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REVIEW

Transcatheter mitral valve therapies: A comprehensive review

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ABSTRACT

Less invasive approaches offer an optimal treatment option for patients with severe mitral regurgitation who is not a candidate for surgical intervention. Favorable outcomes of transcatheter aortic valve replacement have produced great interest in the development of novel minimally invasive transcatheter technologies for repair and replacement of the mitral valve. In this review we aimed to provide an overview of the current transcatheter technologies used to treat mitral regurgitation and help clinicians in selecting the optimal therapy for their patients. We also wanted to provoke clinicians and researchers on how these technologies could be further developed in the future.

Keywords: Mitral regurgitation, mitral annuloplasties, minimally invasive surgeries

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INTRODUCTION

Valvular heart disease influences many patients worldwide and this burden will escalate further as the population ages. Mitral regurgitation (MR) is the most prevalent form of this disease, affecting around 10% of the elderly population [1,2]. Accordingly, the number of patients with MR requiring hospitalization or intervention is expected to rise severely in the following decades. To date, the principal opportunities for treatment of this entity are medical therapy and surgical intervention, surgery being considered the gold standard. However, surgery is contraindicated in almost 50% of patients with severe symptomatic MR due to associated comorbidities or underlying ventricular dysfunction which left many high-risk patients with an only medical treatment option. If untreated, severe MR is associated with poor outcomes which the mortality rate reaching up to 50% at 5-years follow-up. On the contrary, early intervention, performed before the occurrence of the adverse effects of long-standing volume overload on the left ventricle, may have result in excellent long-term outcomes [3-7]. Thus, there is a considerable need for a less invasive approach to offer an optimal treatment option to this subset of vulnerable patients.

Favorable outcomes of transcatheter aortic valve replacement (TAVR) over the last decade have produced great interest in the development and implication of novel minimally invasive transcatheter technologies specifically designed for repair and replacement of the mitral valve. However, the anatomy and pathophysiology of the mitral valve are completely different and more complex compared to the aortic valve. The annulus, leaflets, and cords of the MV, as well as the papillary muscles and the ventricle itself, make up this dynamic system. Its operation is reliant on the ventricular function as well as leaflet apposition and coaptation [8]. Thus, the engineering process behind mitral transcatheter technologies is relatively slow compared to the TAVI approach. Nevertheless, transcatheter technologies to treat MR are evolving and there are many studies ongoing that investigating the safety and efficacy of these technologies. These technologies mainly focus on devices used for leaflet repair, annular reduction, chordal implantation, and valve replacement [5,9]. Current transcatheter mitral valve devices which are in use or under clinical evaluation can be seen in Tables 1 and 2, respectively. There are also many

Table 1. Transcatheter mitral valve repair technologies

Transcatheter repair device	Trials	Status	Outcome
Direct Leaflet Repair			
MitraClip	Everest II, ACCES-EU, TRAMI and COAPT, MITRA-FR		Guideline recommended therapy
PASCAL	CLASP IID/IIF	Enrolling	Estimated to be completed in 2028
Direct Annuloplasty			
Cardioband	EFT (In human)	Enrolling	Sustained +2 MR in 1y follow-up
Millipede IRIS	EFT (In human)	Enrolling	NA
AMEND	EFT (In human)	Enrolling	reductionof the jet area and antero-posterior diameter
Mitral Bridge	CE mark clinical trial	Enrolling	No or trace MR in 6m follow-up
Mitralign Bident System	EFT	Enrolling	NA
Coronary Sinus Annuloplasty			
Carillon Mitral Contour System	AMADEUS, TITAN		Reduction of MR and decrease of the LV size at 1m follow-up
Monarc	EFT		Reduction in MR, reduced LV dimensions, improved LV function at 1y follow-up
ATRO system	MAVERIC EU/US		Reduction of annular dimension at 1y follow-up
Synthetic Support Chords			
DS1000 System	RCT	Enrolling	Estimated to be completed in 2027
Artificial Papillary Muscle			
Mitral Butterfly	Animal study	Proof of concept	100% procedural success and no device-related events in 90d follow-up
Left Ventricular Remodeling Devices			
Coapsys annuloplasty system	RCT	Enrolling	persistent survival advantage over mitral repair in 4y follow-up
PS^3 System	EFT (In human)		Reduction of MR grade with no procedural events
Ancora Device	EFT (In human)	Enrolling	Estimated to be completed in 2024

EFT:Early feasibility trial; RCT:Randomized controlled trial; MR:Mitral regurgitation; LV:Left ventricle; NA:Not available

