing tools. This review aims to discuss the historical development, working mechanisms, present and future clinical applications of zinc-finger nucleases, transcription activator-like effector nucleases, clustered regularly interspaced short palindromic repeats, and prime editors, while presenting the ethical aspects of using these genome editing tools. ***Keywords:*** Zinc-finger nucleases, transcription activator-like effector nucleases, clustered regularly interspaced short palindromic repeats, gene editing

INTRODUCTION

In the last decades, a multitude of novelties was introduced to the field of genetics including the first gene transfer into mammalian cells, cloning of the insulin gene, production of human recombinant lysosomal enzymes, and sequencing the human genome (1-4). Completion of the Human Genome Project provided researchers with a deeper understanding of the role of genetics in physiology and evolution of humankind, the entirety of the human genome, and how genes function, along with the possibility to do systematic research on causes of diseases, which significantly altered the practice of medicine (5, 6). Following this enlightenment, several gene therapy techniques aiming to mitigate the disease-causing effects of genetic conditions have been developed, namely zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR) (7-9). These are programmable site-specific nucleases that have shown therapeutic potential; however, the applications of these technologies are still being perfected. Although CRISPR/CRISPR associated protein 9 (Cas9) systems are reported to have shown superiority over transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) on certain aspects, effects of off-target mutations remain untackled and several ethical questions remain unanswered (7, 10).

Zinc-Finger Nucleases

Induction of a double-strand break (DSB) in a specific genomic target sequence, followed by the generation of desired modifications during subsequent DNA break repair enabled researchers to specifically add or delete genetic information to study gene function in different organisms. ZFNs are artificially engineered restriction enzymes with a hybrid heterodimeric protein structure that can be customized to cleave a targeted DNA region and they are among the first tools of genome editing technology (Figure 1). The FokI endonuclease domain induces double-strand breaks (DSBs) whereas zinc-finger domains are responsible for DNA identification (11). The FokI cleavage domain resides within a 5 or 6 base pair (bp) long spacer sequence, with engineered Cys2His2 zinc-fingers and a linker protein that creates a connection between FokI and zinc-fingers (12, 13).

FokI, a separable enzyme, requires to be dimerized for cleavage; however, the dimer interface cannot maintain enough strength. The best way to overcome this problem is to construct a second set of fingers at the opposite position. Each monomer determines a half site with three or four Cys2His2 zinc-fingers that were first detected in the genus Xenopus, and each zinc-finger binds to a 3 bp sequence of DNA (12, 13). Dimerization appears as a powerful disadvantage; however, cleavage gets activated when there is enough specificity, and two domains can reconstruct it (14).

Address for Correspondence: Eylül Şenödeyici, Trakya University School of Medicine, Edirne, TURKEY
e-mail: senodeyicieylul@gmail.com
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ORCID iDs of the authors: EŞ: 0000-0002-4132-1594; DKŞ: 0000-0001-9865-0930; BRA: 0000-0002-7488-7623; AD: 0000-0002-8587-8941; SS: 0000-0003-1512-8456; GFA: 0000-0002-1870-5776; SD: 0000-0002-0964-5513.

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Studies on ZFN presents hope for a cure for infections such as the human immunodeficiency virus (HIV), human papillomavirus, hepatitis B virus (HBV), and malaria (15-19). In 2009, Kim et al. (20) tested 315 modularly assembled ZFN pairs at 33 sites in the CC chemokine receptor 5 (CCR5) gene, which is vital for curing acquired immune deficiency syndrome (AIDS). Knocking out CCR5 leads to providing insistent T-cells for HIV infection and insertions at CCR5 are considered safe sites for gene therapy. Deleting the CCR5 gene concludes with the repair of the luciferase gene in human embryonic kidney cells (HEK293). Therefore, the activity of the luciferase gene is an indicator of cleavage success.

Context-dependent specificity is noted as one of the most prominent downsides of ZFNs, meaning that the nucleases' capability of cleaving the target sequence is affected by the adjacent sequences in the genome and the target sequence. Instability and genome fragmentation may be caused by the occurrence of these non-specific cleavages. Additionally, ZFN induced non-specific and off-target cleavages may also cause toxicity to cells, making this technique undesirable for several practices (11).

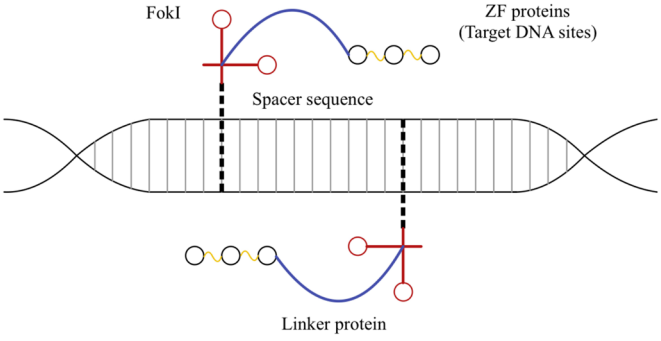

Figure 1: Structure of zinc-finger nuclease.
ZF: *Zinc-finger*

Transcription Activator-Like Effector Nucleases

Transcription activator-like effectors (TALEs) are a family of proteins regulating plant genome expression by binding to specific genes (21). These proteins are obtained from *Xanthomonas* bacteria, which contains pathogenic species for rice, pepper, tomato, wheat, and citrus (21-23). *Xanthomonas* bacteria increase the susceptibility of the plant cells to infection by secreting transcription activator-like effector (TALE) proteins to the cells' cytoplasm. TALE proteins are capable of imitating the eukaryotic transcription factors, thus activating the gene expression on the target DNA sites. Therefore, it is possible to increase, reduce and completely suppress gene expression by using TALEs. TALE proteins' genome modification activity was first demonstrated in 2007, and the code for TALE proteins to recognize the target DNA was deciphered a year later in 2008 (22). This advancement drew many researchers' attention to TALE proteins, deeming this technology worthy of being declared as the "Method of the Year" by Nature Methods in 2011 (24). Transcription activator-like effector nucleases' (TALENs) capability of editing endogenous genes in plants, nematodes, zebrafish, rats, human somatic cells, and pluripotent stem cells proves their versatility as a genome editing tool (25). Additionally, TALENs can be used for increasing crop yields, increasing the quality of agricultural products, and thus leading to a higher market share (21).

Similar to the ZFN system, the TALEN system consists of one DNA binding domain and one FokI catalytic domain (Figure 2) (26). Comparing with ZFN the TALEN system provides opportunities for replacement or inserting mutations at the specific genomic sequences of targeted genes with higher targeting flexibility, simplicity, and efficiency (27). Moreover, TALEs are highly sensitive to mismatches, blocking recognition after three to four mismatches and limiting the DNA binding capacity of TALENs (28). In order to cut the target area, a pair of TALENs that bind to both strands of DNA are required. The TALENs that are attached to either strand of DNA cannot function without being dimerized. These two TALENs must communicate with each other to perform nuclease activity since a single TALEN is not enough to induce a DSB. A 12-25 bp long spacer sequence between two adjacent TALENs is required. Once the dimerization process is completed, FokI nucleases induce DSBs at the targeted DNA sequence (21, 23). TALENs have lower off-target effects compared to other genome editing tools (29). Web-based tools are available that help researchers design pairs of TALENs to target specific gene sequences. Commonly used ones are TALE-NT and E-TALEN (21).

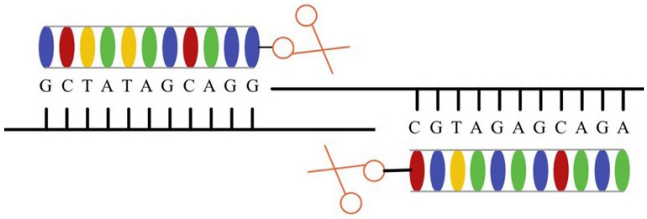

Figure 2: Structure of transcription activator-like effector nuclease.

The DNA binding domain of the TALEN system contains monomers, which are tandem repeats of 33 to 35 amino acids (26). Each monomer binds to one nucleotide that is present in the target DNA sequence. Being composed of only 20 amino acids, the last tandem repeat located in the 3' end is named a "half-repeat" (22). The 12th and 13th amino acids are highly variable and are called "repeat variable residues" (28). Repeat variable residues define the specificity of TALE proteins by recognizing specific nucleotides, while the repeat variable residue's DNA binding specificity is defined by the amino acids that match with these nucleotides.

The requirement of a T nucleotide before the 5' end of the target DNA sequence limits the site selection of TALEN regardless of its overall convenience and simplicity. However, there are two ways to overcome this limitation: site selection can either be made by altering the length of the spacer sequence or selecting the mutant variants of the TALEN N-terminal domain that are capable of binding to A, G, or C (23).

In a study conducted by Mussolino et al. (27), TALEN was reported to be able to successfully modify up to 45% of the transfected cells' CCR5 and interleukin-2 receptor subunit gamma loci. In the same study, a significantly lower rate of nuclease-associated cytotoxicity was reported for TALENs in comparison to ZFNs, indicating a higher specificity rate for TALENs. A study conducted by Sun et al. (30) reports that the TALEN system can potentially be used for curing sickle cell anemia by correcting the genetic mutation in the human beta-globin (HBB) gene that is responsible for this disease. Likewise, Bloom et al. (31) demonstrated TALEN's therapeutic potential against chronic HBV infection. TALEN has also been used in pigs for stopping the encoding of low-density lipoprotein receptors by inactivating the low-density lipoprotein receptor gene, which provided researchers with further understanding of familial hypercholesterolemia (32). This genome editing tool has immense therapeutic potential, tackling the adverse effects or the incidence of off-target mutations.

Clustered Regularly Interspaced Short Palindromic Repeats

The clustered regularly interspaced short palindromic repeats (CRISPR) system is one of the defense mechanisms of prokaryotic organisms against viruses, making it possible for prokaryotic cells to memorize the invader's nucleic acid molecules and create an immune response. However, viruses have managed to counter this system by using random point mutations in their genetic material, more specifically, by creating point mutations in the protospacer adjacent motif (PAM) sequence or protospacer sequence. Bacterial populations with higher spacer diversity are less prone to being tricked by viruses that use point mutations (33).

The description of the CRISPR-Cas system was first disclosed in 1987 while analyzing the Escherichia coli genome sequence responsible for phosphate metabolism (34). However, the acronym CRISPR was not introduced until 2002 (35). Thereafter, similar sequences were reported in halophilic archaea and other bacteria (34). In 2005, spacer sequences were found to have originated from bacteriophage genomes (36). Also, the PAM sequence was identified in the same year (35). Subsequently, it was suggested that the CRISPR system might be an adaptive immune defense against bacteriophages for prokaryotes. This suggestion was proved experimentally two years after the initial hypothesis (34). In 2008, it was shown that small and individual CRISPR RNAs (crRNAs) were produced transcribing CRISPR arrays that guide Cas enzymes. Researchers began understanding the mechanisms and fundamental functions of the CRISPR systems more clearly by 2010 (36). In 2011, trans-activating crRNA (tracrRNA) was identified, and it was discovered that tracrRNA and crRNA fuse to guide the Cas9 enzyme (37). In the following year, single guide RNA (sgRNA) was produced by merging crRNA and tracrRNA (37, 38). In 2013, genome editing in mammalian cells was achieved by using the CRISPR-Cas9 tool (37). Studies on cancer immunotherapy and treatment for blindness were initiated in the following years in the United States of America (38).

Although the history of clinical trials on genome editing date back to the 1960s, substantially more efficient and reliable techniques were introduced in 2012 when Jinek et al. (39) published their manuscript about the discovery of CRISPR-Cas9. Ultimately, Emmanuelle Charpentier and Jennifer A. Doudna were awarded the Nobel Prize in Chemistry 2020 for pioneering the development of this revolutionary genome-editing tool that would allow scientists to "alter the code of life" (40).

As discoveries about the CRISPR systems accumulate, their classification has evolved to make it simpler (Figure 3) (34). Presently, CRISPR systems are divided into two classes, each containing three distinct types: class I uses a multiple-effector protein complex, whereas class II uses a single effector Cas protein to convey immunity against foreign nucleic acids by cleaving them. Each CRISPR system has different functions and characteristics, although the effects of the type IV CRISPR system on either of the nucleic acids are not yet known (Table 1) (41).

The signature protein for the type II CRISPR system, which was deemed worthy of the Nobel Prize in Chemistry 2020, is Cas9. Cas9 is a multi-domain protein responsible for binding the crRNA with the target sequence and cleaving the DNA (33). Two domains in Cas9 protein, named HNH and RuvC, break the opposite strands of DNA. While the HNH domain breaks the targeted strand of DNA, the RuvC domain breaks the complementary strand (34, 41). The type II CRISPR system essentially uses the Cas1, Cas2, and Cas9 enzymes along with tracrRNA and CRISPR array. While this set is sufficient for the type II-C system, type II-A and II-B systems require additional Csn2 or Cas4 genes, respectively (35). The CRISPR Cas9 system requires a sgRNA which can be designed by the association of a target sequence containing crRNA and tracrRNA. The sgRNA guides the Cas9 enzyme to find the PAM sequence and attach to the targeted area, causing a DSB (35, 42). Trans or transgenic DNA can be created by adding a donor piece between the two edges. The break in DNA will then be repaired by the cell. However, this technique renders the triggering of indels possible (38). The signature protein for the type III system is Cas10, and the PAM sequence is not required for this type (43).

The working principle of all CRISPR systems can be subclassified into three stages: adaptation (of spacer acquisition), expression (or crRNA biogenesis or maturation), and interference (Figure 4) (33, 44). Prokaryotic DNA contains a CRISPR locus, which consists of CRISPR and spacer parts (45). The length of the CRISPR sequence generally ranges from 28 nucleotides (nt) to 37 nt although

Table 1: Function and domains of Cas and ancillary proteins.

Cas Enzymes	*CRISPR Type Association*	*Function*
Cas1	I, II, some III, IV, possibly VI	DNA nuclease
Cas2	I, II, some III, V, some VI	RNA nuclease
Cas3	I	DNA nuclease, helicase
Cas4	Mostly I, II, V	DNA nuclease
Cas5	I, III, IV	Ribonuclease that turns pre-crRNA into crRNA
Cas6	Mostly I, some III	Ribonuclease that turns pre-crRNA into crRNA
Cas7	I, III, IV	Contains RNA recognition motif (RRM) and binds crRNA, generally present in multiple copies
Cas8	Mostly I	Large subunit of effector complex of type I
Cas9	II	DNA nuclease
Cas10	Some I, mostly III	Large subunit of effector complex of type III
Cas12 (Cpf1)	V	DNA nuclease, processes crRNA
Cas13 (C2c2)	VI	RNA nuclease, processes crRNA
Csm/Cmr	III	RNA nuclease, DNA nuclease (cleaves one strand)
RNase III	II	Processes tracrRNA, assists crRNA maturation

CRISPR: *Clustered regularly interspaced short palindromic repeats,* ***Cas:*** *CRISPR associated protein,* ***DNA:*** *Deoxyribonucleic acid,* ***RNA:*** *Ribonucleic acid,* ***crRNA:*** *CRISPR ribonucleic acid,* ***tracrRNA:*** *trans-activating CRISPR ribonucleic acid,* ***RNase:*** *Ribonuclease*

the minimum and maximum lengths are 23 nt and 55 nt. The length of the spacer sequence generally ranges from 32 nt to 38 nt; however, it can be as low as 21 nt and as high as 72 nt (46).

In the adaptation stage, the prokaryotic cell cuts off the protospacer from the invader's mobile genetic elements and adds this piece into its spacer DNA sequence between CRISPR systems, in order to deactivate the invader's mobile genetic elements more easily and faster in case of another encounter with the same invader. This process can be defined as "memorizing the invader's nucleic acid" (33, 45). The PAM sequence, which can be interpreted by its name, is located next to the protospacer, meaning that the prokaryotic cell can detect the PAM sequence along with the protospacer part. Protospacer detection with PAM sequence is only possible for types I and II (33). In this process, Cas1 (a DNA nuclease) and Cas2 (an RNA nuclease) enzymes are used, and these two proteins play a role in almost all CRISPR systems, with III-C, III-D, and IV being exceptions (33, 42). Both enzymes are responsible for cutting off the invader's nucleic acid in the adaptation stage (33, 46).

In the expression stage, the CRISPR locus in the microorganism's DNA is transcribed into precursor CRISPR RNAs (pre-crRNAs), which need to be maturated for activation. Cas proteins are needed for the maturation of the crRNAs containing the "memorized" sequences in the adaptation stage (44).

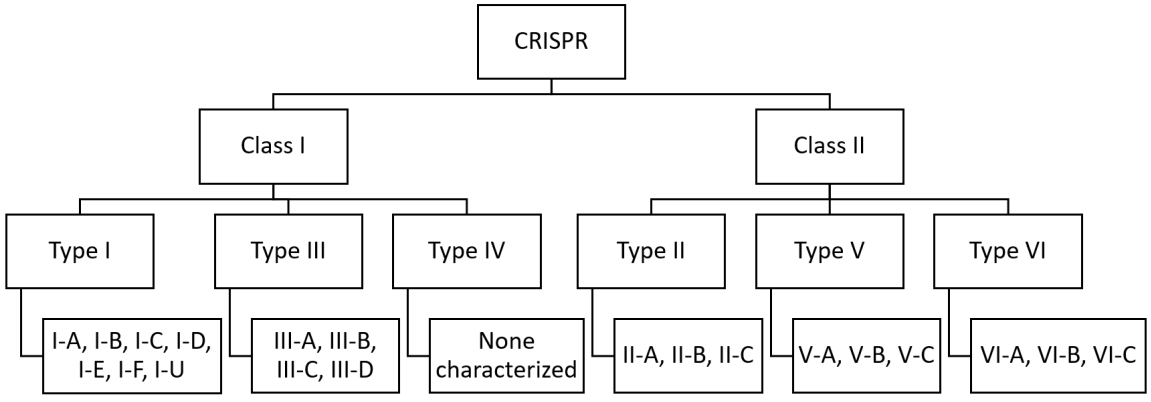

Figure 3: Classification of CRISPR systems.
CRISPR: *Clustered regularly interspaced short palindromic repeats*

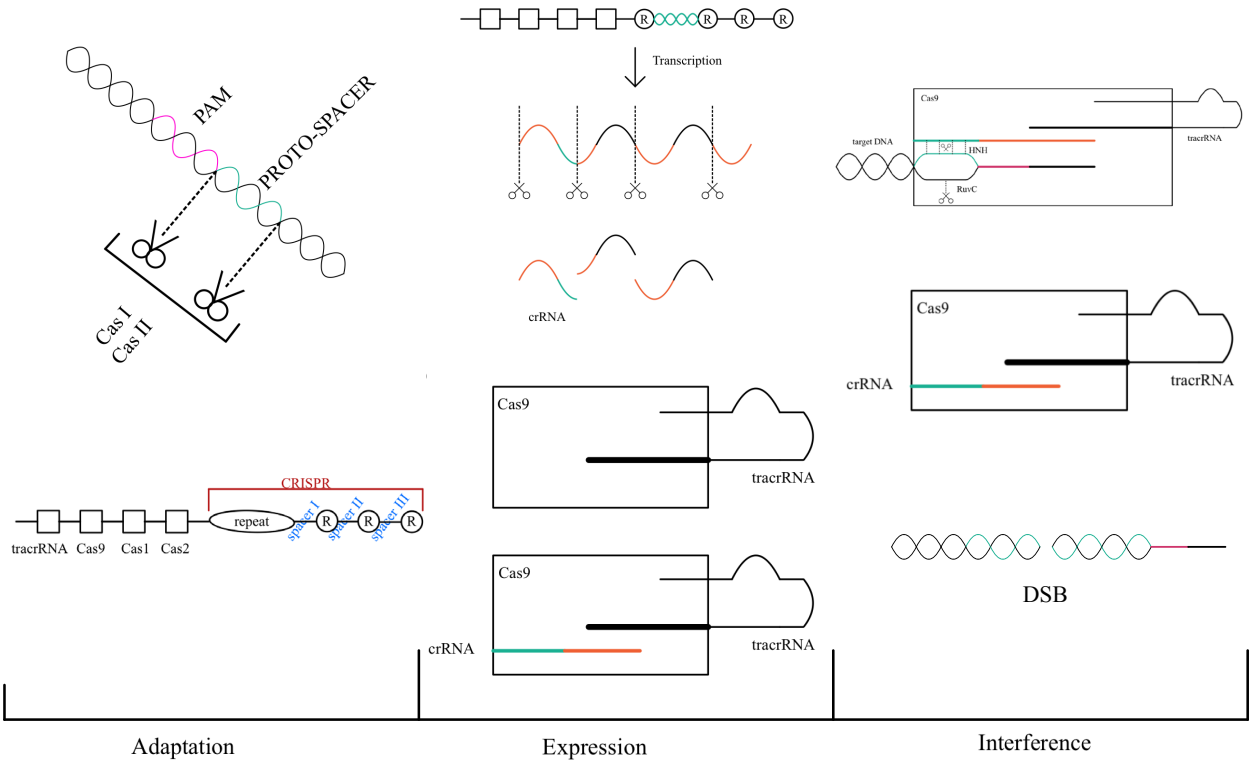

Figure 4: The working principle of clustered regularly interspaced short palindromic repeats systems.
CRISPR: *Clustered regularly interspaced short palindromic repeats,* ***crRNA:*** *CRISPR RNA,* ***tracrRNA:*** *Trans-activating CRISPR RNA,* ***DSB:*** *Double-strand berak,* ***PAM:*** *Protospacer adjacent motif,* ***Cas:*** *CRISPR associated protein*

In the interference stage, which is the last stage of immune reaction, crRNAs guide the Cas proteins to detect and interfere with the specific sequences. While class I CRISPR systems constitute a cascade complex (CRISPR-associated complex for antiviral defense), single effector proteins are sufficient for class II systems for interference. To avoid self-targeting, different CRISPR systems use different mechanisms. Self-targeting is avoided by recognizing the PAM sequence located upstream of the protospacer in types I and V, while in type II it is avoided by the recognition of the PAM sequence located downstream of the protospacer. Mature crRNA in the type III system determines whether the immune response will be self-targeting or not. To proceed with the degradation process by the complex, the 5' tag of crRNA must not be paired with the target (33).

The principle of using the CRISPR tool for diagnostic purposes is "nucleic acids are indicators for diseases". This diagnostic method is based on identifying the disease-related nucleic acid sequences and cleaving them (47). The simplicity of the Type II system allows it to be used widely for diagnosis (42). Bacterial and viral sequences that are derived from infectious organisms or oncogenic mutations can be targeted. CRISPR systems not only identify pathogens but also repair the disease-causing genes on the chromosomes (47).