Table 2. Transcatheter mitral valve replacement technologies

Transcatheter replacement device	Trial	Status	Outcome
CardiAQ/EVOQUE	RELIEF EFT (In human)	Enrolling	92% procedural success and 45% mortality
CardioValve	AHEAD EFT (In human)	Enrolling	Estimated to be completed in 2022
Intrepid	APOLLO (RCT)	Enrolling	NA
Tendyne	SUMMIT (RCT)	Enrolling	NA
Sapien M3	EFT (In human)	Enrolling	88% procedural success and 2.9% mortality
Tiara	TIARA I-II	Enrolling	94% procedural success and 11.3% 30-day mortality
FORTIS	EFT	Stopped	Reports of valve thrombosis

EFT:Early feasibility trial; RCT:Randomized controlled trial; NA:Not available

interventions under evaluation to take patent for transcatheter mitral valve therapy (Table 3). Figure 1 also shows a schematic illustration of some of the repair/replacement technologies.

The aim of this review is to provide a contemporary overview of the current transcatheter technologies used to treat MR and try to guide clinicians in selecting the optimal therapy for their patients, and also to provoke clinicians and researchers on how these technologies could be further developed.

A literature search of the Medline database was performed to obtain related studies discussing

Table 3. Summary of devices under evaluation to take patent for transcatheter mitral valve therapy

Method	Patent no	Assignee	Adjusted expiration date
Transcatheter Delivery System and Method with Controlled Expansion and Contraction of Prosthetic Heart Valve	US35065610P	Medtronic	2031
Transcatheter mitral valve prosthesis	US8579964B2	Neovasc Tiara, Edwards Lifesciences Cardiaq LLC	2032
Sequentially deployed transcatheter mitral valve prosthesis	US9713529B2	Neovasc Tiara	2032
Percutaneous mitral valve replacement and sealing	EP2739214A2	Mitraltech, Cardiovalve	2032
Transcatheter prosthetic heart valve delivery device with passive trigger release	EP2563277A1	Medtronic Inc	2031
Percutaneous heart valve delivery systems	US9668859B2	California Institute of Technology CalTech	2035
Device and Method for Mitral Valve Regurgitation Treatment	US20160235529A1	Sinomed Cardiovita Technology	2034
Stented transcatheter prosthetic heart valve delivery system	CN102548508A	Medtronic	2030
Perivalvular sealing for transcatheter heart valve	US20160361163A1	Edwards Lifesciences Corp	2032
Transcatheter valve structure and methods for valve delivery	EP2538880A1	Medtronic	2031
Transcatheter heart valve with micro-anchors	US20130268066A1	Edwards Lifesciences	2028
Valve replacement systems and methods	CA2870554A1	Caisson Interventional LLC	2033

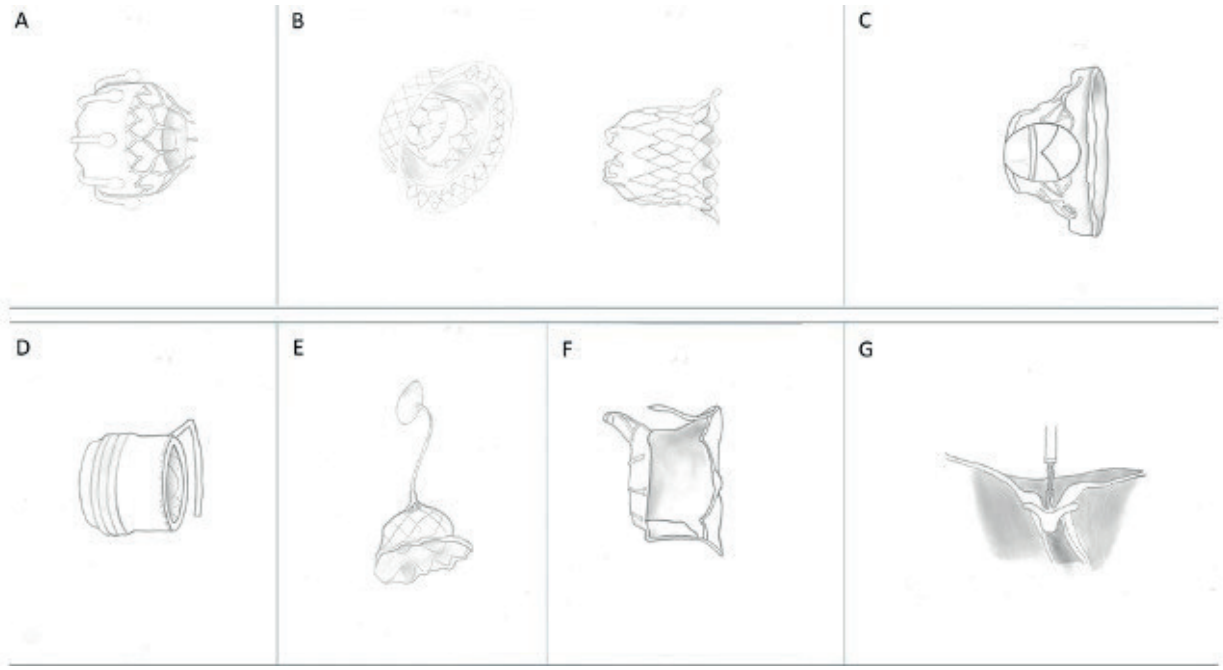

Figure 1. Schematic illustration of some mitral valve replacement/repair technologies
A:CardiAQ; B:Intrepid top/lateral view; C:CardioValve; D: Sapien M3; E: Tendyne; F: Tiara; G: MitraClip

novel transcatheter technologies and ungoing experimental studies used to treat MR. Fundamental concepts were extracted from these articles and combined appropriately. Main concepts also validated with supporting literature.

Transcatheter Repair

Direct Leaflet Repair

MitraClip (Abbott Vascular)

The MitraClip is a V-shaped polyesterdevice covered with a cobalt-chromium clip. In the first step, the clip is positioned over the regurgitation jet. Secondly, the clip arms are opened perpendicularly through the coaptation line and the device is placed into the left ventricle (LV). The leaflets are then grabbed between the clip arms as the clip is subsequently retracted. And finally, the delivery mechanism is released and the arms are closed [10]. To date, edge-to-edge leaflet repair using MitraClip is the solely guideline-recommended transcatheter treatment for MR.

EVEREST II study (randomized controlled trial) showed similar rates of death and Grade 3+ or 4+ MR at 1- and 4-year follow-up in patients who underwent MitraClip compared to surgical mitral valve replacement [11]. Both ACCESS-EU and TRAMI studies (multicenter registries) demonstrated an upgrade of NYHA functional class in patients treated for functional MR with MitrClip at 1-year and 3-month follow-up, respectively [12,13]. COAPT trial (randomized controlled trial) also showed that the rate of all-cause hospitalization and all-cause mortality was statistically significantly less (35.8% and 29.1%, respectively) in patients who underwent this therapy compared to patients who had medical therapy alone [14]. In contrast, the MITRA-FR study, in which patients were randomly assigned to Mitraclip repair plus medical treatment or medical treatment alone demonstrated similar all-cause mortality or heart failure re-hospitalization at 1-year follow-up in both arms. Nevertheless, MitraClip succeeded in a reduction of MR to Grade 2+ or less in 92% of patients at the time of hospital discharge [15].

PASCAL (Paddle, Spacer, Clasps, Alfieri Stitch) (Edwards LifeSciences)

Similar to the MitraClip, the spacer of this device is positioned between the MV leaflets. It catches, grasps and stabilizes the leaflets. A multicenter, randomized, controlled trial (CLASP IID/IIF) was conducted to assess the PASCAL device's efficacy and safety. This randomized study is now enrolling at 57 sites and is estimated to be completed in 2028 [16].

In summary, data derived from studies regarding percutaneous edge-to-edge repair devices demonstrated that these devices are safe and potentially useful in patients with severe MR. Nevertheless, these devices may not be effective in patients with secondary MR with LV dilation, in cases in which leaflet motion is severely restricted, and if there is substantial annular calcification, or multiple jets.

Direct Annuloplasty

Cardioband (Edwards Lifesciences, CA)

Cardioband is a C-shaped polyester device. It's placed through a transfemoral route followed by the insertion of multiple stainless steel screwanchors to secure the band from trigone to trigone. Following the release of the anchors, the adjustment device is attached and slowly cinched to reduce mitral annular size under transesophageal echocardiography (TEE). The adjustment device is removed once the MR has been sufficiently reduced, and the Cardioband device is left in position. It has the potential to be used primarily and in conjunction with the edge-to-edge repair or transcatheter MV replacement devices. In the feasibility trial, early after Cardioband implantation, 93% of patients had ≤ 1+ MR. The majority of these patients also remained to have ≤ +2 MR in the 1-year follow-up [8,17].

Millipede IRIS (Boston Scientific, MA)

This device consists of an adjustable nitinol zigzag-shaped semi-rigid circumferential annular ring, screw anchors, and collars. After the ring has been expanded, it is held in a supra-annular position by the anchors at each of the inferior zigzags. The ring can be cinched by moving the collars and the size of the annulus is reduced [18]. Its first in human procedures were promising and the EFS study is currently enrolling.

AMEND (Valcare Medical, Israel)

This device is a semi-rigid D-shaped nitinol annuloplasty ring which is covered by polyethylene terephthalate fabric. it is secured onto the mitral annulus with 4 zones of anchors. Once fastened, the ring is dragged anteriorly, lowering the annulus' anterior-posterior diameter [19]. The antero-posterior diameter decreased by 20%, and the jet area was reduced by 74% in the first in-human

multicenter clinical trial. The EFS study is currently enrolling.