By using different characteristics of the CRISPR systems, several methods were developed for diagnostic purposes. These methods include diagnostic assay based on sgRNA, CRISPR interference, and serotyping/subtyping using CRISPR. The CRISPR interference method requires a catalytically-dead Cas9 (dCas9), which is a modified form of Cas9 that lacks endonucleolytic activity. A diagnostic assay based on sgRNA takes advantage of the specificity of the CRISPR technology. Since any sequence located next to the PAM sequence can be targeted, it is possible to detect almost any locus of interest by editing the sgRNA. The differentiation is of great importance, because specific strains may cause certain conditions, with one example being the Brazil strain of the Zika virus, which is associated with fetal microcephaly. CRISPR technology is being used for developing a paper diagnostic kit that can be used to distinguish between African and American Zika virus strains, despite the difference being a single base in the PAM sequence (42).

The effects of fluorescent probes can be increased by cleaving them from non-specific RNA with Cas13a, which is an RNA nuclease (42, 43). A molecular detection platform has been developed using this technique and named specific high sensitivity enzymatic reporter unlocking (SHERLOCK). This platform can be used to detect cell-free cancer mutations, single-nucleotide polymorphisms, viral strains, and bacterial pathogens (42). A similar platform using Cas12a named DNA endonuclease-targeted CRISPR trans reporter (DETECTR) can be used to diagnose various viral agents (47). A recently developed SARS-CoV-2 DETECTR reportedly has 95% accuracy and requires around 40 minutes to detect COVID-19 (37).

Although CRISPR technology has immense potential for therapeutic application, some challenges still need to be overcome. Presently, the primary obstacle appears to be delivering the necessary enzymes and the donor DNA into the cell. Some alternatives to solve this problem include (i) using viral vectors to deliver the DNA sequence that encodes both guide RNA and enzymes, (ii) using lipid nanoparticles to deliver the mRNA that encodes guide RNA and Cas9, and (iii) preformed ribonucleoprotein included Cas9 and guide RNA.

The clustered regularly interspaced short palindromic repeats tool can be used for ex vivo based therapy by treating the selected cells of the organism and inserting them back with autologous transplantation. In order to cure immune-based or blood-based diseases, either hematopoietic stem or progenitor cells or immune cells need to be extracted from blood and bone marrow to be genetically edited (48). Vertex Pharmaceuticals and CRISPR Therapeutics offer a treatment named CTX001 for sickle-cell anemia and β-thalassemia using such a technique (37). However, editing stem cells or progenitor cells is not always necessary. For rheumatoid arthritis, editing regulatory T cells may be preferred instead of editing progenitor T cells (48).

Because of the minimal access to the targeted organs, in-vivo based therapy is less common. One of the targetable tissues for in-vivo based therapy is the eye. EDIT-101 is an alternative therapy for Leber congenital amaurosis caused by a mutation in the CEP290 gene with no known cure and results in childhood blindness. In this therapy, CRISPR/Cas9 is delivered into the patient's retina with the intronic IVS26 mutation. This mutation causes aberrant splicing on a specific protein. This splicing may be curable by using the CRISPR/Cas9 tool (37).

The first-ever phase 1 clinical trial using the CRISPR tool approved by the United States Food and Drug Administration has aimed at cancer immunotherapy by editing autologous T cells. This therapy was planned against several types of cancer without treatment options or with relapsed tumors, such as melanoma, multiple myeloma, myxoid/round cell liposarcoma, and synovial sarcoma. In this ex-vivo based therapy, α and β chains are knocked out on endogenous T cell receptors, which generate an immune response, and programmed cell death protein 1, which weakens the immune response. Later, edited genes are delivered back into the patients using lentivirus as a vector. The gene that encodes NY-ESO-1-specific T cell receptor has been demonstrated to be highly upregulated in the relapsed tumors (37).

Despite being prompt, economic, practical, and efficient, many challenges remain to be tackled until the CRISPR tool is available for widespread use (49). Toxicity is one of these disadvantages since DSBs created by CRISPR often trigger the apoptosis pathway of the cell. However, DSB risk may be reduced by using dCas9. Immunotoxicity is another phenomenon that often raises concerns. In a study conducted by Charlesworth et al. (38), more than 50% of the human subjects were found to possess pre-existing anti-Cas9 antibodies. Similar to the other gene-editing technologies, the off-target effects of CRISPR are another major concern (11). The frequency of off-target effects in CRISPR is equal to or greater than 50% (38). All of these drawbacks led researchers to conduct further studies in order to perfect this genome-editing tool. Various strategies have been developed to reduce RNA-guided endonucleases' off-target effects, namely Cas9 paired nickase, dCas9, CRISPR-based cytosine and adenine base editors, ribonucleoprotein delivery, truncated gRNAs, and prime editors (50, 51).

After CRISPR: Prime Editing

Effective and precise correction of most disease-causing gene variants using the ZFN, TALEN, and CRISPR technologies and other tools that produce DSBs is often hindered by excess byproducts and off-target mutations (52). In 2019, Anzalone et al. (52) from the Liu Lab introduced prime editing, a new genome editing tool that "promises to be a cut above CRISPR" and does not produce DSBs, thus having a lower off-target mutation rate (53). This novel genome-editing tool can correct targeted insertions, deletions, and all possible base-to-base conversions in comparison to CRISPR base editing, a genome-editing technology that targets cytosine and adenine for irreversible conversion without DSBs, which is capable of installing the four C→T, G→A, A→G, and T→C mutations, rendering the mediation of all single nucleotide variations (SNV) possible (52, 54). It is anticipated that SNV correction will be a major focal point of precision medicine for years to come (54).

Fundamentally, the prime editor structure consists of reverse transcriptase (RT) merged with Cas9n (an RNA-programmable nickase), along with a prime editing guide RNA (pegRNA) (Figure 5). Presently, there are four prime editing modifications: prime editor 1 (PE1), prime editor 2 (PE2), prime editor 3 (PE3), and prime editor 3b (PE3b). The structure of PE1 comprises a nickase Streptococcus pyogenes Cas9 (SpCas9) merged with Moloney murine leukemia virus (M-MLV) RT and a pegRNA. PE2 utilizes a mutant variant of M-MLV RT and is a modification of PE1. When PE2 is modified by adding another nick on the opposite DNA strand, PE3 is formed. The fourth modification, PE3b, is formed when sequential double nicking is induced by the installation of the determined mutation on the targeted DNA sequence, nicking the non-edited DNA strand only after the conversion of the other strand to the edited sequence has been completed (55, 56).

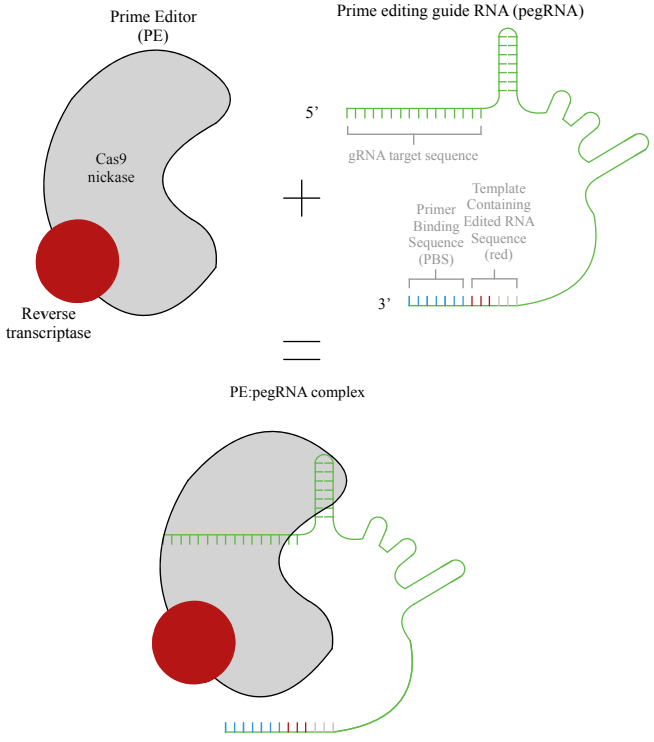

Figure 5: Structure of prime editors.

Prime editors do not produce DSBs, with PE3 creating two nicks and the other prime editors creating one nick. The absence of DSBs is associated with lower indel mutations, often at an undetectable level (55). Prime editing also offers several choices of different primer binding sites, pegRNA induced-nick locations, sgRNA induced second nick locations, and RT template lengths, along with the freedom to choose which DNA strand will be edited first. Allowing a wide range of gene modifications including insertions, deletions, and transversions, up to 89% of pathogenic human genetic variants may be potentially corrected with prime editing (54).

Despite showing immense therapeutic potential, more research is required for further understanding and more efficient use of prime editors. Although the working principles of CRISPR base editing are beyond the scope of this review, it is worth mentioning that base editing has been reported to outperform prime editing when using adenine base editors for correcting the pathogenic G→A mutations in the ATP binding cassette subfamily B member-11 gene that causes bile salt export pump deficiency and the mutations of the serpin family A member-1 gene that causes alpha-1 antitrypsin deficiency (57). However, prime editors may be more favorable when multiple adenine and cytosine bases are present in the targeted DNA site and bystander edits are undesirable (52). Anzalone et al. (52) used PE3 to deploy the HBB (E6V) mutation causing sickle cell disease in the HEK293T cell line with 44% efficiency and 4.8% indels. To revert this mutation to wild type HBB, they treated the cell line with PE3 and a programmed pegRNA, with 26-52% efficiency and 2.8% indels. Anzalone et al. (52) also tested prime editing to correct the mutation that causes Tay-Sachs disease, deploying a 4 bp insertion into hexosaminidase A by utilizing PE3 with 31% efficiency and 0.8% indels. In order to revert the cells to the wild type phenotype, PE3 was used, with the application resulting in ≥20% editing, compared to the application of PE3b, which resulted in 33% efficiency and 0.32% indels. Prime editing has also been reported to be 7.1% more effective than CRISPR base editing on post-mitotic, terminally differentiated mice cells (52).

Ethical Questions Regarding Genome-Editing

Ethical and regulatory issues regarding the use of genome editing tools are being discussed globally and many guidelines, rules, and principles to advance the usage of these technologies are being accepted. Current standards and guidelines are not sufficient for the safe use of these applications, especially for editing the human genome. Since these novel technologies have not been thoroughly tested on editing the human genome, many unpredictable complications may occur during and after application (8). Current issues present mental, cultural, and philosophical conflicts for researchers, clinical specialists, strategy producers, patients, and the general public (54).

Presently, the CRISPR-Cas technique is a practical option for genome alteration in a wide variety of organisms, including humans, where this method has been tried for treating or preventing severe genetic defects. The CRISPR-Cas method can be exceptionally favorable in basic and pre-clinical settings to distinctly explain and further improve the application itself to be relevant in clinical search. However, a significant matter of concern about the CRISPR-Cas method is its potential utilization in human embryo germline editing, as the debate on precisely when an embryo accomplishes "personhood" is yet to be concluded (8).

During the 2015 Napa Valley meeting, a group of developers, scientists, and ethicists from the United States National Academy of Sciences, Engineering and Medicine (NASEM) discussed the legal, ethical, and biomedical aspects of using gene editing methods without sacrificing the benefits of discoveries on the CRISPR-Cas9 mechanism (8). In the follow-up meeting held in February 2017, NASEM declared supporting somatic genome editing, excluding any enhancement. The committee has also decided that the alteration of a germ-line in order to create new individuals who might potentially transfer the altered genome to their off-springs could be allowed under particular conditions, being: (i) treating severe genetic defects in the absence of an alternative approach or treatment, under meticulous supervision, (ii) somatic and germ cells cannot be edited with the intent of enhancement, (iii) genome editing can be done for basic research, and (iv) somatic genome editing can be used for the treatment of severe diseases (58).

In November 2018, Chinese researcher He Jiankui declared that he had used CRISPR to edit human embryos by causing a 32 bp deletion on the CCR5 gene, aiming to make the embryos resistant to AIDS by rendering the associated gene dysfunctional. Two of these edited embryos were successfully implanted, resulting in pregnancy and birth by cesarean section. Although Jiankui expected to be "hailed as a hero", in reality, he was condemned by the scientific

community and his work was defined as "premature and unethical". The additional effects of the absence of a functional CCR5 gene are currently unknown, and whether Jiankui managed to successfully induce the 32 bp deletion on either of the twins is a matter of controversy (59).

Editing human embryos has long been among the greatest ethical concerns of the CRISPR genome-editing system, and the "Jiankui Affair" may have further fueled the ongoing debate. One reason for this concern is the possibility that any damage done to the embryonic genome that causes mutations and off-target effects can be transferred to future generations as well (60). While many scientific community members support fundamental search on CRISPR in somatic cells, a significant number of researchers consider the CRISPR tool to be not extensively developed to insert hereditary substitutions in the human genome (61).

Different countries have prepared restrictive rules and guidelines for human germline gene editing for reproductive purposes. These guidelines diverge widely around the world, placing germline genome editing on a spectrum of limitations ranging from directly "banning" to "outlawing in any circumstances" around the world (Table 2) (8).

Table 2: Legal perception of human germ-line editing in various countries (8).

Country	*Restrictive*	*Legal prohibition*	*Prohibition by guidelines*	*Ambiguous*
United States of America	X			
United Kingdom		X		
Japan			X	
China			X	
Ireland			X	
India			X	
Russia				X
Argentina				X
South Africa				X
Chile				X
Slovakia				X
Colombia				X
Greece				X
Iceland				X
Peru				X

In many countries, biotechnological policies are superannuated and the genome editing policies regarding the use of such tools for clinical, reproductive, and agricultural purposes are poorly discussed. The rapid discoveries on novel genome editing tools, especially the recent advancements on the CRISPR method, render the existing moral guidelines and regulatory policies requiring further discussion and reconsideration to reach a global consensus on the ethical aspects of human genome editing. Participants' privacy, well-being, safety, and dignity should be the number one priority of these discussions (8).

CONCLUSION

Precise and effective genome modification is of immense value for genetic engineering. The rapid development of such technologies makes it possible for researchers to use an arsenal of ever-expanding techniques for genome editing (58). TALENs and ZFNs were the pioneering genome editing tools; however, they do not offer the highest specificity due to their substantial off-target effects. With the discovery of CRISPR-Cas9, genome editing has become more incentivized as a result of providing higher efficiency and applicability. This recent technology appears to be accepted as the "new level" of genome editing (62).

Compared to other preceding programmable gene-editing technologies such as TALENs and ZFNs, the CRISPR tool comes into prominence due to its simplicity and lower cost, making it more readily available for research communities (58). Only a short guide RNA sequence is required to be changed for redirecting the site-specific cleavage, which may also be turned into an enzyme that nicks to simplify homology-directed repair with lower mutagenic activity (58, 63). The high effectiveness and accuracy of the Cas9 protein derived from the type II CRISPR system allows several applications in numerous fields of science (58).

In contrast to the ZFN and TALEN systems in which the DNA recognition sites are dependent on the artificial proteins that require an interaction between the protein and DNA, the DNA recognition function of the CRISPR/Cas system depends on RNA-DNA coactions, presenting some advantages over ZFNs and TALENs. This feature provides a simple design for altering any genomic target, more predictable off-target regions, and the prospect of modifying multiple genomic regions concurrently (11).

The high probability of undesirable off-target effects during gene editing appears to be the most prominent disadvantage of the ZFN technology. Being structurally similar to ZFN, the TALEN system also suffers from high rates of undesirable mutations in the target site. Being an RNA-guided nuclease, Cas9 has a higher sequence specificity owing to Watson–Crick base pairing between the target DNA sequence and its gRNA, in addition to the direct connection between Cas9 and PAM. Although ZFNs' and TALENs' effectiveness for certain purposes has been acknowledged, these tools require new proteins to be synthesized for every new target DNA site. Contrarily, the Cas9 protein maintains the same structure, regardless of which DNA sequence is targeted. Only the short sequence of gRNA needs to be altered to redirect the site-specific cleavage (58). However, all three of these genome editing tools induce DSBs, the ultimate risk factor for off-target mutations. Prime editing developed by Anzalone et al. (52) generates mere nicks on the target sequence rather than DSBs, which may eventually rid the researchers of this "necessary evil" of genome editing.

Even though these genome editing technologies promise countless benefits, hopes of treatment for serious diseases with their immense therapeutic potential, and the power to utterly "alter the code of life", significant ethical and biosafety issues should not be ignored (58). The Jiankui affair may have greatly altered how researchers perceive genome editing by adding more questions to be answered to an already controversial topic. This incidence clearly indicates that many regulatory rules and perhaps decades of further studies are required before the human genome can be safely edited. However, the answer to the ultimate question of whether we should edit the human genome is yet to be given.

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THE ROLE OF IRON IN HEART FAILURE: A LITERATURE REVIEW

Berkin Ersoy, Yaren Yamak

University of Hamburg School of Medicine, Hamburg, GERMANY

ABSTRACT

Iron has a key role in the energy generation of the cell. Different organs utilize iron for their diverse needs. The heart is an organ that demands high energy. Nearly one-third of stable heart failure patients and half of the hospitalized patients, regardless of their heart failure status, have anemia. The potential cause of anemia in heart failure patients is found to be multifactorial. Iron deficiency anemia is associated with the reduced number of red blood cells, which transport oxygen into the tissues of the human body. Therefore, in iron deficiency, mitochondria produce less energy. Since iron deficiency has a very important role in the prognosis of heart failure, since the beginning of the 2000s, researchers have conducted several studies and trials, investigating the effect of correcting iron deficiency on the clinical and symptomatic outcomes of heart failure. The most recent results of those studies and trials suggest that intravenous iron therapy in heart failure with reduced ejection fraction patients is associated with improved exercise capability, quality of life, and New York Heart Association class. Ongoing trials such as Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure 2 are aimed more into investigating hospitalization and mortality rates of patients on intravenous iron therapy. ***Keywords:*** Iron, anemia, heart failure

INTRODUCTION

According to the study conducted by Shahar et al. (1), heart failure (HF), whether acute or chronic, is known to be one of the most frequent reasons for hospitalizations worldwide, with more than 2000 per 100.000 people aged between 75 to 84 years. Iron deficiency (ID) on the other hand, is a common comorbidity in HF patients, regardless of sex and potential anemia. According to Klip et al. (2), 50% of the patients with HF suffer from ID. ID causes anomalies in mitochondrial function and cellular energy metabolism and therefore worsens the prognosis of HF patients. Moreover, HF patients show reduced exercise capacity and lower quality of life (QoL) (3). Since the beginning of the 2000s, researchers have conducted several studies and trials, investigating the effect of correcting ID on the clinical and symptomatic outcomes of HF. In this literature review, we illustrate iron metabolism in the human body and its pathophysiology during HF and give a comprehensive overview of the studies and trials investigating the role of iron in HF.

IRON METABOLISM AND ITS PATHOPHYSIOLOGY IN HEART FAILURE

Iron homeostasis, systemic iron regulation, iron stock

Iron homeostasis is the resorption, absorption, distribution, and elimination of the biometal iron. It is essential to satisfy the metabolic needs of the body or to carry out specialized functions. Humans have 3.5-5 g iron in their bodies (4). Approximately 65% of it is allotted to hemoglobin, 10% is used as functional iron (e.g. myoglobin and ferrous enzymes), and 20% is used as stocked iron in the body as ferritin (5). Ferritin is mainly stocked in the liver. Only 0.2% of the iron in the body is bound to transferrin in circulating form (6).

Iron uptake and absorption

Cells that need iron express transferrin receptor 1 (TfR-1) and transferrin receptor 2 on their membrane surfaces. Transferrin binds to them and the complex is taken up into the cell by endocytosis and is processed in the lysosomes. From there the iron can be integrated into its target proteins (7).

The resorption rate of iron in the gut depends on the iron demand of the cells. If there is an iron need in the body (e.g. loss of blood), resorption of the iron through diet can increase up to 30% (8). Another way for the body to produce iron is to destroy the red blood cells and free the iron from hemoglobin (9).

Duodenal iron uptake

In the stomach, iron is transformed from its ferrous form into the ferric form with the contribution of hydrochloric acid and vitamin C, and then transferred to the duodenum. Like other minerals, iron is absorbed in the duodenum. The amount of iron resorbed in the duodenum is an important factor for systemic iron homeostasis (10). In the duodenum, it is either taken up through the duodenal mucosa transporters such as divalent metal transporter 1 (DMT-1) of the enterocytes as Fe^{3+} (non-heme iron) by symport with the protons or as heme iron over heme carrier protein 1 (11). At the basolateral side of the cell, the iron is then delivered into the bloodstream in reduced form as Fe^{2+} (heme iron) by the ferroportin of the mucosa cells (7). The Fe^{2+} is oxidized to Fe^{3+} and then bound to apo-transferrin (5). The apo-transferrin and ferric iron (Fe^{3+}) make up the transferrin.

Address for Correspondence: Berkin Ersoy, University of Hamburg School of Medicine, Hamburg, GERMANY
e-mail: berkinersoy@gmail.com
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ORCID iDs of the authors: BE: 0000-0001-7111-648X; YY: 0000-0002-2115-3482.

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Systemic iron homeostasis

The iron concentration in the organism is regulated by the protein hepcidin, which binds to ferritin in the small intestine and initiates its degradation (12). If the blood iron concentration is too low, the liver decreases hepcidin production. As a result, the release of iron into the bloodstream is increased (13). On the contrary, if the hepatocyte cells are provided with enough iron, they increase the hepcidin production in the liver. Hepcidin also controls the release of iron from the cells of the reticuloendothelial system and macrophages (14).

Cellular iron homeostasis

The regulation of cellular iron homeostasis depends on whether the cells have an iron deficit or iron repletion (4). On the cellular level, iron homeostasis is regulated by the expression of transferrin receptor (TfR), apo-ferritin, and 5-aminolevulinic acid (ALA) synthase, the key enzyme of the heme biosynthesis (15). Having different stages and levels of manifestation, the best-understood mechanism of iron homeostasis is the iron-dependent binding of iron regulatory proteins (IRPs) (16). In the case of a cell sufficiently provided with iron, the IRPs bind onto the iron-responsive elements (IREs) in the 5' end of the untranslated region of the ferritin messenger ribonucleic acid (mRNA), which has a blocking effect on the molecule, so that the message cannot be translated when there is no need for iron storage. Contemporaneously, the IRPs bind on the 3' uridine triphosphates of the TfR-1 mRNA which ensures the protection of the message from degradation in the cytoplasm, therefore increasing the expression of the receptor on the cell membrane (16). Whereas there is no need for extra iron in the cell, the IRPs do not dock onto IREs, which then allows ferritin translation. This process exposes the TfR-1 mRNA for degradation in the cytoplasm so that the iron uptake can be limited, and cellular iron storage is supported. The messenger ribonucleic acids of TfR, apo-ferritin, DMT-1, ferroportin 1, ALA synthase 2, and hypoxia-inducible factor 2α contain IREs, which makes them important players in cellular iron homeostasis (16, 17).

Cellular iron metabolism

Intracellular iron can be utilized, imported by mitochondria, or bounded to ferritin in the cytoplasm (15). Free iron is harmful to cells since it can catalyze the generation of cytotoxic reactive oxygen species (18). To prevent this from occurring, iron is stored in the cells as ferritin (19).

Iron in mitochondria

Iron has a key role in the energy generation of the cell, like the enzymes that are responsible for energy production in the citric acid cycle -such as cytochrome oxidase. In mitochondria, iron is used for heme synthesis and the generation of iron-sulfur clusters in the electron transport chain (20).

Heme synthesis

Organisms produce the tetrapyrrole porphyrin ring of heme from the forerunner ALA, via an eight-step enzymatic pathway (21). ALA is generated in the mitochondria of eukaryotes by a condensation reaction of succinyl-coenzyme A and glycine, catalyzed by ALA synthase (22). The molecule is then exported to the cytosol, where it is enzymatically converted into intermediate mediators. In the final reaction of the heme biosynthesis pathway, ferrochelatase catalyzes the insertion of Fe^{2+} into the mediator protoporphyrin IX (23). Synthesized heme is then exported to the cytosol to be incorporated into hemoproteins (21).

With that in mind, it is safe to say that iron deficiency can cause interruption of heme synthesis in erythroid cells and cause necrosis both in erythroid cells as well as in non-erythroid cells (24). That may result in microcytic anemia or iron deficiency anemia in general.

Iron regulation in the heart

Different organs utilize iron for their needs. When it comes to the cardiomyocytes, both iron deficiency and iron overload can cause pathologies in cardiac function. Ferroportin, as discussed previously, plays an essential role in iron uptake at the systemic level. However, it is also proven to be found in other cell tissues with no known contribution to systemic iron regulation (25).

Iron and cardiovascular disease – pathology relations

The heart is an organ with high energy demand. Iron deficiency is found in 30-50% of patients with HF thereby causes severe symptoms and poor prognosis (2, 26).

It remains unclear, whether iron also plays a role in atherosclerosis. Potential disease mechanisms might be iron-driven endothelial cell dysfunction, oxidative stress, monocyte adhesion, and plaque instability (27). However, experimental data argue against any significant pathologic relation of macrophage iron in atherosclerotic changes (28).

Anemia and heart failure

Anemia is a frequent comorbidity in patients with HF and affects nearly one-third of the stable patients and one-half of the hospitalized patients, irrespective of the HF subtype (29-33).

Compared to non-anemic patients with HF, anemic HF patients are associated with increased age and clinical manifestations of diabetes and chronic kidney disease (CKD). Anemic HF patients are also found to have increased blood pressure, increased levels of neurohormones, proinflammatory cytokines, and the use of diuretics. Moreover, those patients are expected to have lower QoL and exercise capacity, which is determined by the 6-minute walking time. All these findings suggest that anemia is a chronic disease that can develop with symptoms such as low levels of bone marrow function, decreased erythropoietin synthesis, and insufficient iron utilization (34).

The potential cause of anemia in HF patients is found to be multifactorial. Reduced hematinic vitamins such as vitamin B12 and folic acid can be seen in a minority of HF patients (35).

Levels of erythropoietin are reduced in HF. Erythropoietin is produced primarily in the kidneys, precisely within the renal cortex and outer medulla by peritubular fibroblasts. In patients with HF, renal blood flow (RBF) is reduced, which can increase the production of erythropoietin due to low partial pressure of oxygen, which activates the inducer of the erythropoietin gene transcription, hypoxia-inducible factor-1α (36). The increase of erythropoietin levels correlates with the severity of HF (37).

Angiotensin receptor blockers and angiotensin-converting enzyme inhibitors are commonly used drugs in HF treatment. Angiotensin II (AII) reduces RBF and increases oxygen demand, which stimulates erythropoietin production. Secondly, AII stimulates bone marrow progenitor cell production directly. As both of the previously named drugs inhibit the availability of AII, they cause a modest reduction in hemoglobin by reducing the production of erythropoietin and erythroid progenitors, as well as by blocking the disintegration of the hematopoiesis inhibitor N-acetyl-seryl-aspartyl-lysyl-proline (38).

Androne et al. (39) reported that nearly 50% of the patients introduced for cardiac transplant had hemodilution-induced pseudo-

anemia, despite having normal plasma volume. Hemoglobin analysis shows true anemia, determined by the volume of red blood cells, in the majority of the anemic HF patients (40).

Another major factor for anemia in HF patients is the proinflammatory state they are found to be in. As discussed before, proinflammatory cytokines such as but not limited to tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), and C-reactive protein are increased in HF. IL-6 and TNF- α activate GATA binding factor 2 (a protein that binds GATA sequence in target gene promoters) and nuclear factor-κB, which act as inhibitors of erythropoietin production (41). Furthermore, IL-6 stimulates hepatocytes for increasing hepcidin synthesis and as a result, blocks duodenal iron uptake (42). By the same token, IL-6 reduces the expression of ferroportin, and therefore, iron release into the bloodstream is prevented (42).

As HF can cause anemia, it appears that the mechanism also works the other way around. In another study, when hemoglobin was elevated up to 5.5 g/dL with erythropoietin in patients with moderate anemia and CKD, cardiac output, and left ventricular ejection fraction (LVEF) shortening decreased progressively and anti-proportionally to the hemoglobin increase (43). Thus, the evidence argues that increasing hemoglobin levels in heart failure with reduced ejection fraction (HFrEF) patients can be associated with reduced LVEF, boosted left ventricle afterload, and extended vascular resistance, which would explain the inverse association of hemoglobin and LVEF (31, 44, 45).

A meta-analysis of 33 studies, involving more than 150.000 patients with HF, showed that anemia increased the relative risk of death by 100% (46). On the other hand, it is unlikely that there is a linear association between hemoglobin level and mortality. A majority of the increased risk arises at low hemoglobin (47, 48). A J-shaped relation between hemoglobin and mortality rate has been shown in the normal population, as well as in patients with acute coronary syndrome, coronary heart disease, and HF (49-51). The hemoglobin range of 13 to 16 g/dL provides the lowest mortality risk, with increased risk below or above this interval.

As already discussed, the pathomechanism of anemia as a comorbidity in HF patients is multifunctional. The hemodynamic, renal, and neurohormonal alterations are triggered when the oxygen delivered to metabolizing tissues is decreased. This leads to elevated workload in myocardial cells, which later on may cause diverse left ventricular remodeling and hypertrophy of the myocardium (30, 52).

Within 148 patients with stable chronic HF, Opasich et al. (31) determined a specific cause of anemia in 43%, an iron deficiency in 5%, and insufficient erythropoietin production, proinflammatory cytokine activation, or defective iron utilization notwithstanding adequate iron storage indicating anemia of chronic disease with functional iron deficiency in 57% of all patients. Regardless of the anemic status, iron deficiency is a comorbidity in HF, which cannot be ignored. Iron deficiency anemia results in a decreased number of red blood cells (24). Therefore, one may tire easier than normal and show poor resilience.

According to previous studies HF patients are often iron deficient. Current guidelines of the European Society of Cardiology recommend iron supplementation in HFrEF patients for the improvement of symptoms (53). Clinical trials of intravenous iron supplementation in HF patients usually define ID as having a ferritin level between 100-300 μg/L with transferrin saturation (TSAT) <20% or ferritin level less than 100 μg/L, and these trials have demonstrated improvement in symptoms and functional outcomes (54).

OVERVIEW OF CLINICAL TRIALS

Therapy with erythropoiesis-stimulating agents

The Reduction of Events with Darbepoetin alfa in Heart Failure (RED-HF) trial was conducted to investigate erythropoiesis-stimulating agents' (ESAs) usage on anemic patients with HFrEF. The study was published in 2013 (55). Previous to the RED-HF trial, 13 smaller studies investigated the potential benefits of ESAs. As reported in the meta-analysis by Kotecha et al. (56), ESAs led to improvement of symptoms, improved 6-minute walk distance (6MWD), improvement in New York Heart Association (NYHA) class, QoL, and an increase in hemoglobin by 2 g/dL. Furthermore, erythropoiesis-stimulating agent therapy was shown to reduce HF-related hospitalization (56). However, the RED-HF trial, being a pivotal double-blind placebo-controlled trial, did not support the promising results of these 13 small studies. In the RED-HF trial, the non-placebo group received darbepoetin alpha to increase and maintain the hemoglobin level of 13 g/dL (57). The trial had a median follow-up time of 28 months and thereafter darbepoetin usage resulted in no benefits concerning primary outcomes, such as mortality rate, from any cause, and hospitalization for worsening HF. Compared to the placebo group, darbepoetin increased the hemoglobin level by around 1.5 g/dL throughout the trial and therefore a marginal improvement of QoL was seen in the darbepoetin alfa group. On the other hand, in the darbepoetin alfa group, a statistically significant increase in the occurrence of thrombotic and embolic events was seen (p=0.01) (57).

Oral iron therapy

Since the RED-HF trial demonstrated that treating anemia by increasing hemoglobin levels in HFrEF patients did not significantly improve the prognosis of HF, the focus shifted to replacing the deficient iron. The oral iron replacement was first investigated in a study by Silverberg et al. (58). Iron Repletion Effects on Oxygen Uptake in Heart Failure trial, being a phase 2 double-blind randomized controlled trial, is the biggest study, which investigated the outcomes of oral iron preparations on HF patients. Within the trial, the non-placebo group received oral iron polysaccharide 150 mg twice daily (59). After 16 weeks of follow-up in the non-placebo group, there were no statistically significant changes neither in the primary endpoint (peak VO2) nor in the secondary endpoints (6MWD, QoL, etc.) (60). These findings were not coherent with the findings of intravenous iron trials on similar populations (61). The reason for non-beneficial outcomes of oral iron preparations is suspected to be elevated hepcidin levels of patients with HF, which then inhibits the absorption of iron in enterocytes by reducing transmembrane ferroportin. As mentioned above, reduced transmembrane ferroportin limits the transport of iron from enterocytes to blood (3).

Intravenous iron therapy

Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure (FAIR-HF) trial was the first large trial in which the outcomes of intravenous iron preparations on patients with HFrEF were investigated. The trial was conducted between 2007 and 2008 and the results were published in 2009 (62). A total of 459 patients with NYHA class II or III, LVEF less than 45%, and ID (ferritin level between 100-300 μg/L with TSAT <20% or ferritin less than 100 μg/L) were enrolled into the study and randomized 2:1 into the non-placebo and placebo groups (63). In the correction phase, the non-placebo group received weekly ferric carboxy-malt-

ose (FCM), the equivalent of 200 mg of iron. In the maintenance phase (8 or 12 weeks after the beginning), the frequency was reduced to every 4 weeks with the same amount of FCM until the end of the study (63). In terms of primary endpoints, at week 24, one-half of the patients in the FCM group were found to be much or moderately improved according to the Patient Global Assessment (PGA), whereas only 28% of the patients in the placebo group found to be as such. There was a reduction in NYHA class in the FCM group when compared to the placebo group, which was also statistically significant ($p<0.001$). The improvements of the FCM group were regardless of potential anemia (62).

The Ferric Carboxymaltose Evaluation on Performance in patients with Iron Deficiency in Combination with Chronic Heart Failure (CONFIRM-HF) trial was conducted between 2011 and 2013 and the results were published in 2015 (64). The design of the trial showed similarities to FAIR-HF, but the FCM doses were higher (the equivalent of 500-1000 mg of iron), therapy duration (52 weeks) was longer, and randomization was done in a 1:1 ratio in 304 participating patients (65). In terms of the primary endpoint of the CONFIRM-HF, patients in the FCM group at week 24 and onwards had an improved 6MWD, when compared to the placebo group ($p>0.01$). There were also improvements in the secondary endpoints (NYHA class, QoL, PGA) (64). Furthermore, CONFIRM-HF indicated that FCM therapy might cause a reduction in the risk of hospitalization due to worsening HF. This finding has once again shifted the focus from symptomatic improvement to clinical improvements such as but not limited to hospitalization for any reason or due to cardiovascular events and mortality rate.

After CONFIRM-HF, in 2017, the results of the Effect of Ferric Carboxymaltose on Exercise Capacity in Patients With Iron Deficiency and Chronic Heart Failure trial were published (66). The inclusion criteria were nearly identical when compared with other past trials, additionally including hemoglobin levels lower than 15 g/dL, brain natriuretic peptide greater than 100pg/mL or N-terminal-pro hormone brain natriuretic peptide greater than 400pg/mL, and peak VO2 of 10 to 20 mL.kg-1.min-1 .172 patients were enrolled in the trial and were randomized in 1:1 ratio into FCM and placebo groups. The FCM group received the same dose (the equivalent of 500-1000 mg of iron) as in the CONFIRM-HF trial for 24 weeks. The primary endpoint changes in peak VO2 levels were investigated, which showed no significant improvement in the FCM group. In terms of secondary endpoints, PGA and NYHA class, there were significant improvements in the FCM group ($p<0.05$ for both results) (66).

Most recently in late 2020, the results of a Randomized, Double-Blind Placebo-Controlled Trial Comparing the Effect of Intravenous Ferric Carboxymaltose on Hospitalizations and Mortality in Iron Deficient Subjects Admitted for Acute Heart Failure (AFFIRM-AHF) trial were published (67). The trial had the highest sample size (n=1132) compared to the other FCM in HF trials. In this particular trial, eligible patients were hospitalized for acute HF with accompanying ID (ferritin levels lower than 100 μg/L, or between 100-299 μg/L with transferrin saturation <20%) and LVEF of less than 50%. Patients were randomized before their hospital discharge 1:1 into FCM or placebo groups. The FCM group was given intravenous FCM according to their weight and ID status for 24 weeks, which was equivalent to 500-1000 mg of iron in each dose (68). The patients were also followed up after their hospital discharge with no longer than 6 week-periods. The AFFIRM-AHF trial used the rate of recurrent HF hospitalizations and cardiovascular mortality up to 52 weeks after randomization as its primary outcomes. During the follow-ups for 52 weeks, a total of 293 (52.5%) primary outcome events occurred in the FCM group, whereas 372 (67.6%) events were recorded in the placebo group (67). Although this was only borderline significant ($p=0.059$), the findings are very promising for the FCM therapy, since the trial showed that fewer days were lost due to HF hospitalization in the FCM group (369 days per 100 patient-years) compared to the placebo group (548 days per 100 patient-years). Furthermore, there was no significant difference between the two groups about cardiovascular death (67).

As the trials were conducted, meta-analyses using individual patient data of several FCM in HF trials were reported. Anker et al. (61) published a meta-analysis in 2018, which was based on 4 randomized control trials (RCTs) (FER-CARS-01, FAIR-HF, EFFICACY-HF, and CONFIRM-HF). In their meta-analysis, they focused on the clinical outcomes such as but not limited to recurrent cardiovascular hospitalizations and cardiovascular death. The data of a total of 839 patients were examined. When compared with the placebo groups, patients in FCM groups had a lower rate of recurrent cardiovascular or HF hospitalization, and cardiovascular mortality. Moreover, there was no increase in adverse events associated with the FCM groups (61). In a recent meta-analysis by Khan et al. (69), the data of AFFIRM-AHF was examined along with the 4 RCTs included in the meta-analysis of Anker et al. (61). Khan et al. (69) included a total of 1947 patients from 5 RCTs and the outcomes of interests were HF hospitalizations, all-cause mortality, and cardiovascular mortality. The findings of this meta-analysis showed that FCM therapy, when compared to the placebo groups, statistically significantly reduced the time to first heart failure hospitalization or death for cardiovascular causes (hazard ratio= 0.76; 95% confidence interval (CI)= 0.63-0.90; I2 =555). In addition, FCM therapy was indicated to reduce the risk of recurrent HF and cardiovascular hospitalization. On the other hand, there was no significant reduction in all-cause (odds ratio (OR)= 0.97; 95% CI= 0.73-1.28; I2 =0%) or cardiovascular (OR= 0.93; 95% CI= 0.69-1.27; I2= 0%) mortality rates (69).

The currently ongoing trial Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure 2 intends to investigate the effect of intravenous FCM in HFrEF patients, taking the rate of recurrent hospitalizations due to heart failure and cardiovascular death during follow-up as its primary endpoints. The trial is planned to have a total of circa 200 patients, who had present chronic HF for at least 12 months from the initial diagnosis (70).

CONCLUSION

With iron being a crucial biometal complex in homeostasis, iron deficiency is a common finding in HF patients regardless of their anemia status. HF patients with ID show more severe symptoms and have an impaired prognosis. Throughout the last 20 years, several studies evaluated the role of iron supplementation in HF. They demonstrated that intravenous iron therapy in HF patients is associated with improved exercise capability, QoL, and NYHA class. The beneficial effects of intravenous iron therapy on hospitalization and mortality rate are also shown partially in several trials, but further trials and studies are needed to have a better understanding of this aspect of the topic.

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ASSESSMENT OF THE AWARENESS AND OPINIONS OF TURKISH MEDICAL STUDENTS TOWARDS VIOLENCE AGAINST WOMEN: A QUESTIONNAIRE-BASED STUDY

Fatih Erkan Akay[1], Ilgın Kılıç[2], Nur Gülce İşkan[1], Begüm Söyleyici[1], Ece Şenyiğit[1], Gülsüm Önal[3]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]University of Liverpool School of Medicine, Liverpool, ENGLAND
[3]Department of History of Medicine and Medical Ethics, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: This study aims to evaluate the opinions of medical students about violence against women, the education they receive during medical school regarding violence against women, and how they would manage a case of violence victims if they were to encounter it in their medical careers. ***Methods:*** In this questionnaire-based study, 610 medical students from 37 medical schools were approached, and their opinions towards violence against women were evaluated through an online questionnaire. Demographic data such as age and gender were also gathered. Students were grouped according to their gender (male and female) and study levels; 1st, 2nd, and 3rd grades were grouped as pre-clinical, whereas students in 4th, 5th, and 6th grades were grouped as clinical. In comparing parametric variables, the Student's t-test was used, whereas, in non-parametric variables, the Mann-Whitney U test was used. The Chi-square test was used in the comparison of categorical variables. ***Results:*** There was a statistically significant difference within both groups (gender and study levels) for questions 5 (Which of the following would you describe as violence? [Multiple answers are allowed.]) and 13 (Do you think female physicians are exposed to violence more?). Our results also demonstrate a statistically significant difference for questions 15 (Is it obligatory for a physician to keep a legal report for a female patient who has been admitted to the emergency department as a victim of violence), 16 (It is not obligatory for a physician to keep a legal report for a female patient who has been admitted to the emergency department as a victim of violence without her consent.), and 17 (A physician is not obliged to complete the physical examination or continue the diagnosis-treatment process of a female patient who has been a subject of violence without her consent.) between pre-clinical and clinical groups, where the clinical group gave the most correct answers. ***Conclusion:*** In conclusion, our results gave us a perspective that clinical students' awareness and management of violence against women is higher than pre-clinical students'. These results may be attributed to two factors; lectures on violence against women or the experience students attain during their clinical practices. Female students were keener in participating in our study. Further prospective studies with equal gender distribution investigating the possible impact of lectures about violence against women on physicians' management of similar situations are needed.
Keywords: Violence, medical students, awareness

INTRODUCTION

Violence against women (VAW) has been an ongoing issue in the world and has been accepted as a form of human rights violation in the last two decades (1). The United Nations defines VAW as an act of "gender-based violence that results in or is likely to result in, physical, sexual, or mental harm or suffering to women, including threats of such acts, coercion or arbitrary deprivation of liberty, whether occurring in public or private life" (2). VAW is the primary cause of female mortality and morbidity, affecting 35% of women globally (3). VAW is present in every country in the world, and it significantly affects Turkey (1). According to a questionnaire study conducted in Turkey in 2009, 42% of women that are 16 to 60 years old have experienced a form of physical or sexual violence by their partners, most commonly by their husbands (4). Similarly, according to a more recent study conducted by Hacettepe University in 2014, it was found that four women out of ten in Turkey have been subjected to physical violence by their partners, and nearly half of these victims would classify the physical violence they have received as "severe" (5). The study shows that 44% of women in Turkey have been subjected to emotional violence/abuse by their partners (5). When asked, 43% of the female study participants stated that "If a woman does not share the same opinion about something with their partner, she should not argue and stay silent", which shows the effects of physical, sexual, and emotional VAW and how this can affect women's thoughts towards their equality in relationships (5).

With the emergence of the coronavirus disease 2019 (COVID-19) pandemic and most families having to stay at home, VAW has increased significantly (6). Many countries including France and Cyprus have reported a 30% increase in VAW cases since the beginning of the countries' national lockdowns (6). This increase can go up to 50% in some countries, like Argentina (6). A study conducted in Turkey shows that VAW has increased by 27.8% in Turkey since the beginning of the COVID-19 pandemic (7).

Address for Correspondence: Fatih Erkan Akay, Trakya University School of Medicine, Edirne, TURKEY
e-mail: erkanfatih48@gmail.com
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ORCID iDs of the authors: FEA: 0000-0001-7598-1016; IK: 0000-0001-7393-7839; NGİ: 0000-0001-6924-2532; BS: 0000-0001-6888-623X; EŞ: 0000-0002-0108-976X; GÖ: 0000-0002-1981-6611.

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With the increasing cases of VAW and femicides, which is defined by the World Health Organization (WHO) as the murder of a woman, the importance of the treaties that aim to prevent VAW have significantly increased (8). Examples of these treaties are the "Universal Declaration of Human Rights" adopted in 1948, "The Convention on the Elimination of All Forms of Discrimination Against Women" adopted in 1979, "Inter-American Convention on the Prevention, Punishment, and Eradication of Violence against Women" in 1994, and the most recent human rights treaty for the prevention of VAW, "The Council of Europe Convention on preventing and combating VAW and domestic violence", also known as the Istanbul Convention (9). Initially, Turkey was the first country to ratify the treaty on 14 March 2012 (10). In March 2021, the Turkish government announced Turkey's withdrawal from the Istanbul Convention (11).

Medical doctors play a vital role in the management of VAW, as most of the time, they are the first contacts for the victims of violence, and appropriate care and management is expected (12). VAW being recognized in a hospital setting gives rise to the opportunity for femicide prevention and appropriate management with therapeutic or conservative measures (13). However, the recognition of violence in victims can be challenging due to a lack of violence-related training in medical curriculums in medical schools across the globe (14). A study conducted in the United Kingdom shows that only 25% of medical schools provide teaching on sexual assault in their undergraduate medical curriculum (15). This lack of education in medical schools can cause less sensitive approaches towards gender-based crimes and sexual violence and can affect future doctors' abilities to manage such a situation (15). Thus, this affects the care that VAW victims get in hospital settings (15). Including educational sessions that focus on the key points of care for sexual violence and VAW victims, and addressing any potential myths about VAW may help medical students to better manage these types of patients (16).

The aim of this questionnaire-based study is to evaluate the opinions of medical students about VAW, the education they receive on VAW, and how they would manage a case of a victim of violence if they were to encounter it in their medical careers.

MATERIAL AND METHODS

This study was approved by the Scientific Research Ethics Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2021/167). In this questionnaire-based study, 610 medical students from 37 different medical schools were approached and their opinions towards VAW were evaluated through an online questionnaire using Google Forms in the Turkish language. Demographic data including age, gender, and medical school grades were obtained. Students were grouped according to their gender (male and female) and study levels; 1st, 2nd, and 3rd grades were grouped as pre-clinical whereas students in 4th, 5th, and 6th grades were grouped as clinical (Table 1). Students could select multiple answers for questions 5, 8, 10, 12, 14, and 20.

The answers given to the questionnaire were compared using SPSS, version 23.0.0. Numbers, percentages, mean, standard deviation, median, and interquartile range (IQR) were used as descriptive statistics. In question 5, 1 point was given in each selection and a total score was calculated for the analysis. The Shapiro-Wilk test was used to determine whether the data were distributed normally or non-normally. Answers given to questions 5, 6, 9, 11, 15, 16, 17, 18, and 19 were compared between pre-clinical and clinical groups. Answers given to questions 5 and 13 were compared between genders. Answers given to other questions were given as numbers and percentages. In the comparison of parametric variables, Student's t-test was used whereas, in non-parametric variables, Mann-Whitney U test was used. In the comparison of categorical variables, the Chi-square test was used. P-value of <0.05 was considered to be statistically significant. Students were not obliged to answer all the questions; hence some of the results may have varied between each other.

RESULTS

A total of 610 medical students from 37 medical schools in Turkey participated in this questionnaire-based study. Demographic data comprised of 412 (67.55%) female, 174 (28.53%) male, 1 (0.16%) non-binary students with 20 (3.27%) not specifying their gender. The median age was 21 years (range: 17-29). Other demographic data involving grade and its division into clinical and pre-clinical years are summarized in Table 2.

There was a statistically significant difference within both groups (gender and clinical/pre-clinical) for question 5 "How many of the following would you define as 'violence'?" ($p<0.001$) (Table 3). The distribution of answers chosen is shown in Figure 1 with 'get beaten' 608 (99.8%) as the most selected option. There was a statistically significant difference found between male and female students for question 13 'Do you think women healthcare workers are exposed to more violence?' ($p<0.001$).

Between the true or false questions regarding the approach towards a patient who is a victim of violence; question 15 'Is it obligatory for a physician to keep a legal report for a female patient who has been admitted to the emergency department as a victim of violence?', question 16 'It is not obligatory for a physician to keep a legal report for a female patient who has been admitted to the emergency department as a victim of violence without her consent,' and question 17 'A physician is not obliged to complete physical examination or continue the diagnosis-treatment process of a female patient who has been a subject of violence without her consent,' were statistically significant between the pre-clinical and clinical groups (Table 4) where clinical students gave the most correct answers. The p-value for questions 15, 16 and 17 are 0.018, 0.007, and 0.050, respectively.

Two-hundred forty-three (98%) students considered psychological/mental violence as an emergency that should be sought medical help for. Followed sequentially by physical [n=238 (96%)], and sexual violence [n=235 (94.8%)], and economic [n=65 (26.2%)]. and cyberviolence [n=65 (26.2%)]. The most selected answer [n=549 (90.4%)] for defining the origin of violence was "social gender constructs". Followed sequentially by "neurochemical and psychiatric factors" [n=405 (66.7%)], "hormonal" [n=128 (21.1%)] and "genetic factors" [n=119 (19.6%)]. It should be noted that multiple answers could be selected in these two questions. The distribution of answers for questions 8, 10, and 14 are shown in Figures 2, 3, and 4, respectively.

Table 1: The questionnaire used in the study.

	Questions	*Answers*
1	*Your age?*	
2	*How would you describe your gender?*	Male / Female / Do not want to specify / Other
3	*Name of your medical school.*	
4	*What is your grade?*	Preparatory year / 1 / 2 / 3 / 4 / 5 / 6
5	*Which of the following would you describe as violence? (Multiple answers are allowed.)*	To belittle / Demand obedience / Exposure to aggressive behaviors / Jealousy-Suspicion / Force into sexual acts / To punch / To say 'ugly' / To exploit service / Condemn-Criticize / Quick-temperedness / Get threatened / Force to take care of the children / Get beaten / Inhibit or hinder economic independence / Verbal abuse / Physical abuse / Confiscate partner's money / Disallow to work
6	*What is the most up-to-date international document on combating violence against women and domestic violence? (Open-ended question)*	
7	*Do you think that there should be lectures on violence against women in the curriculum of medical schools?*	Yes / No
8	*If your answer to question 7 is 'Yes', please specify the reason. (Multiple answers are allowed.)*	It is a common problem / Violence against women, domestic violence and neglect have a significant effect on health and society / It is needed for clinicians to be aware of, recognize, understand, and seek help for violence against women, domestic violence, and abuse / It is needed to enable clinicians to provide an appropriate approach and management to women and those affected by domestic violence or abuse
9	*Do you think the lectures on violence against women in the curriculum of medical schools are sufficient?*	Yes / No
10	*If your answer to question 9 is 'No', please specify the reason. (Multiple answers are allowed.)*	There is not enough time / It is not seen as a medical problem / It was only recently decided as an important issue to consider and cover in medical school / This issue can be undertaken in other faculties
11	*Do you think violence is a medical problem?*	Yes / No / Depends on the type of violence
12	*If your answer to question 11 is 'depends on the type of the violence', which type of violence, victims should seek for help? (Multiple answers are allowed.)*	Physical violence / Psychological-emotional violence / Sexual violence / Economic violence / Cyberviolence
13	*Do you think female physicians are exposed to violence more?*	Yes / No
14	*What would you do as a doctor if a female patient who has been subjected to violence is admitted to your hospital? (Multiple answers are allowed.)*	I would notify judicial authorities like prosecutors, law enforcement officers, etc. / I would notify administrative authorities such as the chief physician, local health authorities / I would notify institutions and non-governmental organizations that have violence support lines available / I would call the hospital social services / I do not know what to do
15	*Is it obligatory for a physician to keep a legal report for a female patient who has been admitted to the emergency department as a victim of violence?*	Yes / No
16	*It is not obligatory for a physician to keep a legal report for a female patient who has been admitted to the emergency department as a victim of violence without her consent.*	True / False
17	*A physician is not obliged to complete the physical examination or continue the diagnosis-treatment process of a female patient who has been a subject of violence without her consent.*	True / False
18	*If the initiation of the judicial process would cause harm to the patient who has been subjected to violence, the notification may be postponed until the patient's health conditions become appropriate.*	True / False
19	*Do you think physicians play a role in preventing violence against women?*	Yes / No
20	*How would you describe the origin of violence against women? (Multiple answers are allowed.)*	Genetic factors / Hormonal factors / Neurochemical-psychiatry problems / Social gender constructs

Table 2: Summary of the demographic data of the participants.

	n (%)
Gender	
Female	412 (67.55)
Male	174 (28.53)
Non-binary	1 (0.16)
Prefer not to say	20 (3.27)
Missing data	3 (0.49)
Age (years)*	21 (3)
Grade	
1	154 (25.24)
2	90 (14.75)
3	175 (28.68)
4	60 (9.88)
5	55 (9.0)
6	71 (11.64)
Missing data	5 (0.81)
Study level	
Pre-clinical	419 (68.7)
Clinical	186 (30.4)
Missing data	5 (0.81)
Total	610 (100)

**Non-parametric values were given as median (IQR).*

Table 3: Comparison of answers given by female versus male study groups.

Question Number	*Female*	*Male*	*P-value*
5*	18 (2)	16.50 (4)	***<0.001***
13*			***<0.001***
Yes	333 (80.8)	91 (52.3)	
No	79 (19.2)	82 (47.1)	
Missing data	0	1 (0.6)	

**Data is expressed as median (interquartile range).*
***Data is expressed as number and percentages.*
Statistically significant values are marked in bold.

Table 4: Comparison of the answers given by clinical versus pre-clinical study groups.

Question Number	*Pre-clinical Students' Answers*	*Clinical Students' Answers*	*P-value*
5*	17 (2)	18 (2)	***0.004***
6**			0.507
True	215 (51.3)	90 (48.4)	
False	204 (48.7)	96 (51.6)	
9**			0.588
Yes	394 (94)	172 (92.5)	
No	25 (6.0)	14 (7.5)	
11**			0.559
Yes	269 (64.2)	127 (68.3)	
No	16 (3.8)	5 (2.7)	
Depends on the type of violence	134 (32.0)	54 (29.0)	
15**			***0.018***
Yes	319 (83.3)	166 (90.7)	
No	64 (16.7)	17 (9.3)	
16**			***0.007***
True	155 (40.4)	51 (28.7)	
False	229 (59.6)	127 (71.3)	
17**			***0.050***
True	260 (67.7)	135 (75.8)	
False	124 (32.3)	43 (24.2)	
18**			0.067
True	289 (74.9)	118 (67.4)	
False	97(25.1)	57 (32.6)	
19**			0.417
Yes	307 (75.2)	129 (72.1)	
No	101 (24.8)	50 (27.9)	

**Data is expressed as median (IQR).*
***Data is expressed as number and percentages.*
Statistically significant values are marked in bold.

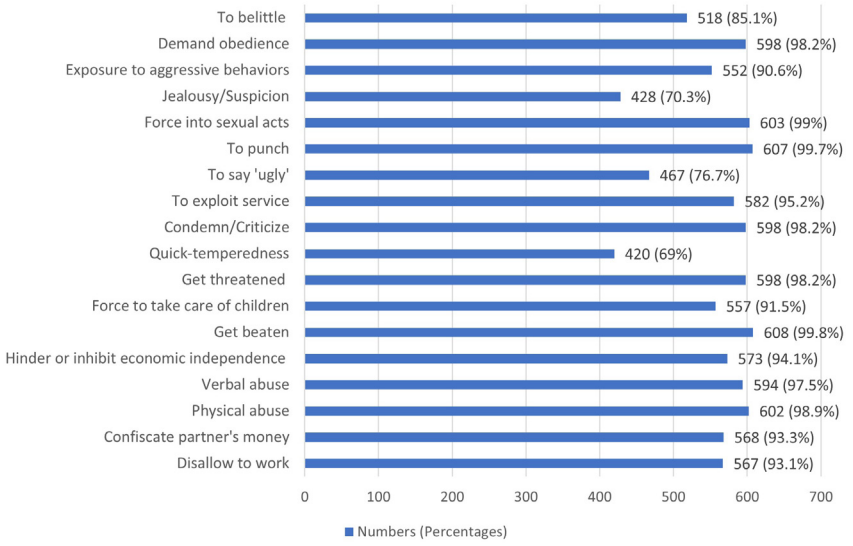

Figure 1: Distribution of answers chosen for question 5 "How many of the following would you define as 'violence'?".

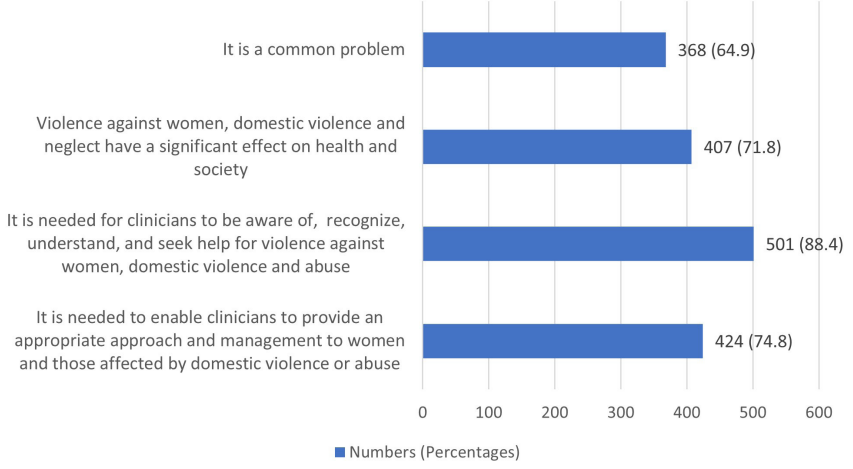

Figure 2: Distribution of answers selected for question 8 "If your answer to question 7 is 'Yes', please specify the reason. (Multiple answers are allowed.)".

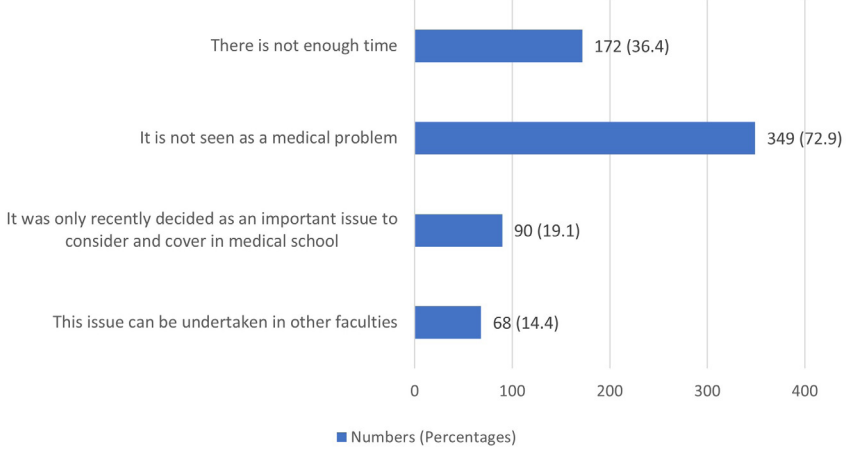

Figure 3: Distribution of answers selected for question 10 "If your answer to question 9 is 'No', please specify the reason. (Multiple answers are allowed.)".

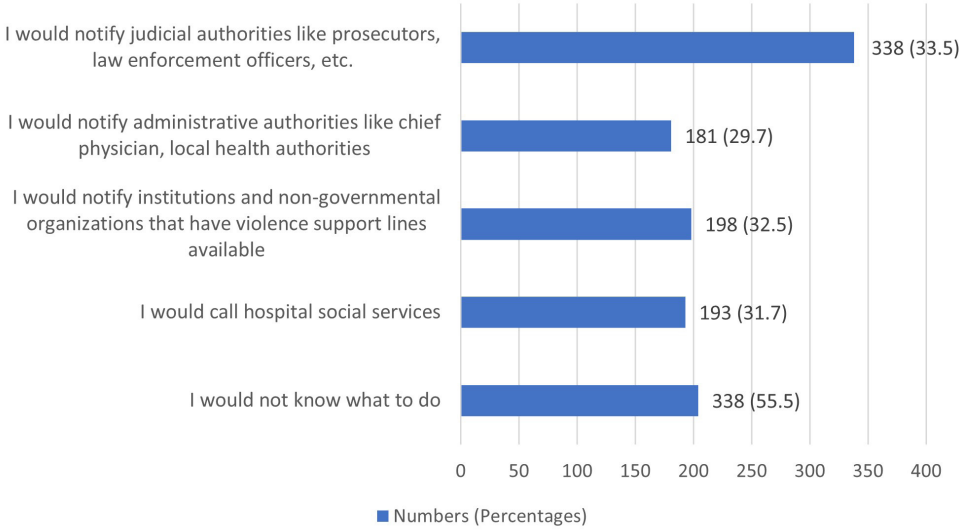

Figure 4: Distribution of answers selected for question 14 "What would you do as a doctor if a female patient who has been subjected to violence is admitted to your hospital? (Multiple answers are allowed.)".

DISCUSSION

Violence against women is a significant public health problem and a violation of human rights. Estimates published by WHO indicate that about 30% of women worldwide have been subjected to either physical and/or sexual intimate partner violence or non-partner sexual violence in their lifetime (17). Thus, having sufficient education about VAW in the medical school undergraduate curriculum plays a cardinal role in medical students to recognize and manage victims of violence (18). Therefore, our study aimed to evaluate the attitude of medical students towards VAW and the education they receive on it, and how they would manage VAW cases.

In a study conducted by Ernst et al. (18), students were given a general-knowledge survey about VAW before and one month after 3 hours lecture about VAW. They demonstrated a statistically significant difference between male and female students, indicating a lack of knowledge about VAW in the male student population. In this manner, our study also resulted in a statistically significant difference between male and female students when asked to define violence. However, since female students [412 (67.7%)] constituted most of our study population, it could have rendered a biased nature in our results. Two other studies, with equal distribution of male and female participants, similarly showed a less sensitive approach in male student groups towards rape victims (19, 20). William et al.'s (19) questionnaire-based study about rape myths revealed how male students were more likely to accept such myths. In our study, 603 (99%) of students defined "forcing into sexual acts" as a type of violence, exhibiting an overall awareness concerning this topic.

Looking at question 5 from a different perspective, there was a statistically significant difference found between pre-clinical and clinical students, in which clinical students had a higher score in defining violence, indicating that lectures throughout clinical curriculums increase awareness towards VAW. Similarly, Kennedy et al. (16) and Ernst et al. (18) have shown that courses about violence significantly improve the survey results of medical students. This is especially important for future doctors who will embark on their careers. In this regard, when the students were asked whether they think lectures on VAW should be included in their curriculum, 568 (93.3%) of the participants answered, "Yes" with the most agreeable reason being "It is needed for clinicians to be aware of, recognize, understand, and seek help for violence against women, domestic violence, and abuse" [n=510 (88.4%)]. According to the study conducted by Potter et al. (21), nearly 90% of medical students have stated that providing a formal education about VAW should be mandatory in medical schools. In the mentioned study, the most selected answer for the question "Why do you think there should be formal teaching on domestic violence and abuse in the curriculum?" was "Violence against women is a common, hidden, and important problem."

A study conducted by Manuel et al. (14) indicates that the undergraduate curriculums in medical schools focus more on diseases and treatments rather than managing potential complex effects of some conditions, including psychological manifestations. In a similar study, Çalıkoglu et al. (22) reported that less than half of their participants [173 (48.5%)] felt competent in recognizing and managing VAW victims. Likewise, our study shows how 204 (33.4%) participants felt unsure and did not know how to manage a situation where they would encounter a female patient who was a VAW subject. Following the questionnaire, 500 (83.6%) students believe that the available courses in their medical school curriculum on VAW are insufficient, expressing their reasoning by selecting the answer "It is not seen as a medical problem" the most [349 (73.9%)]. Although 398 (65.4%) participants consider VAW a medical problem, WHO reports VAW as a universal issue, an entity that needs to be grasped and taught more in medical schools (10).

Even though there was a statistically significant difference demonstrated in male [n=91 (56.9%)] and female [n=333 (80.8%)] students for question 13 "Do you think female physicians are exposed to violence more?", it has been reported that male physicians are subjects of physical [n=65 (45.8%)] and verbal [n=132 (93%)] violence more than female physicians (23). However, Demirci et al.'s (24) results demonstrated how female physicians are more prone to verbal/sexual violence. Our results indicate how female medical students are more concerned about working in the field as future doctors.

When asked whether physicians play a role in preventing VAW, 438 (74.2%) students answered, yes. Further questioning their actions in a possible situation, 487 (85.6%) students stated that they

would report the given situation to legal authorities. The reason why a high percentage of students selected these answers might be to evade potential legal consequences that they might face. However, this might contradict the "non-maleficence" principle of medicine since the victim's safety should come first and legal action might sometimes result in harm rather than a benefit. Moreover, the answer given for question 14 demonstrates a perception of healthcare workers playing an active role in preventing VAW. This demeanor may also lead to averting femicides after all, which is the greatest endpoint violence can get.

Case-based management of students based on their study levels was evaluated in our study. There was a statistical significance found for questions 15, 16, and 17, where students in the clinical group showed a higher percentage in answering the questions correctly. These results provide that having taken lectures on VAW; medical students attain a broader perspective in managing similar cases. In a study done in Ireland, Kennedy et al. (16) evaluated attitudes of medical students towards victims of sexual violence. They found that the educational curriculum significantly increases undergraduate medical students' awareness when it comes to issues involving patient care (16).

Taking a closer look at answers given for question 20, it is evident that there still lies a major misconception defining the origin of violence. Despite 549 (90.4%) students selecting the option for the cause of violence as "social gender constructs", 405 (66.7%) students picked the answer "neurochemical/psychiatric problems" as a factor for violent behavior as well, representing a misunderstanding which leads to the justification of problems that are not related with VAW. 128 (21.1%) students have also chosen the answer "hormonal factors" while 119 (19.6%) students chose "genetic factors" to define the origin of violence. In addition to theoretical views revealing violence as a learned and adaptive behavior, studies have been focusing on trying to uncover its pathophysiological background through a neurochemical and neurophysiological objective (25). The focal point has been the neurochemistry and genetics of serotonin and dopamine. The emergence of aggressive behavior is associated with a faulty gene that controls the production and transportation of serotonin, or defective genes that prohibit serotonin bind to its receptor (25). DNA polymorphisms are found to be closely related to aggressive behaviors. (26). The best-known DNA polymorphism is in the tryptophan hydroxylase (TPH) gene (25). TPH is the enzyme responsible for accelerating serotonin synthesis (26). Low levels of serotonin catabolites were detected in the cerebrospinal fluid of people exhibiting aggressive behavior with different alleles of this gene (26). Furthermore, the incidence of aggressive behaviors in psychiatric patients was found not to be different from "normal" population groups. Nonetheless, it is challenging to elucidate the origin of violence with a single reason since it has a multifactorial background (25).

Our limitation in this questionnaire-based study was the unequal distribution of participants in study groups. Our study population primarily consisted of female students, which could have led to biased answers. Further studies with an increased number of participants and equal distribution of numbers in groups could result in more accurate findings.

In conclusion, our results gave us a perspective that clinical students' awareness and management of VAW is higher than pre-clinical students'. These results may be attributed to two factors; lectures on VAW or the experience students attain during their clinical practices. Yet, many studies show that education may significantly impact students' awareness about VAW. As previously stated, female students were keener on participating in our study. This demonstrates a dichotomy between gender and interest in issues like VAW. Further prospective studies, with equal gender distributions, investigating the possible impact lectures on VAW can have on physicians' management of similar situations are needed.

Ethics Committee Approval: This study was approved by the Scientific Research Ethics Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2021/167).
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Author Contributions: Concept: FEA, IK, NGİ, BS, EŞ, GÖ. Design: FEA, IK, NGİ, BS, EŞ, GÖ. Supervision: GÖ. Resources: FEA, IK, NGİ, BS, EŞ, GÖ. Materials: FEA, IK, NGİ, BS, EŞ, GÖ. Data collection and/or processing: FEA, IK, NGİ, BS, EŞ, GÖ. Analysis and/or Interpretation: FEA, IK, NGİ, BS, GÖ. Literature Search: FEA, IK, NGİ, BS, EŞ, GÖ. Writing Manuscript: FEA, IK, NGİ, BS, EŞ, GÖ. Critical Review: FEA, IK, NGİ, BS, EŞ, GÖ.
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ANALYSIS OF CLINICAL RELATIONSHIP OF VISUAL ACUITY WITH OPTICAL COHERENCE TOMOGRAPHY AND PERIMETRY PARAMETERS IN PRIMARY OPEN-ANGLE GLAUCOMA

Mustafa Ömer İzzettinoğlu[1], Vuslat Gürlü[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Ophthalmology, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: This study aims to analyze the clinical relationship of visual acuity with optical coherence tomography and perimetry in primary open-angle glaucoma. ***Methods:*** This retrospective cross-sectional study was conducted with patients who were diagnosed with primary open-angle glaucoma in the ophthalmology department of Trakya University School of Medicine between January 2010 and January 2021. Perimetric data of patients such as visual field index, mean defect, pattern standard deviation, short-term fluctuation, corrected pattern standard deviation; results of optical coherence tomography such as average retinal nerve fiber layer thickness, average C/D ratio, and thickness of retinal nerve fiber layer quadrants, and records of examinational findings were statistically tested to evaluate the statistical relationship. Numbers, percentages, mean and standard deviation were used as the descriptive statistics. ***Results:*** Initially 80 eyes of 49 patients diagnosed with primary open-angle glaucoma met the inclusion criteria. 22 patients were female and 27 patients were male. Thinner mean retinal nerve fiber layer, inferior and superior quadrants were observed in eyes with lower visual acuity. It was observed that eyes with lower visual acuity had a worse visual field index and mean defect. ***Conclusion:*** In tertiary clinics that receive various numbers of patient referrals, perimetry can be seen as more useful and accurate in primary open-angle glaucoma detection and monitoring. It provides us with better and more accurate results for glaucoma management in the later stages. It is recommended that both optical coherence tomography and perimetry be used for disease monitoring, as this allows doctors to better monitor disease progression. In addition, since optical coherence tomography is an objective test and is less likely to require a patient response, it should be kept in mind that advanced and severe glaucoma may occur when performing perimetry, especially in patients with poor cooperation. ***Keywords:*** Glaucoma, perimetry, optical coherence tomography, visual field, epidemiology

INTRODUCTION

Primary open-angle glaucoma (POAG) is a chronic, degenerative optic neuropathy that causes selective loss of retinal ganglion cells, and is one of the leading causes of blindness worldwide. Even though POAG often occurs with elevated intraocular pressure (IOP), many POAG cases were reported to progress with a normal range of IOP values (1, 2). The condition of retinal ganglion cell loss leads to progressive enlargement of the optic nerve cup and thinning of the neural rim (3). Glaucoma is the leading cause of blindness, seen in 60 million people around the world (3). According to the survey data of 2013, POAG accounts for 44 million people across the world and is estimated to hit 53 million people by the year 2020 (4). According to recent studies in the United Kingdom, POAG is reported to be the most common type of glaucoma (5). Studies suggest that POAG is the second most common cause of blindness in the United States, approximately affecting 2.5 million people (6).

Optic disc findings frequently are detected before the deficit of the visual field appears. An enlarged cup-to-disc (C/D) ratio greater than 0.5, C/D ratio asymmetry of 0.2 or more between two eyes or a high asymmetry of the cup in one eye are considered to be diagnostic findings. The destructions caused by glaucoma are clinically revealed by OCT and perimetry tests (6). Perimetry, also known as visual field testing, is a crucial diagnostic tool that maps out the visual field of the patient which makes it necessary for the diagnosis and management of POAG (7). Typical visual field changes in POAG include asymmetry of visual field between two eyes, temporal wedge, paracentral scotoma, general constriction of peripheral field, and increased variability of responses in an area which later developed field defects (7). The perimetry threshold for the detection of visual loss is estimated to reliably stand at 40-50% ganglion cell loss. Thus, in POAG, typical optic nerve head structural changes occur earlier than functional change (visual field loss) (7). It should be noted that there is the phase of characteristic glaucomatous changes and increased tendency of damages to the retinal nerve fiber layer (RNFL) that comes before the perimetrical defects are detectable (7).

This retrospective study aims to analyze the clinical relationship of best-corrected visual acuity (BCVA) with optical coherence tomography and perimetry on patients with POAG in the ophthalmology department of Trakya University School of Medicine.

MATERIAL AND METHODS

This study was approved by the Scientific Research Ethics Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2021/104). This retrospective cross-sectional study analyzed 80 eyes of 49 patients who were diagnosed with POAG

Address for Correspondence: Mustafa Ömer İzzettinoğlu, Trakya University School of Medicine, Edirne, TURKEY
e-mail: omer.iz@icloud.com
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ORCID iDs of the authors: MÖİ: 0000-0001-6736-7294; VG: 0000-0001-5650-2075.

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in the Ophthalmology Department of Trakya University Hospital between January 2010 and January 2021. The study was carried out under the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Demographic data such as age and gender, accompanying systemic comorbidities such as hypertension and diabetes, risk factors such as high IOP levels, and the duration of onset POAG, the usage of topical prostaglandins, beta-blockers, carbonic anhydrase inhibitors and alpha agonists, initial and following clinical findings were obtained from the medical records of the patients. All patients underwent complete ophthalmologic examination at each visit including BCVA determined by the Snellen chart, anterior segment biomicroscopic examination, IOP measurement with Goldmann applanation tonometer, and detailed fundus examination obtained with 78 diopters non-contact lens.

The following findings were documented at the clinical visits: visual field parameters such as VFI, mean deviation, pattern standard deviation, optical coherence tomography (OCT) results such as mean RNFL thickness, mean C/D ratio, thicknesses of RNFL quadrants, surgical procedures that the patients underwent, the usage of topical anti-glaucomatous agents and types of anti-glaucomatous agents that have been used.

Initially, 80 eyes of 49 patients diagnosed with POAG met the inclusion criteria of having BCVA with positive light perception and above, absence of corneal pathologies, ocular-vascular diseases (central retinal artery occlusion, retinal venous occlusion), accompanying macular pathologies such as a macular hole and macular degeneration. POAG patients who have poorer BCVA than positive light perception, onset corneal pathologies, ocular-vascular diseases, and macular pathologies which could cause unreliable fundus examination results were excluded from this cross-sectional study.

Perimetric data of patients such as visual field index (VFI), mean defect (MD), pattern standard deviation (PSD), short-term fluctuation (SF), corrected pattern standard deviation (CPSD); results of OCT such as mean RNFL thickness, mean C/D ratio, and thickness of RNFL quadrants, and records of examinational findings were statistically tested to evaluate the statistical relationship.

Numbers, percentages, mean and standard deviation were used as the descriptive statistics. The variables were tested for normal distribution by the One-sample Kolmogorov-Smirnov test. The Chi-square test was used for qualitative comparison. Quantitative data were compared with the Independent Sample t-Test. The bivariate Pearson Correlation test was performed to measure the strength and direction of linear relationships between pairs of continuous variables. A p-value of <0.05 was set for statistical significance. The data were analyzed with IBM SPSS version 20.

Table 1: Patient characteristics.

	Number of Patients [n (%)]
Age (years)*	63.1 ± 10.2 (43-84)
Duration of onset glaucoma (months)*	55.5 ± 45.3 (1-204)
Gender	
Female	22 (44.9)
Male	27 (55.1)
Affected eye	
Right	40 (50.0)
Left	40 (50.0)
Comorbidity	
Hypertension	15 (18.8)
Diabetes Mellitus	12 (16.3)
Hyperthyroidism	2 (2.5)
None	49 (59.9)

*Data were expressed as mean ± SD (min-max).

Table 2: Examinational findings.

	Number of Patients [n (%)]
BCVA (Snellen Unit)*	0.86 ± 0.26 (0.0080-1.0)
IOP (mm Hg)*	16.3 ± 3.5 (6-23)
Biomicroscopic findings	
Cataract	3 (3.9)
Nepheline	2 (2.5)
Nevus Filament	1 (1.3)
Pterygium	2 (2.5)
Biomicroscopic C/D ratio*	0.6 ± 0.3 (0.1-1.0)
Lens statement	
Phakic	60 (75)
Pseudophakic	20 (25)

***BCVA:** Best corrected visual acuity, **IOP:** Intraocular pressure, **C/D:** Cup-to-disc*
*Data were expressed as mean ± standard deviation (min-max).

RESULTS

In our study, 22 (44.9%) patients were female and 27 (55.1%) patients were male. The summary of patients' characteristics (mean age, duration of onset glaucoma, gender, accompanying comorbidity, and the number of the right or left eye that was affected) is shown in Table 1.

The mean BCVA for all eyes was 0.86 ± 0.26 Snellen decimals (ranging from 0.0080 to 1.0 Snellen decimals). It was found that bigger C/D ratios due to fundus examination led to eyes in worsened BCVA (p=0.018, r=-0.263, Pearson Correlation Test). Mean IOP was observed to be 16.3 ± 3.5 mm Hg (ranging from 6 to 23 mm Hg) and was not observed to have a noticeable correlation with BCVA (p=0.228). The summary of examinational findings (BCVA, IOP, biomicroscopic findings, lens status, and biomicroscopic C/D ratio) was shown in Table 2.

In terms of perimetry and OCT results, the mean VFI was revealed as 74.2 ± 34.6 % (ranging from 0 to 100%), and the mean thickness of RNFL was found out to be 76.9 ± 17.5 μm. The summary of perimetry and OCT results (VFI, MD, PSD, SF, CPSD, mean C/D ratio, mean RNFL thickness) is shown in Table 3.

When mean C/D ratio and other quadrants of RNFL were observed, mean C/D ratio, nasal and temporal quadrants did not have a significant correlation (p=0.082 for mean C/D ratio, p=0.237 for nasal quadrant, p=0.211 for temporal quadrant). The inferior quadrant thickness had a negative correlation with age (p=0.01, r=-0.273).

The mean thickness of the RNFL and BCVA had a statistically significant linear relationship (p<0.001, r=0.418). Thinner RNFL was also observed in eyes with lower BCVA, indicating that they are positively correlated. VFI and BCVA also had a statistically significant linear relationship (p=0.001, r=0.441). It was observed that eyes with lower BCVA had worsened VFI. Additionally, MD had a statistically significant linear relationship with BCVA (p<0.001, r=0.545). RNFL inferior quadrant (p<0.001, r=0.457) and RNFL superior quadrant (p=0.006, r=0.303) had a moderate positive correlation with BCVA. All of the correlations are presented in Table 4 and Table 5.

The percentages of anti-glaucomatous agents' usage were observed to be 67.5% for prostaglandins, 68.8% for beta-blockers, 61.3% for carbonic anhydrase inhibitors, and 47.5% for alpha agonists. Regarding the operative approach, 11 (13.8%) eyes underwent trabeculectomy, 8 (10.0%) eyes underwent phacoemulsification cataract extraction and 1 (1.3%) eye underwent vitrectomy.

Table 3: Perimetry and optical coherence tomography results.

	Mean ± SD (min-max)
Perimetry	
VFI (%)	74.2 ± 34.6 (0 – 100)
MD (dB)	-9.6 ± 9.1 (-30.9 – 1.1)
PSD (dB)	5.6 ± 3.1 (1.5 – 14.4)
SF (dB)	2.0 ± 1.0 (0 – 5.7)
CPSD (dB)	4.8 ± 3.3 (0 – 14.1)
Optical Coherence Tomography	
Mean C/D Ratio	0.7 ± 0.2 (0.1 – 1.1)
Mean RNFL Thickness (µm)	76.9 ± 17.5 (40 – 115)

VFI: *Visual field index,* ***MD:*** *Mean defect,* ***PSD:*** *Pattern standard deviation,* ***SF:*** *Short-term fluctuation,* ***CPSD:*** *Corrected pattern standard deviation,* ***C/D:*** *Cup-to-disc,* ***RNFL:*** *Retinal nerve fiber layer*

Table 4: The strength and direction of linear relationships between pairs of continuous variables.

	Age		***Duration of POAG***		***BB usage***		***CAI usage***		***Prostaglandin usage***		***AA usage***	
Continuous variables	r	P-value	r	P-value	z	P-value	z	P-value	z	P-value	z	P-value
VFI	-0.141	0.213	0.084	0.0460	-0.526	0.599	-1.510	0.131	-0.927	0.354	-2.531	0.011
MD	-0.165	0.144	0.117	0.300	-0.706	0.480	-1.639	0.101	-0.801	0.423	-2.895	0.004
PSD	-0.118	0.299	-0.002	0.98	-0.140	0.889	-0.523	0.601	-1.998	0.046	-1.600	0.110
The mean thickness of RNFL	-0.176	0.118	0.157	0.165	-0.706	0.480	-0.835	0.404	-1.937	0.053	-2.008	0.003
I Quadrant	-0.273	0.014	0.044	0.696	-0.457	0.648	-0.790	0.429	-1.264	0.206	-2.082	0.037
S Quadrant	-0.122	0.282	0.110	0.333	-0.280	0.779	-0.741	0.131	-1.408	0.159	-3.011	0.003
N Quadrant	-0.185	0.101	-0.048	0.672	-0.336	0.736	-0.702	0.483	-1.347	0.178	-1.572	0.116
T Quadrant	-0.207	0.066	-0.040	0.725	-0.519	0.480	-0.672	0.502	-0.457	0.647	-0.800	0.424
Mean C/D Ratio	-0.135	0.233	0.113	0.319	-1.288	0.198	-1.655	0.098	-0.915	0.360	-4.583	<0.001

VFI: *Visual field index,* ***MD:*** *Mean defect,* ***PSD:*** *Pattern standard deviation,* ***RNFL:*** *Retinal nerve fiber layer,* ***I Quadrant:*** *Inferior quadrant,* ***S Quadrant:*** *Superior quadrant,* ***N quadrant:*** *Nasal quadrant,* ***T Quadrant:*** *Temporal quadrant,* ***BB:*** *Beta blocker,* ***AA:*** *Alpha agonist,* ***CAI:*** *Carbonic anhydrase inhibitor*

Table 5: The strength and direction of linear relationships between pairs of continuous variables.

	BCVA	
Continuous variables	r	P-value
VFI	0.441	0.001
MD	0.545	<0.001
The mean thickness of RNFL	0.418	<0.001
I Quadrant	0.547	<0.001
S Quadrant	0.303	0.006
N Quadrant	134	0.237
T Quadrant	0.141	0.211
Mean C/D Ratio	-0.196	0.082
Biomicroscopic C/D Ratio	-0.263	0.018
IOP	0.136	0.228

BCVA: *Best-corrected visual acuity,* ***r:*** *Pearson correlation coefficient,* ***VFI:*** *Visual field index,* ***MD:*** *Mean defect,* ***RNFL:*** *Retinal nerve fiber layer,* ***I quadrant:*** *Inferior quadrant,* ***S quadrant:*** *Superior quadrant,* ***N quadrant:*** *Nasal quadrant,* ***T quadrant:*** *Temporal quadrant,* ***C/D:*** *Cup-to-disc,* ***IOP:*** *Intraocular pressure*

DISCUSSION

Primary open-angle glaucoma is an asymptomatic, progressive, and degenerative optic neuropathy characterized by enlarging optic disc and loss of visual field. Patients at high risk for POAG include black people older than 40 years, white people older than 65 years, and people with a family history of glaucoma or people with a history of diabetes or severe myopia (-6.00 diopters or more, i.e., farther from 0.00) (6). In our study, the mean age of people with POAG was 63.1 years, many other studies confirming an older age prevalence as well (1, 6, 8-10). It was also seen that hypertension and diabetes were two of the major risk factors for POAG. Other studies with different ethnicities have revealed that blood pressure changes and onset diabetes played a major role in increasing the risk of POAG (11, 12). Therefore, adequate management of blood pressure, blood sugar levels, and HbA1c are crucial for decreasing the risk of POAG.

In our study, mean BCVA results were seen as 0.86, with mean IOP being in the normal range. Our study did not reveal a statistically significant linear relationship between IOP and BCVA. The fact that POAG could occur and progress with normal IOP should not be overlooked. The study with 229 eyes conducted by Omodaka et al. (13) confirmed that IOP had a weak correlation with BCVA,

which is in line with the results of our study. According to the study of Leung et al. (14), better BCVA is more correlated with normal RNFL thickness, especially with the thickness of the temporal quadrant RNFL. On the contrary, our study revealed the temporal quadrant of RNFL to not have a correlation with BCVA, unlike superior and inferior quadrants which have strong correlations with BCVA. This situation could be explained by the earlier and more destructive effects of POAG on inferior and superior quadrants. In addition, it was seen that older patients had thinner inferior RNFL quadrant. It is believed to occur due to the age-related changes in the retinal layer of the globe. Moreover, the mean thickness of the RNFL was within the normal range in our study, which could explain the reason for the better BCVA results.

Visual field loss is also one of the other causes of POAG. Peripheral vision loss is usually the initial defect and is followed by central vision loss (blindness) in POAG. Perimetry should be done in patients with POAG to detect visual field defects. Perimetry is the gold standard investigation tool in which defects are detectable when 40-50% loss of ganglion cells occurs. Therefore, it is believed that structural tests can identify progression in the early stages better while perimetry is more useful in the later stages (15-20). Thus, it is believed that perimetry is more relevant in the later stages of POAG (18). On the contrary, OCT detects even the slightest defects on the optic disc head which gives a higher detection chance of disease progression in the early stages and is still indeed a handy tool for POAG and examination of worsened BCVA. Nonetheless, the fact that moderate BCVA could be gotten despite OCT results even with minimal defects should be noted (7). Therefore, examination of peripapillary RNFL thickness will provide a better chance of detecting early glaucoma than that of perimetry (14, 18). In our study, it was observed that perimetry parameters were more accurate in POAG than the results of OCT in which VFI and MD had better strength to reveal the defects than the parameters of OCT such as mean RNFL thickness, superior and inferior quadrants of RNFL. These results could be attributed to the fact that the patient group in our study was referred to our tertiary clinic from another one. Additionally, the study of Banegas et al. (16) supported the functionality of OCT in progression detection of pre-perimetric glaucoma, while perimetry is more reliable in later stages. Given these facts, we believe that worsened BCVA is better explained with perimetry in the tertiary clinics.

The main limitation of our study is that our clinic is considered a tertiary center which meant that the majority of our patients were already on anti-glaucomatous medications at the time of their referral, indicating that their POAG was relatively under control. This left us with better parameters of POAG such as BCVA, IOP, RNFL thickness, C/D ratio, which were used for investigation and management.

In conclusion, OCT has higher sensitivity in the detection of progression than perimetry in the early stages, which gives OCT the ability of progression detection within a shorter follow-up time in early POAG. On the other hand, perimetry provides us better and more accurate results for POAG management in the later stages. Therefore, our study has revealed that in tertiary clinics that receive various numbers of patient referrals, perimetry is more useful and accurate in POAG detection and monitoring. Using both OCT and perimetry for disease monitoring is advisable, as this provides us better disease progression tracking with a better chance than using one method without the other.

Ethics Committee Approval: This study was approved by the Scientific Research Ethics Committee of Trakya University School of Medicine (Protocol Code: TÜTF-BAEK 2021/104).
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TMSJ
TURKISH MEDICAL STUDENT JOURNAL

THE INVESTIGATION OF MEDICAL STUDENT JOURNALS

Fatih Erkan Akay[1], Beliz Koçyiğit[1], Berfin Tan[1], Ceren Yüksel[1], Eylül Şenödeyici[1], Elif Çalışkan[1], Janset Özdemir[1], Pınar Tuncer[1], Necdet Süt[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Biostatistics and Informatics, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: The aim of our study is to examine and evaluate data of medical student journals from around the world for the year 2020. ***Methods:*** In this observational study 20 medical student journals were examined. Data analyzed comprised of the year of foundation, country of origin, number of issues per year, types of articles published, and the total number of articles published in a year. Issues that were published in 2020 only were taken into consideration. ***Results:*** In 2020, the majority of medical student journals were based in the United States of America, followed by Canada. There were only four indexed (Scopus, Science Citation Index Expanded, PubMed) medical student journals; three were from the United States of America, and one from Canada. In comparison with other journals, the Yale Journal of Biology and Medicine had the most published issues. They also have the most crowded editorial board. Overall, the median of people on the editorial boards and advisory boards were 17.5 and 14, respectively. The median for issues per year was 2. The median number of publications in medical student journals in 2020 was 23. Among them, 13.1% were original research articles, 10.8% review articles, 9.4% case reports, 4.9% editorials, and 2.9% letters to the editor. The remaining 59.0% were publications that fell into the "others" category. ***Conclusion:*** In conclusion, the majority of medical student journals are based in North America and Europe, and a substantial amount of the published articles falls into the "others" category. Considering the lack of effective guidance and regulations with relevance to indexing. Medical student journals face certain challenges regarding visibility, accessibility, and publishing articles. However, provided that the editors of medical student journals remain keen, motivated, and focused, medical student journals will contribute to the scientific community by creating a supportive and intellectual environment for aspiring researchers where they can enhance their understanding of scientific research and publishing skills. ***Keywords:*** Medical students, medical student journal, publications

INTRODUCTION

Medical students played a significant role throughout the development of basic and clinical sciences in history. In recent years, there has been an increase in schools encouraging their students to carry out scientific research, which sets a prerequisite for their graduation (1). Students may attain knowledge about critical reading and evidence-based medicine through these compulsory and voluntary studies (2, 3).

Medical student journals (MSJs) are establishments arising from the desire of medical students to create platforms for other students to share their articles and findings (3). MSJs can also set examples by encouraging students to conduct and publish scientific research themselves. Therefore, student journals are seen as a unifying element between young researchers and the scientific world.

There are MSJs reported from all around the world. Some are run and published by editorial boards that exclusively consist of medical students (2, 4). The scope and the working principles of these journals vary within each other. While some of them publish only articles related to health and medicine, some have hybrid content where art and culture-related articles are published as well. Their primary purpose is the dissemination of knowledge gained through scholarly work. Another contribution of MSJs is to close the gap between clinical and experimental studies in the publication field (5).

Medical student journals' role and possible effect are often discussed as being prime examples of medical students' interests in research or developing research abilities (6, 7). The first issue of a medical student journal (MSJ) was published in 1923 (2). Since then, the number of journals has been increasing over the past years, and the best-observed leap has been in the last fifteen years (2). Although most of these journals are not indexed in MEDLINE, the peer-review process conducted by students is as stringent as any peer-review carried out by faculty members (4, 8).

A recent study described that students who publish works in MSJs during their undergraduate years are more likely to succeed in their future careers (7). Moreover, graduating medical students will enroll in future research activities with a more meticulous demeanor if guided properly by adequate role models during their undergraduate studies (9). MSJs could provide the necessary motivation and guidance to undergraduate students by being a stepping stone to the field of publication and research. The aim of this study is to examine MSJs from the year 2020 around the world.

MATERIAL AND METHODS

In this observational study, the data collection was made by searching the term "medical student journal" on search engines like Google© (www.google.com), Yahoo! (www.yahoo.com), and Bing (www.bing.com), and on bibliometric databases such as

Address for Correspondence: Fatih Erkan Akay, Trakya University School of Medicine, Edirne, TURKEY
e-mail: erkanfatih48@gmail.com
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Available at https://tmsj.trakya.edu.tr

ORCID iDs of the authors: FEA: 0000-0001-7598-1016; BK: 0000-0001-6056-0219; BT: 0000-0002-9256-7631; CY: 0000-0003-2456-7282; EŞ: 0000-0002-4132-1594; EÇ: 0000-0003-4556-8698; JÖ: 0000-0001-7774-5068; PT: 0000-0001-6778-9868; NS: 0000-0001-6678-482X.

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Web of Science, PubMed, and Scopus. The term "medical student journal" refers to journals that publish actively and periodically, whose editorial boards consist mostly or exclusively of medical students. 23 medical student journals were included after this initial data screening. Due to lack of data, 3 of them were attempted to be contacted via email but no response was received. They were excluded from the study due to insufficient data.

Data used for the classification of the journals were the year of foundation, country of origin, number of issues per year, types of articles published, number of articles per issue, and the total number of articles published in a year. Issues that were published only in 2020 were taken into consideration while analyzing the types and numbers of articles. Data concerning the editorial board included the number of editors in the board and the number of professors in the editorial advisory board.

Percentages, mean and standard deviation were used as the descriptive statistics. The number of issues per year, the number of people on the editorial board, the number of people on the editorial advisory board, publication in 2020, and the total number of publications in 2020 were presented as mean, standard deviation, minimum and maximum, median and interquartile range. Distribution of publications in journals by article types and the overall number of articles published in journals are presented as numbers and percentages. Along with the general characteristics of journals, issue per journal, the number of people in the editorial board, the number of people in the editorial advisory board, and the total number of publications in 2020 were also presented as percentages. A p-value <0.05 was set for statistical significance. The data were analyzed with IBM SPSS version 23.0.

RESULTS

In this study, twenty MSJs were evaluated based on their publications in the year 2020. Our study shows an increase in MSJs considering there were only four in the 1900s, three of them being in Canada and one in the United States of America (USA). Therefore, the number of MSJs considerably increased in the 2000s (Figure 1)

The summary of journals' characteristics (years of foundation, country, indexes, issues per year, number of people on the editorial and editorial advisory board) are presented in Table 1. The majority of MSJs were from the USA, followed by Canada. Overall, 80% of journals were from North America and Europe. There were only six MSJs indexed in medical indexes, four were from the USA, and the other two were from Canada and Turkey. 70% of the journals were not indexed. In comparison with other journals, the Yale Journal of Biology and Medicine had the most published issues in the year 2020. They also had the most crowded editorial board.

Overall, the median number of people on the editorial and editorial advisory boards were 14 and 18.3, respectively. The median number of yearly publications was 2 issues, with most of the journals publishing 2 issues per year. Table 2 shows statistics of the journals' issues per year, the number of people on the editorial board, the number of people on the editorial advisory board, and the total number of publications in 2020.

Table 3 shows the number of journals' publications and publication type's percentage distributions in 2020, with the International Journal of Medical Students having the most publications (82 publications). With just 5 publications per year, American Medical Student Research Journal published the fewest. The type of publications published by the journals varied according to their "aims and scope". The median number of publications in MSJs in 2020 was 23. Among them, 13.1% were original research articles, 10.8% review articles, 9.4% case reports, 4.9% editorials, and 2.9% letters to the editor. The remaining 59.0% were publications that fell into the "others" category. The "others" category comprised of interviews, approaches to patient perspective, fine arts, reflections, short communications, news, symposium pieces, clinical images, book reviews, profiles, clinical reports, solving statics, teachable moments, ethics challenge, feature articles, research spotlights, clinical practices, staff reviews, abstracts, emerging trends, focuses on medical humanities, research news, technical reports, creative writing pieces, quizzes, discussion starters, sound pieces, education reflections, visual pieces (digital effects), poetry, viewpoint, images in medicine, picture quizzes, study resources, and eye spy. Medical Student Research Journal was excluded from this table due to inadequate information.

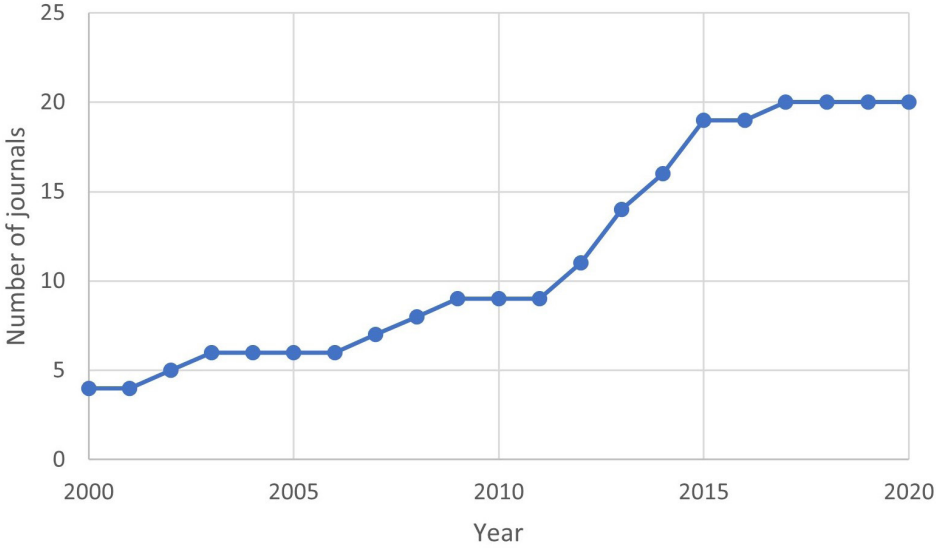

Figure 1: Number of journals in the last 20 years.

Table 1: Summary of journals' general characteristics.

Name	*Year of foundation*	*Country*	*Indexed in*	*Issue per year (n)*	*People on the editorial board (n)*	*People on the editorial advisory board (n)*
University of Toronto Medical Journal	1923	Canada	Scopus	3	49	10
Yale Journal of Biology and Medicine	1928	USA	Scopus / SCIE	5	78	15
Dalhousie Medical Journal	1936	Canada	N/A	2	15	N/A
University of British Columbia Medical Journal	1962	Canada	N/A	2	N/A	N/A
New Zealand Medical Student Journal	2002	New Zealand	N/A	2	15	5
McMaster University Medical Journal	2003	Canada	N/A	1	13	N/A
Medical Student Research Journal	2007	USA	N/A	3	20	1
Royal College of Surgeons in Ireland Student Medicine Journal	2008	Ireland	N/A	1	N/A	N/A
Australian Medical Student Journal	2009	Australia	N/A	2	30	22
University College Dublin Medical Student Journal	2012	Ireland	N/A	1	10	N/A
Journal of Asian Medical Students Association	2012	Asian-Pacific Region	N/A	2	14	3
Medical Student Press Journal	2013	USA	N/A	2	10	2
International Journal of Medical Students	2013	USA	BASE / Bibliotheca Alexandrina Information for Africa / DOAJ / EZB / HINARI / ICMJE / IMBIOMED / J Gate / Journal Guide / Journal Seek Database / OCLC WorldCat / Publons / Pubshub / Research Bible / The Open Access Digital Library / Ulrich's International Periodical Directory / Summon by Serial Solutions	3	63	N/A
American Medical Student Research Journal	2013	USA	N/A	1	12	38
Turkish Medical Student Journal	2014	Turkey	CABI: CAB Abstracts and Global Health / Türk Medline / Scilit	3	28	50
Harvard Medical Student Journal	2014	USA	PubMed	1	33	14
The McGill Journal of Medicine	2015	Canada	N/A	2	52	N/A
Amsterdam Medical Student Journal	2015	Netherlands	N/A	4	13	54
Florida Medical Student Review Journal	2015	USA	PubMed	1	20	18
British Student Doctor Journal	2017	UK	N/A	3	14	6

N/A: *Not available,* ***USA:*** *United States of America,* ***UK:*** *United Kingdom,* ***BASE:*** *Bielefeld Academic Search Engine,* ***CAB:*** *Center for Agriculture and Bioscience,* ***CABI:*** *Center for Agriculture and Bioscience International,* ***DOAJ:*** *Directory of Open Access Journals,* ***EZB:*** *Elektronische Zeitschriftenbibliothek,* ***HINARI:*** *Health InterNetwork,* ***ICMJE:*** *List of Publications that follow the International Committee of Medical Journal Editors,* ***IMBIOMED:*** *Mexican Index of Latin American Biomedical Journals,* ***J Gate:*** *The e-Journal Gateway,* ***OCLC:*** *Online Computer Library Center,* ***SCIE:*** *Science Citation Index Expanded*

Table 2: Descriptive statistics of the journals.

	Descriptive Statistics	
	Mean ± SD (min-max)	*Median (IQR)*
Issues per year	2.2 ± 1.1 (1-5)	2 (2)
People on the editorial board	27.2 ± 20.2 (10-78)	17.5 (24)
People on the editorial advisory board	18.3 ± 18.0 (1-54)	14 (26)
Total number of publications in 2020	29.5 ± 23.0 (5-82)	23 (30)

Table 3: The number of articles published in journals and their percentage distribution by type.

Name	Total number of publications in 2020 (n)	Original research article (%)	Review article (%)	Case report (%)	Editorial (%)	Letter to the editor (%)	Others (%)
University of Toronto Medical Journal	39	0	2.6	5.1	0	0	92.3
Yale Journal of Biology and Medicine	77	14.3	26.0	7.8	0	0	51.9
Dalhousie Medical Journal	21	0	9.5	0	9.5	0	81.0
University of British Columbia Medical Journal	32	0	15.6	3.1	6.3	0	75.0
New Zealand Medical Student Journal	56	0	0	0	19.6	0	80.4
McMaster University Medical Journal	14	21.4	21.4	28.6	7.1	0	21.4
Royal College of Surgeons in Ireland Student Medicine Journal	25	4.0	12.0	8.0	4.0	0	72.0
Australian Medical Student Journal	10	20.0	10.0	0	0	0	70.0
University College Dublin Medical Student Journal	10	0	10.0	0	0	0	90.0
Journal of Asian Medical Students Association	52	3.8	5.8	0	0	0	90.4
Medical Student Press Journal	7	14.3	0	0	0	14.3	71.4
International Journal of Medical Students	82	15.9	8.5	9.8	4.9	7.3	53.7
American Medical Student Research Journal	5	80.0	0	20.0	0	0	0
Turkish Medical Student Journal	23	47.8	26.1	17.4	0	8.7	0
Harvard Medical Student Journal	7	14.3	42.9	14.3	0	0	28.6
The McGill Journal of Medicine	27	0	0	0	7.4	0	92.6
Amsterdam Medical Student Journal	20	0	15.0	0	5.0	5.0	75.0
Florida Medical Student Review Journal	14	7.1	0	64.3	14.3	14.3	0
British Student Doctor Journal	40	5.0	0	0	15.0	5.0	75.0
Median (IQR)	23 (30)	5 (15.9)	9.5 (15.6)	3.1 (14.3)	4 (7.4)	0 (5)	72 (52.4)

DISCUSSION

Of the medical students who were involved in research and publications, 84% had intentions of continuing a research career during their lifetime, which is linked to long-term success in academia (5, 10, 11). The publication process is vastly competitive and time-consuming in leading peer-reviewed journals. This lengthy process is often topped with heart-breaking rejection which results in up to 70% of students' works remaining unpublished (7, 12). Considering the declining number of physician-scientists, a supportive and encouraging environment is needed, which may be filled by student-run journals (13). MSJs are of chief value to encourage and develop aspiring researchers. MSJs play a vital role in maintaining a heightened interest in research (14). Therefore, the parameters of these journals should be thoroughly investigated. We hereby and evaluate journals run by medical students in predetermined features.

The oldest MSJ found was the University of Toronto Medical Journal in 1923. The amount of MSJs showed a subtle increase from then until 2005, and an inclination happened after that year. Among all MSJs, 7 of them were based in the United States, 5 were from Canada, 5 were published in Europe, 2 were active in the Asia-Pacific region and 1 journal was based in Australia. Since Yasir et al.'s study (2) in 2016, only two new MSJ started publishing, which are Florida Medical Student Review Journal and British Student Doctor Journal. These values may indicate a higher need for more MSJs in some areas throughout the world, as they are platforms for visibility and bridges for medical students to the research field. Active and periodical publishing is a criterion that indicates the consistency of a journal, and all MSJs reviewed in this research had a pre-set amount of issues to be published annually. The MSJs evaluated in this study, published a median of 2 issues per year, at a maximum of 5 and a minimum of 1 issue. However, issues released in a year are not the sole parameter to determine a journal's activity as articles published in an issue differ from journal to journal.

Malin et al. (15) stated that researchers interacting, communicating between, and elaborating on each other's ideas were necessary to build a research community. In this manner, a median of 17.5 students took part in the editorial processing of MSJs. For example, University College Dublin Medical Student Journal was found to have the least number of students (n=10) on the editorial board, whereas the highest number (n=78) was in the Yale Journal of Biology and Medicine.

The number of articles published in the year 2020 displayed great variety. The median publication number was 23, with a minimum of 5 and a maximum of 82 articles. Only 13.1% of articles published were original articles, 10.8% were review articles, and 9.4% were case reports. These values clarify that there is room for improvement in MSJs in terms of original articles, review articles, and case reports published, possibly due to the lack of proper guidance and mentorship from the relevant researchers.

Meaningful mentorship and in-depth guidance are crucial in the academic career and progress of blooming researchers. A study by Angel-Isaza et al. (16) pointed out that physician-scientists were looked up to the most and taken as role models by 57% of their researcher population. Another study run by Bonilla-Escobar et al. (5) reported 30% of medical students engaged in research are demotivated by lack of mentorship. Of all 20 MSJs, 13 of them had an established advisory board comprising a median of 14 members. Journals that were indexed in an academic bibliographic database all had at least 10 members as their academic advisors. These data may indicate that the guidance of mentors may lead to MSJs which are higher reputed and better-known.

Being indexed in search engines is one of the most prominent challenges that MSJs face. Among all 20 journals that were included in the study, 2 were indexed in PubMed, 1 was indexed in Scopus, 1 was indexed in both Scopus and SCIE. The lack of indexing for the majority of MSJs negatively affects their visibility. Being highly selective, the terms of eligibility for indexing in such search engines often cannot be tailored to fit the needs of MSJs. This may be due to the lack of general knowledge among the scientific community about the hardships MSJs face, such as the constant changes in the editorial board line-ups due to the very nature of being students and the lack of expertise of the all-student editorial boards in relevant medical fields. Additionally, being few in numbers may have deprived MSJs of much-needed regulations about eligibility for indexing and being evaluated in a distinct category of MSJs, rather than being assessed amongst world-class scientific journals.

However, there were some limitations in our study. The journals were evaluated in a certain timeframe; therefore, the data may not present an overall look throughout the history of journals that have been discussed. The journals that do not take place on search engines (Google©, Yahoo!, and Bing) were excluded from the study. Medical student journals present on these engines and databases but lacking necessary data were also excluded from the study.

Subsequently, an increased number of undergraduate researchers and pre-trained scientists in the medical field may accelerate novel discoveries by reducing the amount of time spent grasping the principles of conducting scientific research after graduation. Although the first MSJ is nearly a century old, a substantial number of MSJs began their publishing life in the new millennium, with the pioneer being New Zealand Medical Student Journal. The increased steepness of this slope may be linked to medical students' rising awareness of the necessity of gaining experience in scientific research, and possibly the realization that once they graduate, they will be the successors of the current physician-scientists.

In conclusion, the majority of MSJs are based in North America and Europe, and a substantial amount of the published articles falls into the "others" category. Considering the lack of effective guidance and regulations with relevance to indexing, MSJs face certain challenges regarding visibility, accessibility, and publishing articles. However, provided that the editors of MSJs remain keen, motivated, and focused, MSJs will contribute to the scientific community by creating a supportive and intellectual environment for aspiring researchers where they can enhance their understanding of scientific research and publishing skills.

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LENGTH OF HOSPITAL STAYS OF PATIENTS OPERATED DUE TO GLIOMA TUMOURS IN NEUROSURGERY CLINICS AND INTENSIVE CARE UNITS

Irmak İrem Özyiğit[1], Fatih Erkan Akay[1], Elif Cengiz[1], Janset Özdemir[1], Pınar Tuncer[1], Eylül Şenödeyici[1], Sarper Kızılkaya[1], Ahmet Tolgay Akıncı[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Neurosurgery, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: To evaluate the relationship between the length of hospital stay and age, gender, and disease characteristics among glioma patients who went under surgery in Trakya University School of Medicine. ***Methods:*** The data of 51 glioma patients over 18 years of age, who have been followed up during 2019-2020 in Trakya University School of Medicine, Department of Neurosurgery were analyzed. Patients' data comprised of sex, age, tumour location, grades of the tumours, the presence of isocitrate dehydrogenase mutation, whether the patients were hospitalized in the intensive care unit or the neurosurgery clinic, duration of hospitalization, and whether radiotherapy and chemotherapy was received. Length of hospital stay was evaluated separately as intensive care unit and the neurosurgery clinic. ***Results:*** Out of 51 patients diagnosed with glioma, 18 (35.3%) were female, and 33 (64.7%) were male. The length of neurosurgery clinic and intensive care unit stays were not associated with radiotherapy and chemotherapy approaches. There was a statistically significant difference between the male and female patients in terms of the number of days stayed in the neurosurgery clinic. ***Conclusion:*** In conclusion, gender affected the length of neurosurgery clinic stays with a longer duration for female patients in our clinic. A waste number of parameters, including social ones, affect hospital stays. To reveal predictors of postoperative hospitalization thoroughly and overcome the study's limitations, further prospective studies with larger sample sizes are needed. ***Keywords:*** Glioma, hospitalization, isocitrate dehydrogenase, retrospective study

INTRODUCTION

Gliomas are the most frequent form of primary intracranial neoplasms, accounting for 81% of malignant brain neoplasms (1, 2). With an incidence rate of 6 per 100,000 individuals in the United States and a peak between the ages of 45-60 years, gliomas typically appear in adults (2, 3). Glioma is a general term that refers to neuroepithelial tumours that are mitotically active with a propensity to infiltrate diffusely, arising from supporting cells of the central nervous system (2, 4).

Based on microscopic similarities in their supposed cell origins, gliomas are classified into two subtypes: diffuse gliomas are the most common type characterized by broad infiltrative growth into the surrounding parenchyma and develop mainly in elderly patients (5). These gliomas, among which glioblastoma is the most common and deadliest representative, have been described as diffuse astrocytomas (5). The latter ones are non-diffuse gliomas, which are more circumscribed, considerably rarer, and develop mainly in younger patients (4, 5). Pilocytic astrocytoma and ependymomas are the most common varieties of this group (4, 5). Gliomas can also be classified based on their cell morphologies, mitotic activities, and molecular markers as low grade, high grade, and atypical (2).

The majority of gliomas are not inherited and occur spontaneously (3). Less than 5% of gliomas are considered hereditary (3, 4). Potential risk factors have been studied, and in most cases, the cause of the tumour is unknown (3). The only well-established risk factor for brain neoplasms is exposure to high or moderate ionising radiation doses (2, 4, 6). It has been shown that low-grade gliomas can progress into high-grade gliomas by changing their genetic makeup, probably due to their exposure to toxicokinetics (2). Furthermore, several studies suggest that having an atopic disease or allergy can decrease the risk of developing glioma (4, 6).

While gliomas can appear in all lobes of the brain, mostly in the frontal lobe (23.6% of gliomas), they can be seen in the brain stem, cerebellum, and spinal cord as well (4). Clinical manifestations depend on the localisation of the mass; however, the most common symptom is headache followed by seizures, nausea, vomiting, change in vision, tingling sensations, and difficulty in ambulation (2). The patients may be neurologically intact or show different degrees of focal weaknesses, sensory deficits, or altered mental status due to the mass effect of tumour growth in severe cases (2).

Clinical diagnosis is commonly achieved using computed tomography and magnetic resonance imaging scans (2, 7). Due to its poor prognosis, gliomas require urgent treatment, challenging both the physician and the patient (7, 8). Current standard treatments for

Address for Correspondence: Irmak İrem Özyiğit, Trakya University School of Medicine, Edirne, TURKEY
e-mail: iiozyigit@gmail.com
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ORCID iDs of the authors: İİÖ: 0000-0003-2443-0155; FEA: 0000-0001-7598-1016; EC: 0000-0002-5902-2904; JÖ: 0000-0001-7774-5068; PT: 0000-0001-6778-9868; EŞ: 0000-0002-4132-1594; SK: 0000-0002-7868-1585; ATA: 0000-0002-9937-076X.

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gliomas involve maximum surgical resection followed by chemotherapy (generally temozolomide) and radiotherapy (2, 7, 8). The treatment success depends on several factors including the time of diagnosis, new-onset or recurrence of the disease, the performance status, and the age of the patient (9). Treatment choice mainly depends on the patient's age as the elderly have lower survival rates and higher risks of toxicity (10).

Patients who undergo craniotomy for primary brain neoplasms are hospitalized, ranging from 4 to 10 days after surgery, and the median duration of stay for a single acute care visit is five days (11-14). The length of hospital stay depends on the postoperative complications and the patients' neurological conditions (13). Furthermore, most elderly patients spend their limited time hospitalized (14). Considering the heavy burden of the disease, the predictors of hospitalization in glioma patients are far from clear, and further research is recommended on factors affecting the length of hospital stay (4, 15).

This retrospective study aims to evaluate the relationship between the length of hospital stay and age, gender, and disease characteristics among glioma patients who went under surgery in Trakya University School of Medicine.

MATERIAL AND METHODS

The Institutional Research Ethics Committee approved this retrospective study (Protocol Code: TÜTF-BAEK 2021/169). The digital medical records of patients were screened. Our study group consisted of 51 glioma patients over 18 years of age, who have been followed up during 2019-2020 in Trakya University School of Medicine Department of Neurosurgery. Since all patients who suit the inclusion criteria in our center were included in the study, no sampling methods were used. Patients' data comprised of sex, age, tumour location, tumour grade according to the 2016 World Health Organization (WHO) Classification of Tumours of the Central Nervous System, presence of isocitrate dehydrogenase (IDH) mutation, whether the patients were hospitalized in the intensive care unit (ICU) or the neurosurgery clinic (NRC), duration of hospitalization, and whether radiotherapy, and chemotherapy were received.

Patients' ages at the date of admission were recorded in years. Length of hospital stay was evaluated separately for both in ICU and NRC. For categorical parameters, patients' sex, IDH status, tumour location, WHO grades of the tumours, radiotherapy and chemotherapy treatments were noted.

The data were analyzed using SPSS 23.0.0.0 program. A p-value of <0.05 was set for statistical significance. Numbers, percentages, median, and interquartile range (IQR) were used as the descriptive statistics. Shapiro-Wilk test was used to test the normality of variables. Mann-Whitney U test was used to assess non-parametric variables, which were expressed as "median (IQR)". Fisher-Freeman-Halton exact tests were used in the comparison of tumour locations and WHO grades between males and females.

Median values of length of stay in ICU and NRC were analyzed and compared between patients grouped according to their gender, IDH mutation status, chemotherapy, and radiotherapy parameters. Tumour locations and WHO grades of the tumours were analyzed and compared between female and male patients.

RESULTS

In this retrospective study, out of 51 patients diagnosed with glioma, 18 (35.3%) were female and 33 (64.7%) were male. The median (IQR) age of the patients at admission was 60 (15) years. The median (IQR) stay at NRC was 12 (10) days, while the median (IQR) stay at ICU was 2 (1) days.

Out of 51 patients, 8 (15.7%) patients received chemotherapy (2 patients received only chemotherapy, 6 patients received both treatments), and 32 (62.7%) patients received radiotherapy (26 patients received only radiotherapy, 6 patients received both treatments). 3 (5.9%) patients' data regarding chemotherapy were missing. Duration of NRC and ICU stays were shorter in the patients who received radiotherapy compared to patients who did not receive radiotherapy. Age and duration of ICU and NRC hospitalization parameters were not normally distributed.

Patients who received radiotherapy had no statistically significant difference in terms of NRC and ICU stays than the ones who did not receive radiotherapy (p= 0.184, p= 0.179, respectively). In a similar trend, patients who received radiotherapy stayed in the NRC and ICU for fewer days than the patients who did not receive radiotherapy. Furthermore, there was a significant difference between the male and female patients regarding the number of days stayed in NRC (p= 0.006). In our study, the IDH mutation marker was also examined and it was found that the majority (70.6%) of our patients had a negative IDH status. IDH negative patients stayed in ICU for a median of 12 days while IDH positive patients stayed in ICU for 2 days. The data regarding IDH status of 5 (9.8%) patients were missing. The summary of patients' statistics for the parameters of gender, radiotherapy, chemotherapy, and IDH are presented in Table 1.

Location-based classification showed that the parietal lobe of the brain was the most common location of glial tumours with a prevalence of 25.5%, in male patients. The patients were also classified according to the WHO classification. A summary of the statistical distribution of location and grade of the tumours with regards to gender is shown in Table 2.

Table 1: Summary of patients' statistics for gender, radiotherapy, chemotherapy, and IDH parameters.

	Patients [n (%)]	***Age* (years)***	***NRC* (days)***	***ICU* (days)***
Gender				
Female	18 (35.3)	58.5 (20.2)	16.5 (8.5)	2 (2)
Male	33 (64.7)	60 (13)	9 (7)	2 (0)
p-value		0.567	***0.006***	0.171
Radiotherapy				
Not received	19 (37.3)	55 (14)	15 (11.5)	2 (0.5)
Received	32 (62.7)	64.5 (12.5)	10.5 (8.3)	2 (2)
p-value		0.088	0.184	0.179
Chemotherapy				
Not received	40 (78.4)	58.5 (16.2)	12.5 (11.3)	2 (2.25)
Received	8 (15.7)	61.5 (12)	8 (5.75)	2 (0.5)
p-value		0.857	0.157	0.731
IDH mutation				
Negative	36 (70.6)	64.5 (13.5)	12 (8)	2 (0.5)
Positive	10 (19.6)	55.5 (5.5)	12.5 (13.75)	2 (3.25)
p-value		0.136	0.650	0.291
Total	51 (100.0)	60 (15)	12 (10)	2 (1)

IDH: *Isocitrate dehydrogenase,* ***NRC:*** *Neurosurgery clinic,*
ICU: *Intensive care unit*
**Data were expressed as "median (IQR)".*
Significant value is marked as bold.

Table 2: Distribution of location and grade (according to WHO classification of glial tumours of the brain) of the tumors by gender.

	Female [n (%)]	*Male [n (%)]*	*P-value*
Tumour locations			0.851
Frontal	6 (11.8)	7 (13.7)	
Temporal	5 (9.8)	8 (15.7)	
Parietal	6 (11.8)	13 (25.5)	
Occipital	1 (2.0)	4 (7.8)	
Cerebellar	0 (0.0)	1 (2.0)	
WHO grades			0.558
II	1 (2.0)	2 (3.9)	
III	2 (3.9)	1 (2.0)	
IV	12 (23.5)	24 (47.1)	
Missing	3 (5.9)	6 (11.8)	

WHO: *World Health Organization*

DISCUSSION

Although gliomas are the most frequent malignant brain neoplasms, there are not many studies on the hospitalization of glioma patients. Therefore, in this study, duration of NRC and ICU stays, whether patients received radiotherapy and chemotherapy, location and WHO grades of tumours and IDH mutation statuses were primarily analyzed.

Gliomas are commonly diagnosed in elderly individuals, similar to the rest of the brain tumors (4). The median (IQR) age in our study was 60 (15) years, which is in line with the results of Rahman et al. (15), which reported a median age of 59 (range: 23-90) years. Similar median age results were found in other studies (13, 17). However, studies of Ben Nasr et al. (16) and Jan et al. (18) revealed slightly younger mean values of age, possibly due to the inclusion of patients under 18 years old in their studies. Furthermore, results similar to our study were obtained by Rahman et al. (15), and Jan et al. (18) regarding gender. In our study, 35.3% of the patients were female, whereas female patient percentages of the mentioned studies were 39.3% and 41.6%, respectively (15, 18). These two studies included higher numbers of patients than our study.

To categorise gliomas, the 2016 WHO classification was used from grade I through grade IV. In our study group, there was no patient with a grade I glioma. There were 24 (47.1%) male and 12 (23.5%) female patients with a diagnosis of grade IV glioma, each representing the majority in their gender groups. It is known that longer survival durations are associated with grade II and III gliomas, which are accompanied by IDH mutations (4). Since no survival analysis was conducted in our study, we cannot compare it with the current literature. In our research, the number of grade IV tumours was higher, and IDH statuses of patients were mostly negative, which is in line with the aforementioned literature.

In our study, tumours were also categorized based on their locations inside the brain. Six different locations were identified. The most common tumour location was the parietal lobe (25.5%) for male patients while frontal (11.8%) and parietal (11.8%) lobes were the most common locations for female patients. In contrast, in a study by Verlut et al. (17), the most common area of lesions was the frontal lobe in the overall study population. Another study reported frontal (35.2%) and temporal (33.2%) lobes as the most common (15).

Following surgical management of gliomas, radiotherapy and chemotherapy are the main treatment approaches (9). In our study, patients who received radiotherapy outnumbered the patients who received chemotherapy. Also, out of 8 patients with chemotherapy treatment, 6 of them received radiotherapy together with chemotherapy. A phase III trial study by Malmström et al. (10) revealed that temozolomide chemotherapy had better outcomes than standard radiotherapy. However, only elderly patients were included in this study, and this study did not evaluate the length of NRC and ICU stays as an outcome. Contrastly, our study evaluated the length of hospitalization and found that the choice of chemotherapy treatment did not lead to better outcomes in terms of the length of hospitalization. In a study conducted on glioblastoma patients, the number of patients who received radiotherapy alone was higher, which is similar to our results (19).

Duration of NRC and ICU stays were found to be unrelated to the type of treatment in our study. However, there was a difference between female and male patients regarding the lengths of NRC stays. Muhlestein et al. (12) and Dasenbrock et al. (13) also reported a difference between the sexes. Nevertheless, in their studies with much larger samples, female patients stayed a lesser number of days, unlike in our study. We think that many factors affecting hospitalization, including social factors, might have an impact on this result, as well as major limitations of the study, which are small sample size, retrospective construct, and the fact that only stays in NRC and ICU and not other services are accounted for. The median length of stays was 12 days in NRC and 2 days in ICU. A study by Moroney et al. (19) reported a median of 14 days for acute hospitalization after surgery. The majority of the patients were inpatients for a minimum of 14 days in another study, which is higher than our results (14). In another study, the mean of hospitalization was 6.9 days and the duration decreased in patients who received a biopsy (18). However, our study did not specify surgery techniques to compare with this result. In another study, records of inpatients showed less than eight days for 72.2% of their patients, which is less than our results (13).

This study has some limitations. Our analysis included a limited number of patients (n= 51). A more extensive period can be analyzed or multi-centre studies can be conducted in upcoming research studies to meet current articles' results. Further studies including survival analysis, number of emergency visits, and the cost of care per patient are needed to improve our findings.

In conclusion, our study provided general information on hospitalization and its related factors. The length of NRC and ICU stays were not associated with radiotherapy and chemotherapy approaches. However, gender affected the length of NRC stays, with a longer duration for female patients. Locations of tumours varied, while the WHO grades were mainly graded IV in both genders. Further studies are needed to overcome limitations and to reveal predictors of postoperative hospitalization thoroughly.

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CONSTRICTIVE PERICARDITIS: AN OVERLOOKED CAUSE OF ASCITES

Beliz Koçyiğit[1], Irmak İrem Özyiğit[1], Servet Altay[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Cardiology, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: Constrictive pericarditis is a well-known but unusual and rare cause of ascites. The diagnosis of constrictive pericarditis in most patients who have chronic ascites is challenging. We aimed to present a patient with constrictive pericarditis with chronic ascites and pericardial calcifications seen in chest radiography. ***Case Report:*** A 66-year-old male patient presented to the emergency room of Trakya University School of Medicine. The patient had abdominal swelling and ascites for the last 5 years and underwent large-volume paracentesis in his previous emergency admissions. After the electrocardiography of the patient, he was directed to the cardiology department for further examinations. The chest radiography revealed significant pericardial calcifications. Following imaging procedures confirmed radiological findings, and the patient was diagnosed with constrictive pericarditis. Furthermore, his cardiac catheterization findings were consistent with constrictive pericarditis. The patient was recommended to have a pericardiectomy operation and there was a significant reduction in the patient's symptoms after surgery. ***Conclusion:*** The absence of specific symptoms and resemblances of existing symptoms to liver disease make the early diagnosis of constrictive pericarditis difficult. Physicians should consider constrictive pericarditis as a differential diagnosis when dealing with a patient who has chronic ascites.
Keywords: Constrictive pericarditis, ascites, pericardial disease

INTRODUCTION

Constrictive pericarditis (CP) results from pericardial fibrous thickening and reveals itself with restrained diastolic function caused by inelastic pericardium (1, 2). Throughout history, the main cause of CP remains to be tuberculosis and is still highly seen in particular countries, mostly ones that are not fully industrialized and with immunodeficiency-related population profiles (1, 2). In developed countries, the unknown antecedent is the leading cause of CP (1). The prevalent cause of CP in Turkey is tuberculosis, which supports the literature (2).

Ascites, edema, and hepatomegaly are the common symptoms of CP (3). A combination of hepatomegaly and ascites can be observed through the physical examination of the abdomen (2). When ascites and hepatomegaly are present together in a patient, misdiagnosis of a liver-related disease is common (1). Due to the challenges in differentiating by these primary signs, diagnosis of CP can remain unnoticed by physicians (4). Lack of typical cardiological signs causes referrals to non-cardiological specialties, which delay diagnosis and treatment (4, 5).

Though it is difficult to differentiate; anamnesis, biochemical parameters, unexplained examination findings, and imaging techniques can reveal CP when considered altogether (2). Chest radiographs, computed tomography scanning, cardiac magnetic resonance imaging (MRI), cardiac catheterization, and echocardiograms can lead physicians to a final diagnosis if a patient has findings consistent with CP (1-3).

Treatment of CP includes different approaches based on the type of disease such as chronic or pericardial inflammation approaches (2). Anti-inflammatory therapy is needed when patients are present with inflammatory clinical findings, while chronic CP requires pericardiectomy surgery by its progressive nature (1, 2). Patients' symptoms are significantly relieved in the postoperative course (2).

We aim to present a case report regarding a patient with CP with a history of chronic ascites who underwent a pericardiectomy operation. Since the differential diagnosis of CP as a cause of ascites can be challenging, we believe this case will contribute to the existing literature.

CASE REPORT

A 66-year-old male patient presented to the emergency department of Trakya University School of Medicine with the complaint of abdominal swelling. It was known that the patient applied to the emergency service with the complaint of abdominal swelling and ascites for about 5 years. At his emergency admissions, he underwent large-volume paracentesis to remove ascites. The patient was followed up by the gastroenterology department with a diagnosis of cardiac cirrhosis. The patient was a current smoker (30 packs/year) with a history of hypertension, hepatitis B, umbilical hernia operation, and appendectomy. The patient was also using 110 mg twice daily dosing of dabigatran, 5 mg of ramipril, 25 mg of spironolactone, and 40 mg of furosemide. According to the patient's hemogram, his erythrocyte count was 3900000/uL, his hemoglobin was 11.6 g/dL, and his hematocrit was 35.2%. The patient had neutrophilia (5700/uL) and lymphocytopenia (800/uL). The patient's C-reactive protein

Address for Correspondence: Beliz Koçyiğit, Trakya University School of Medicine, Edirne, TURKEY
e-mail: belizkocyigit@gmail.com
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ORCID iDs of the authors: BK: 0000-0001-6056-0219; IİÖ: 0000-0003-2443-0155; SA: 0000-0001-7112-3970.

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level was elevated (1.34 mg/dL), his total protein (6.5 g/dL), and albumin (3.4 g/dL) levels were slightly low. The patient's transaminase levels were within the normal range.

Atrial fibrillation was detected in the electrocardiography and the patient was directed to the cardiology department with the pre-diagnosis of heart failure. On physical examination, palpitations and ascites were detected in the patient, and he was pre-diagnosed with heart failure. Significant pericardial calcifications were observed in the posteroanterior chest radiography of the patient (Figure 1). In the echocardiography, pulmonary pressure was 55 mm Hg and the thickness of the inferior vena cava was 26 mm. Mild tricuspid regurgitation, mild mitral insufficiency, biatrial enlargement, and pulmonary hypertension were detected in the patient. Later on, the patient was directed to cardiac MRI with suspicion of CP. Cardiac MRI showed localized pleural effusions on both sides, prominent on the right, and intra-abdominal diffuse intraperitoneal fluid. Right heart chambers were larger than normal, and the vena cava was wider than normal, 43 mm at the inferior suprahepatic level. There was biatrial dilatation in the heart and the pericardium was measured as 5.2 mm in its most prominent place adjacent to the left ventricle. The thickening of the pericardium is shown in Figure 2. Cardiac MRI results also strengthened the pre-diagnosis of CP. All of these imaging findings supported the diagnosis of CP. Our patient was then referred to the coronary angiography laboratory for right left heart catheterization with the indication of CP. Cardiac catheterization revealed equalization of the end-diastolic pressure of the right and left ventricles, square root sign, and enlargement of the cardiac silhouette. Calcification and thickening were also observed in the pericardium. It was reported that the patient had catheter and fluoroscopy findings consistent with CP.

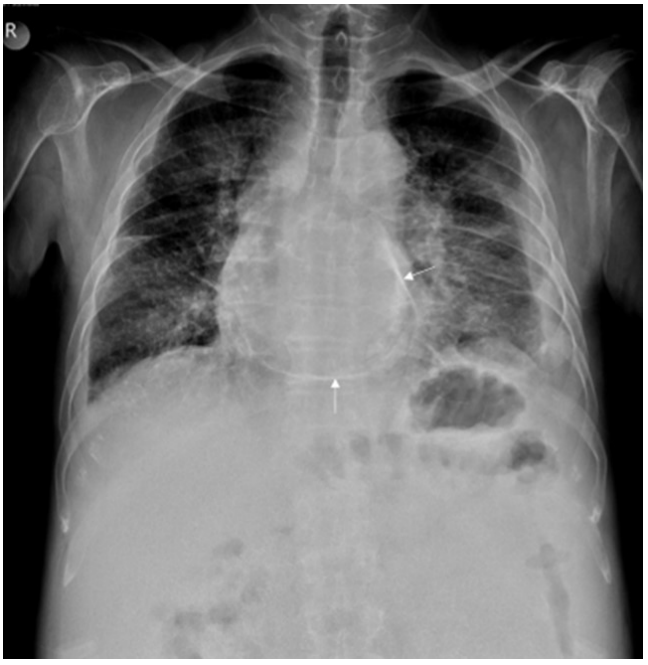

Figure 1: Posteroanterior chest radiography revealing pericardial calcifications (Arrows).

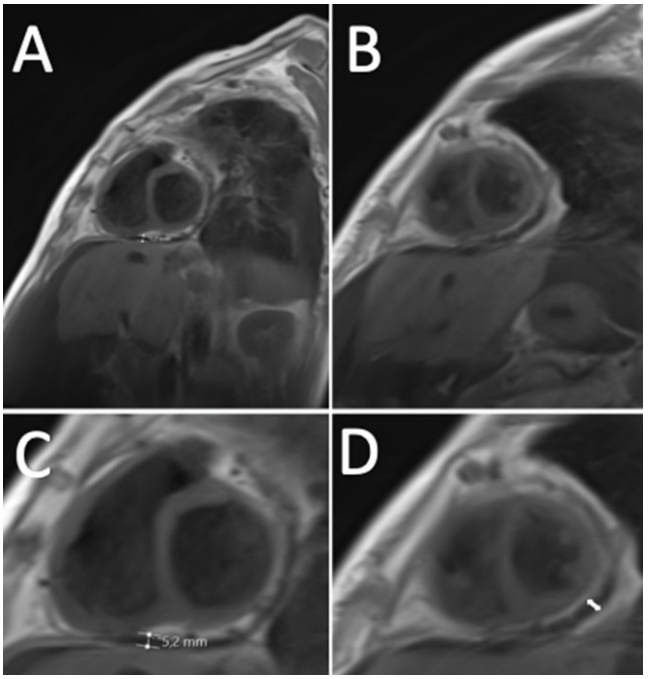

Figure 2: A, B: Cardiac MRI (short axis T1A view) of the heart shows pericardial thickening more pronounced over the left ventricle. C: A closer image of A. Thickness measured as 5.2 mm (Line). D: A closer image of B (Line).

Subsequently, our patient was diagnosed with constrictive pericarditis and he was transferred to the cardiovascular surgery department for a pericardiectomy operation. During surgery, it was observed that the pericardium was adhered to the pleura, heart, and surrounding tissues and was highly calcified. There was a significant reduction in the patient's symptoms after surgery.

DISCUSSION

Historically, tuberculosis has been cited as the most common cause of CP (2). On a global scale, this is still the case, especially in regions where the human immunodeficiency virus and acquired immunodeficiency syndrome are very common (2). While tuberculosis is still the leading cause of CP in developing countries, it is now relatively rare in the United States and Europe (2, 6). The leading etiologies of CP in developed countries are idiopathic, post-cardiac surgery, and radiation (2, 6, 7). In our case, the patient did not have any of these prior conditions, however, he had a history of hepatitis B. Even though it is not as common as the previously mentioned etiologies, hepatitis is one of the pathogens that can lead to CP (8). It is also possible that even though it was not known, he could have had a history of undiagnosed tuberculosis since it is still the leading cause in developing countries.

Constrictive pericarditis may present with signs and symptoms of left heart failure such as dyspnea on exertion, orthopnea, bilateral pleural effusion, pulmonary edema, and with signs and symptoms of right heart failure such as hepatomegaly, ascites, peripheral edema, and hepatic congestion (2, 9). Due to varying clinical manifestations and initial history, patients may be evaluated for liver disease before being referred to cardiological evaluation. Our patient also had an ongoing history of abdominal swelling and ascites and was previously diagnosed with cardiac cirrhosis.

Although the electrocardiogram might be unremarkable when it comes to diagnosis, atrial fibrillation was reported in approximately 20-40% of the patients, which was also present in our patient (10). Pericardial calcifications may be a very helpful finding in chest radiographs, especially in idiopathic cases (2). In a case series with a large number of patients, pericardial calcifications were prevalent in 27% of the patients (11). This was also a crucial finding

in our case since it led us to consider CP as the cause of ascites. The Mayo Clinic echocardiography diagnosis study found that the three prominent variables associated with CP were (i) ventricular septal shift, (ii) medial mitral e' (early diastolic mitral annulus velocity), and (iii) hepatic vein expiratory diastolic reversal ratio (12). In our case, the echocardiography showed lateral e' as 7 cm/s and septal e' as 14 cm/s. It also showed 26% of inferior vena cava collapse which indicated inferior vena cava plethora. Inspiratory reverse flow increase and septal bounce were positive. Although our patient's echocardiography results did not completely meet the Mayo Clinic criteria, some of the findings such as the medial e' velocity and inferior vena cava plethora led us to consider possible CP (10). Some of the characteristic findings in the MRI are pericardial thickening, pericardial calcifications, and pericardial effusions (10). Pericardial thickening and pleural effusions were also present in our patient, which was found to be in line with the literature. Hemodynamic catheterization, which remains the gold standard diagnostic tool for CP, may be used when non-invasive tests are inconclusive or indeterminate (2, 10, 13). Expected findings on cardiac catheterization such as equalization of the end-diastolic pressure of the right and left ventricles and square root sign were present in our patient, and therefore led us to a conclusive diagnosis (10, 13).

Our patient suffered from ascites for about 5 years, which was thought to be caused by liver disease. CP is a well-known but unusual and rare cause of ascites (5, 11). Due to the lack of specific symptoms and liver disease resemblances, the early diagnosis of CP can be challenging (5). Physicians should consider CP as a differential diagnosis when dealing with a patient who has chronic ascites. We hope that this case will help raise awareness among physicians and shine more light on the condition.

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A RARE CASE OF RECURRENT SIGNET RING CELL CARCINOMA PRESENTING WITH THROMBOCYTOPENIA

Sezin Sayın[1], Elçin Kasapoğlu[1], Ali Gökyer[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Division of Medical Oncology, Department of Internal Medicine, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: Signet ring cell carcinoma is a rare type of gastric cancer most commonly seen in the stomach. There is a 60% systemic recurrence rate of gastric cancer after curative resection, and it most commonly recurs in the liver, peritoneum, and lungs. Bone metastasis is extremely rare in gastric cancer. We aim to present a rare case of signet ring cell carcinoma with thrombocytopenia as a primary symptom of bone marrow metastasis years after the patient's primary diagnosis with gastric adenocarcinoma. ***Case Report:*** A 52-year-old male patient was admitted to the Medical Oncology Division of Trakya University Hospital with dyspeptic complaints six years ago, whereupon gastroscopy revealed an ulcerated lesion in the antral region of the stomach, and the pathological biopsy revealed adenocarcinoma. The patient underwent subtotal gastrectomy and lymph node dissection, combined with adjuvant chemoradiotherapy. In the follow-up, a complete blood count revealed thrombocytopenia, and poorly differentiated adenocarcinoma metastasis with a signet ring cell component was demonstrated through imaging studies and pathological examination. Treatment with cisplatin and 5-fluorouracil was initiated after the diagnosis of human epidermal growth factor receptor-2 negative, stage 4 gastric adenocarcinoma. The patient showed clinical and laboratory response to the treatment and will continue with the current regimen. ***Conclusion:*** In this case, the primary tumor was in the stomach, and he had signs of thrombocytopenia only. When the prognosis of the patient was evaluated, it was thought that there was tumor residue at a cellular or clonal level in the stomach after gastrectomy, or an asymptomatic metastasis present and unnoticed in the bone marrow during the surgical treatment. In conclusion, this case shows that physicians should be alert to the changes in blood parameters in terms of recurrence with bone marrow involvement even if there is no visible recurrence in the patient. ***Keywords:*** Bone marrow, gastric cancer, metastasis, signet ring cell carcinoma, thrombocytopenia

INTRODUCTION

Signet ring cell carcinoma (SRCC) of the stomach is a diffuse type of gastric adenocarcinoma, which is a rare condition with a poor prognosis due to being commonly diagnosed at advanced or metastatic stage (1). In gastric adenocarcinoma, neoplastic cells produce mucin that fills the cytoplasm and displaces the cell nucleus to the periphery, creating the signet ring cell pattern (2). According to the World Health Organization classification, if this signet ring cell pattern is seen in more than 50% of the cells, the diagnosis is SRCC, whereas if this pattern is present in less than 50% of the cells, it is only referred to as "adenocarcinomas with a signet ring cell component" (3).

Signet ring cell carcinoma is most commonly seen in the stomach but can also be seen in almost any part of the gastrointestinal tract, or the breast, prostate, and bladder (4). While the overall incidence of gastric carcinomas is decreasing, the incidence of SRCC is increasing even though it is a rare subtype of gastric adenocarcinoma (5). A study conducted by Benesch et al. (1) showed that signet ring cell carcinomas constitute 16.8% of gastric cancer cases. Clinical management of metastatic SRCC is difficult due to the rarity of cases and poor chemosensitivity (6). The most common first-line treatment for advanced SRCC is triplet chemotherapy with docetaxel-5FU-oxaliplatin known as TEFOX (7).

There is a 60% systemic recurrence rate of gastric cancer after curative resection and it most commonly recurs in the liver, peritoneum, and lungs (8, 9). Bone metastasis is extremely rare in gastric cancer, accounting for 0.9-3.8% of overall metastases in gastric cancer patients (10).

In this case report, we aim to present a patient with gastric SRCC who presented with thrombocytopenia due to bone marrow metastasis six years after his primary diagnosis.

CASE REPORT

A 52-year-old male patient was admitted to the Medical Oncology Division of Trakya University Hospital with dyspeptic complaints six years ago, whereupon gastroscopy revealed an ulcerated lesion in the antral region of the stomach, and the pathological biopsy revealed adenocarcinoma. Since distant metastasis was not detected in thoracoabdominal computed tomography, which was performed for staging, the patient underwent subtotal gastrectomy and lymph node dissection. Postoperative pathological examination documented gastric adenocarcinoma located in the antrum, and it was classified as pT1bN2M0, according to the TNM Classification of the Union for International Cancer Control Eighth Edition.

The patient underwent combined treatment with FUFA (combination of calcium folinate and 5-fluorouracil) and radio-

Address for Correspondence: Sezin Sayın, Trakya University School of Medicine, Edirne, TURKEY
e-mail: seziniyas@gmail.com
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ORCID iDs of the authors: SS: 0000-0001-7892-5992; EK: 0000-0001-7888-1053; AG: 0000-0002-1653-6155.

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therapy. Afterward, treatment was continued for 6 months by receiving FOLFOX (combination of folinic acid, fluorouracil, and oxaliplatin) chemotherapy regimen in the following 4 months. After adjuvant therapy, the patient was followed up with gastroscopy and systemic imaging studies, to assess whether recurrence had occurred.

The complete blood count in August 2020 revealed the following values: Platelet count was 83x103/uL, hemoglobin 12.8 g/dL, leukocyte 5x103/uL, and neutrophil 2.6x103/uL. Upon detection of these values, positron emission tomography/computed tomography imaging was performed in September 2020 to evaluate if any recurrence had occurred. There was mild to moderate Fluorodeoxyglucose uptake, commonly observed in the bone marrow with a maximum standardized uptake value of 4.15, and reactive bone marrow activation or metastases were investigated. Pathological fluorodeoxyglucose involvement was not detected in other parts of the body. The pathological examination of the bone marrow biopsy documented poorly differentiated adenocarcinoma metastasis with a signet ring cell component. In addition, molecular analysis was performed on the bone marrow specimen and was negative for programmed cell death ligand-1 and human epidermal growth factor receptor-2; stable status for microsatellite instability was determined.

Cisplatin and 5-fluorouracil treatment was initiated with the diagnosis of human epidermal growth factor receptor-2 negative, stage 4 gastric adenocarcinoma. The thrombocyte count of the patient, who received the 5th cycle, was measured as 130x103/uL at the last follow-up examination during the treatment. The patient, who has shown clinical and laboratory response to the treatment, will continue with the current regimen.

DISCUSSION

Signet ring cell carcinoma of the stomach is a rare form of gastric adenocarcinoma (7). According to Lauren classification, the most frequently used classification of gastric cancer, SRCC is a diffuse-type gastric cancer (11). Diffuse type gastric cancer consists of poorly cohesive and poorly differentiated cells, which explains its malignant nature (11).

Metastasis from primary tumors to the bone is a rare condition, and bone marrow involvement is even rarer (12). According to a study, the incidence of bone marrow metastasis in advanced gastric cancer, confirmed by bone marrow biopsy, is 0.024% (13). Although bone marrow metastases with the primary tumor in the stomach are rare, they are usually seen in young patients and patients with aggressive histology, such as SRCC or poorly differentiated adenocarcinoma (14). In our patient, the primary tumor was in the stomach and had the molecular characteristics of human epidermal growth factor receptor-2 negative, programmed cell death ligand-1 negative, and microsatellite instability stable.

In the study conducted by Ekinci et al. (15), 245 patients with advanced gastric cancer were assessed and the findings were that 5 patients had bone marrow metastases, and the median age of the patients was 45 years. Pain and hemorrhagic symptoms were frequently observed as main complaints along with serum alkaline phosphatase elevation in 28 cases of diffuse carcinomatosis of the bone marrow associated with gastric cancer (16). In addition, bone marrow metastasis of gastric adenocarcinoma is mostly manifested by hematological imbalances such as anemia, thrombocytopenia, and high alkaline phosphatase and lactate dehydrogenase levels (14). In a case presented by Fonocho et al. (17), the patient had new-onset pancytopenia without evidence of systemic disease despite normal blood counts in the postoperative follow-up; thus, carcinomatosis of the bone marrow associated with recurrent gastric cancer was considered. In the study of Takayasu et al. (12), a case presented with weight loss, back pain, bicytopenia without reticulocytosis, and leukoerythroblastosis, which are all compatible with bone marrow infiltration. In our case, the patient only had signs of thrombocytopenia. There were no additional physical examinations or laboratory findings. The patient was 52 years old and was compatible with the findings of the study conducted by Ekinci et al. (15).

When the prognosis of the patient was evaluated, it was thought that there was a tumor residue at a cellular or clonal level in the stomach after gastrectomy, or an asymptomatic metastasis present and unnoticed in the bone marrow during the surgical treatment. Since distant metastasis was not detected in the thoracoabdominal computed tomography, the patient was considered as an early-stage gastric adenocarcinoma, and subtotal gastrectomy and lymph node dissection were performed because the lesion was close to the antrum. In addition, combined therapy with the FUFA regimen was used to increase the effectiveness of radiotherapy given as adjuvant therapy. Because our patient was human epidermal growth factor receptor-2 negative, programmed cell death ligand-1 negative, and microsatellite instability stable; fluorouracil plus cisplatin was chosen as the treatment regimen. Spontaneous recovery of the platelet count during treatment was considered as a response to treatment, and the patient's treatment is ongoing.

In conclusion, even if there is no visible recurrence of gastric cancer in the patient, physicians should be alert to the changes in blood parameters in order to eliminate recurrence with bone marrow involvement.

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A CASE REPORT WITH FIBRIN-ASSOCIATED DIFFUSE LARGE B-CELL LYMPHOMA SECONDARY TO CARDIAC MYXOMA

Fatih Erkan Akay[1], Nurija Bilalović[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Pathology, Sarajevo University Clinical Center, Sarajevo, BOSNIA & HERZEGOVINA

ABSTRACT

Aims: To raise awareness for differential diagnosis of fibrin-associated diffuse large B-cell lymphoma with patients that have sustained chronic inflammation or are immunocompetent with a previous Epstein-Barr virus infection. ***Case Report:*** A 58-year-old male patient was admitted to the Clinical Center of Sarajevo University, Cardiovascular Surgery Department with the symptoms of getting tired quickly accompanied by dyspnea. His echocardiography findings exhibited a large polymorphic clavicle type highly mobile formation in his left atrium with a size of 76x23mm, intermittently prolapsing the annulus of the mitral valve and reaching the middle of the extended left ventricle. After the detection of a cardiac mass, the patient underwent surgery and had a total excision of the mass. Histopathological analysis showed a tumor made of stellate cells that form complex structures resembling wires together with an islet of plasma cells embedded in a myxoid/fibrinoid background. Immunohistochemically, lymphoma cells were positively stained for CD20, CD30, MUM1, and EBER. After excluding all other systemic manifestations of any other diseases, the patient was diagnosed with fibrin-associated diffuse large B-cell lymphoma, as a primary cardiac lymphoma, and myxoma. ***Conclusion:*** In conclusion, we are reporting a very rare case seen approximately 3% of all lymphomas in the Western Population associated with Epstein-Barr virus B-cell Lymphoproliferative disorders, therefore making them harder to diagnose due to limited experience. Albeit being an infrequent disease fibrin-associated diffuse large B-cell lymphoma should be an entity included in the differential diagnosis of the patients that have sustained chronic inflammation or are immunocompetent with a previous Epstein-Barr virus infection. ***Keywords:*** Cardiac myxoma, diffuse large B-cell lymphoma, Epstein-Barr virus infection

INTRODUCTION

Primary cardiac lymphoma (PCL) is a rare entity comprising up to 0.5% of extranodal lymphomas and representing <2% of all primary cardiac tumors (1). Fibrin-associated diffuse large B-cell lymphoma (FA-DLBCL) is a PCL involving only the heart or pericardial sac which is listed in the 2016 World Health Organization (WHO) classification as a provisional entity associated with chronic inflammation and Epstein-Barr virus (EBV) type III latency (2, 3). Nevertheless, systemic manifestations of lymphomas involving the heart have been documented as well, often observed in terminally ill patients, and autopsies, existing in up to 10% of patients (3). It has been most commonly reported in men that are in their mid-sixties (2). Diffuse large B-cell lymphoma (DLBCL) has been demonstrated as the most common histological subtype (3).

On the contrary, primary cardiac neoplasms alone are very rare (1, 4). Unlike FA-DLBCL they predominantly occur in women between the fourth and sixth decade of life (4). They are 80%-90% benign and the most common type is cardiac myxoma (4). The classic triad of embolic events, intracardiac flow obstruction, and constitutional symptoms may be present in most patients, and around 10% are asymptomatic (1, 3, 4). Echocardiography is the first diagnostic modality of choice. The prognosis is excellent for patients that underwent surgical resection of the mass with a great reported postoperative recovery (4).

We report a 58-year-old patient exhibiting mild symptoms with two distinct tumors, one being a primary cardiac lymphoma developed through the base of a chronic inflammation induced by the second tumor, which is a myxoma located in his left atrium. We aim to increase the awareness of FA-DLBCL by Stellate-shape, to be an entity included in the differential diagnosis for the patients that have sustained chronic inflammation and/or are immunocompetent with a previous EBV infection.

CASE REPORT

A 58-year-old male patient was admitted to the Clinical Center of Sarajevo University, Cardiovascular Surgery Department with the symptoms of getting tired quickly accompanied by dyspnea. He suffered from this complaint for over 7 months. His past medical and family history was irrelevant. He was an ex-smoker (30 pack-a-year). On his performed echocardiography, there was a large polymorphic clavicle type highly mobile formation in his left atrium with a size of 76x23mm, intermittently prolapsing the annulus of the mitral valve and reaching the middle of the extended left ventricle (Figure 1). After the detection of a cardiac mass patient underwent surgery for total excision of the mass. His postoperative course was unproblematic, and he was followed in the cardiovascular surgery clinic.

Address for Correspondence: Fatih Erkan Akay, Trakya University School of Medicine, Edirne, TURKEY
e-mail: erkanfatih48@gmail.com
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ORCID iDs of the authors: FEA: 0000-0001-7598-1016; NB: 0000-0002-7125-4863.

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Histopathological analysis showed a tumor made out of stellate shaped cells which form complex structures resembling wires and nest fingers surrounding the varicose veins on a myxoid background. In addition to the myxoid cells, a portion of the tumor stranded with abundant plasma cells, and siderophores intertwined in the fibrin network were observed (Figure 2).

There can be seen an accumulation of large lymphocytes with prominent nucleolus of more abundant cytoplasm, large irregular nuclei, sporadic atypical mitoses, and also myxoma in a fibrinous background. Because of their centrally located nucleolus, lymphoma cells resemble immunoblasts (Figure 2).

Immunohistochemically, lymphoma cells were positively stained on CD20 (Figure 3), CD30 (Figure 3), MUM1, EBER (Figure 2), and had a high expression (80%) of ki-67. Cyclin-D1, CD10, BCL6, FOXp1, CD5, ALK, cMyc, and EMA were all negative. After excluding all other systemic manifestations, together with the morphological findings' patient was diagnosed with two distinct tumors: FA-DLBCL a primary cardiac lymphoma secondary to his initial diagnosis which was a cardiac myxoma.

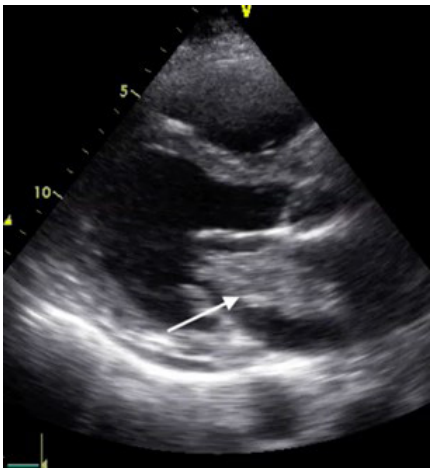

Figure 1: Large polymorphic clavicle type highly mobile formation in the left atrium with a size of 76x23mm (white arrow), prolapsing the annulus of the mitral valve and reaching the middle of the extended left ventricle.

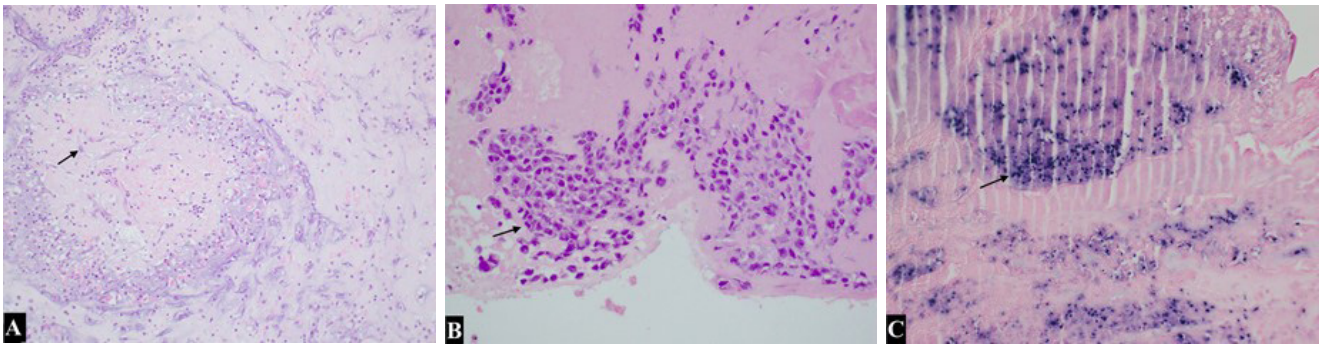

Figure 2: A: Stellate-shaped tumor cells (black arrow) of the myxoma surrounding varicose veins on a myxoid background with an infiltration of abundant plasma cells intertwined in a fibrinoid network (hematoxylin & eosin stain: x40 magnification). B: Plasmacytoid atypical lymphoid cell (black arrow) infiltration embedded in eosinophilic stained fibrinous material site (hematoxylin & eosin stain: x40 magnification). C: EBV-encoded RNA expression (EBER) in lymphoid cells (black arrow) (x40 magnification).

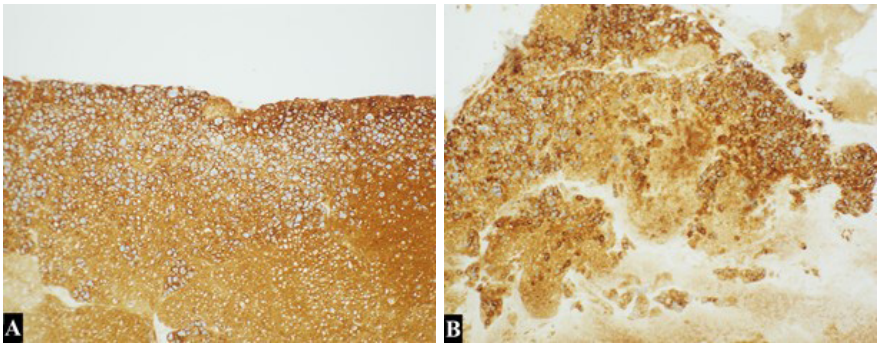

Figure 3: A: Diffuse positive staining for CD20 (x40 magnification). B: Diffuse positive staining for CD30 (x40 magnification).

DISCUSSION

Primary cardiac lymphomas' definition still differs among authors while some exclude extra-cardiac involvement, others permit a changeable amount of tumor outside the heart to be categorized as PCL either (2, 3). There has not been proposed a cutoff to quantify extra-cardiac disease, thus PCL's accepted definition is cardiac involvement of Hodgkin lymphoma (2, 5). Lymphoma involvement is usually in the right atrium and ventricle, found less on the left side. It may also extend to the inferior vena cava, superior vena cava, and jugular veins (6). Involvement can include all three layers of the heart. The most commonly reported age of the patients is the mid-sixties, most being men. Histologically most PCLs exhibit DLBCL being positive for CD20; although, few patients with T-cell and NK-cell origin have been reported as well (7, 8).

Diagnostic features for FA-DLBCL include a cluster of lymphoid cells with prominent apoptotic activity ingrained in a rich fibrinous background exhibiting non-germinal center B-cell phenotype, usually associated with EBV type III or a sustained chronic inflammation in a restricted anatomic space with an indolent clinical course (2, 7).

Pathogenesis is still a topic keeping its ambiguity on track with the need for further research. However, there have been several hypotheses presented. The most commonly accepted one has been the proliferation of EBV-positive B-cells in a localized immunosuppressed area (9). Boyer et al. (9) hypothesized a theory that EBV-positive B-cells are immortalized by the virus and lack the ability to grow autonomously in a restricted anatomic space. This was achieved through 7 of their patients showing type-III EBV latency with low or no expression of lytic protein BZLF-1, which is typically associated with immunodeficiency (10). It has been evident that telomerase-specific reverse transcriptase activity inhibits the EBV lytic cycle resulting in a maintenance of latency in infected cells (11, 12). Another important finding was how 6 (86%) patients out of 7 had positive PD-L1 stain in their immunohistochemical analysis (10). Therefore, by displaying a correlation between studies, we can elucidate how PD-L1 positivity plays a role in immune evasion in EBV-positive DLBCL and plasmablastic lymphoma cells (13). Hence, restricted anatomic space may further enhance the suppression of antitumor immunity in fibrin-associated EBV-positive large B-cell lymphoma. Protection from EBV-specific cytotoxic T cells in an immune-privileged environment could facilitate an unchecked proliferation of lymphoma cells.

Another key element in the development process of FA-DLBCL is chronic inflammation (14). There were cases reported with subdural hematoma, endovascular graft thrombi, adrenal gland pseudocyst and adjacent implanted prosthetic devices; the mutual finding of all cases is that they sustained chronic inflammation in an enclosed space (14). Furthermore, Hubackova et al. (15) has shown how interleukin 6 signaling, which plays a cardinal role in inflammatory processes, regulates the promyelocytic leukemia protein gene expression in both human and cancer cells. In this manner, inhibition of T-cell proliferation through cytokines like interleukin 10 could also provide an immunosuppressive effect resulting in a build-up of EBV-immortalized B-cells in restricted anatomic space (14). In our case, chronic inflammation induced by myxoma in the left atrium could have led to the advancement of tumor in an immune-privileged niche established by a previous EBV infection. Interestingly, there were cases reported with no immunosuppression nor EBV positivity and had no relation with a foreign body or chronic inflammation (9, 10).

The two most common clinical presentations are heart failure and pericardial effusion (1, 3). Due to tumors' location in the heart embolisms, thrombosis and stroke may develop as well. In advanced disease, there were cases reported with superior vena cava syndrome and myocardial infarction (16). Because the mass is not formed by lymphoid tissue, patients do not exhibit any signs of hematological malignancies like lymphadenopathy, organomegaly, or B-symptoms (9).

Diagnosis is difficult due to its unpredictable clinical manifestation; it is usually late or post-mortem (4). After a suspicious cardiac mass, detection of the tumor and pericardial effusion is best made by echocardiography (6). Other visualization techniques like electrocardiography-gated magnetic resonance or positron emission tomography may provide useful information for excluding the presence of other tumors that come in the differential diagnosis (6). Differential diagnosis may include neoplasm involving the heart such as malignant melanoma, angiosarcoma, metastatic carcinoma characterized by proliferation of large atypical cells that can be easily distinguished by immunophenotypic studies. In terms of malignant lymphomas, FA-DLBCL does not exhibit any systemic involvement, its characteristic features are that it originates from a base of chronic inflammation, usually associated with EBV, with lymphoma cells embedded in a fibrinous background. Nevertheless, a definitive diagnosis is made by a biopsy.

Due to its rarity, there has not been established a specific treatment protocol for FA-DLBCL. Different therapy methods such as are stem-cell transplantation, heart transplantation, and chemotherapy (R-CHOP) have been tried, with some of them resulting in complete remission (17). Nonetheless, with reported cases that had favorable outcomes with surgical excision (only according to WHO and other researchers), the question of whether patients must undergo high-dose chemotherapy arises because localized lesion does not have any clinical manifestation of secondary malignancy (1, 4). Thus, our patient underwent surgery where both tumors were resected, no further chemotherapy or radiation was administered.

In conclusion, we are reporting a very rare case seen in approximately 3% of all lymphomas associated with EBV B-cell lymphoproliferative disorders in the Western population, therefore, making them harder to diagnose due to their rarity (2, 7). As stated, pathogenesis is still a topic that requires further research. Albeit being an infrequent disease, FA-DLBCL should be an entity included in the differential diagnosis for patients that have sustained chronic inflammation or are immunocompetent with a previous EBV infection.

Ethics Committee Approval: N/A
Informed Consent: Informed consent was obtained from the patient.
Conflict of Interest: The authors declared no conflict of interest.
Author Contributions: Concept: FEA, NB. Design: FEA, NB. Supervision: FEA, NB. Resources: FEA, NB. Materials: FEA, NB. Data collection and/or processing: FEA, NB. Analysis and/or Interpretation: FEA, NB. Literature Search: FEA, NB. Writing Manuscript: FEA, NB. Critical Review: FEA, NB.
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Contribution	Explanation	Contributing Authors
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DESIGN	Planning the methods to generate hypothesis.	
SUPERVISION	Supervision and responsibility for the organization and course of the project and the manuscript preparation.	
RESOURCES	Supplying financial resources, equipment, space, and	
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DATA COLLECTION AND/OR PROCESSING	Responsibility for conducting experiments, management of patients, organizing and reporting data.	
ANALYSIS AND/OR INTERPRETATION	Responsibility for presentation and logical explanation of results.	
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3. I understand that the case report will be published without my name attached and researchers will make every attempt to ensure my anonymity. I understand, however, that complete anonymity cannot be guaranteed.
4. I have been given a copy of the Participant Information Sheet and Consent Form to keep.

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