Mitral Bridge (HRT-Heart Repair Technologies, CA)

Mitral Bridge is a nitinol bridge with an infra-annular arch that can be implanted transeptally or transapically. It is secured to the annulus by using standard sutures. It reduces the annular dimension, restore the annular saddle form, raise the coaptation height, and thus lowers the MR. The initial clinical study demonstrated promising results at a 6-month follow-up [8,19].

Mitralign Bident System (Mitralign, MA)

Mitralign Bident system implanted directly onto the posterior annulus by placing sutured pledgets through a catheter across the aortic valve. Plication of the sutures leads to a reduction of the annulus. Its use has been described in one patient with functional MR with a resultant decrease of mitral regurgitant volume [20,21].

In general, these devices are potentially beneficial in patients with secondary MR, in which the primary mechanism is the annular dilatation. Besides, these approaches can be used as adjunct therapy to other transcatheter repair or replacement methods.

Coronary Sinus Annuloplasty

Carillon Mitral Contour System (Cardiac Dimensions, WA)

The carillon device is implanted into the deep coronary vein with 2 anchors which are linked with a curved bridge. Its efficacy has been investigated in 2 studies and the results demonstrated a reduction of MR, advance of symptoms, and quality of life measures with an additional decrease of the LV size at 1-month follow-up [22,23].

Monarc (Edwards Lifesciences, CA)

The Monarc system is a nitinol implant that consists of a distal self-expanding anchor, a springlike bridge, and a proximal self-expanding anchor. The safety and EFS study showed a reduction in MR by 1 or more grades in half of the patients, reduced LV dimensions, and improved LV ejection fraction and NYHA class at 12-month follow-up [24].

ATRO system (MVRx)

Two magnetic-tipped catheters—one in the coronary sinus and the other inserted transseptally into the left atrium—along with a septal bridge on the coronary sinus side make up this system. Application of tension to the bridge shortens the septo-lateral annular dimension [25]. The early results of the safety and EFS study demonstrated a reduction of annular dimension with the majority of patients having ≤ 2+ MR at 1-year follow-up [26].

Coronary sinus annuloplasty devices are inserted percutaneously into the CS and improve leaflet coaptation and MR indirectly by constricting the mitral annulus. The major drawback of these devices is the potential to cause coronary artery compromise.

Synthetic Support Chords

DS1000 System (NeoChord, Inc., Minnesota)

The only commercially available repair device so far is the NeoChord DS1000 system, which is now being evaluated for American Food and Drug Administration (FDA) approval. The device is performed transapically through a lateral mini-thoracotomy [27]. A randomized controlled clinical trial comparing this device with the conventional surgical repair is enrolling patients and is expected to be completed in 2027 [28]. A multicenter study showed very promising success rates, in which 97% of patients showing mild post-operative mitral regurgitation. One-year survival and freedom from composite endpoints were reported as 98% and 84%, respectively [29]. In a recent systematic review, Ahmed et al. analyzed the feasibility and outcome of NeoChord device implantation in 6 studies, including 249 patients. Operative success was reached in 96.8% of the patients with no intraoperative mortality and few (~3%) minor morbidities [30].

There are also other devices in development, that have shown promising results with good safety profiles, such as Harpoon (Edwards LifeSciences), V-Chordal (Valtech, Or Yehuda, Israel), Pipeline (Gore Medical, Flagstaff, AZ), MitralStitch (Hangzhou DeJin Medtech Co Ltd., Hangzhou China), CardioMech (Trondheim, Norway), and ChordArt™ (CoreMedic AG, Biel, Switzerland). While Harpoon

is also placed transapically, V-chordal and Pipeline can be implanted transfemorally. ChordArt™ and Cardiomec devices can be implanted in both routes [31,32].

The prosthetic chordal support devices will hold an important place in the transcatheter treatment of MR with solid clinical evidence and many new devices that will be in the clinical practice soon. As in the other transcatheter technologies, the careful patient selection remains the paramount step of performing these devices with optimal outcomes. More importantly, the ability to perform the appropriate combination of leaflet repair, annuloplasty techniques, and ventricular devices together with chordal support devices will progressively improve long-term outcomes.

Artificial Papillary Muscle

Mitral Butterfly (Angel Valve, Vienna, Austria)

Mitral Butterfly is made of a nitinol-stent with ePTFE yarns which act as artificial chordae. It is a concept technology that can hold and capture the entire prolapsing valve leaflet which can be delivered through a transeptal or transaortic approach. A hook coupled with the ePTFE filaments extends into the ventricle and mimics the papillary muscle [31]. A recent animal study reported the procedural success as 100% with no device-related events within 90 days follow-up [33].

Left Ventricular Remodeling Devices

Coapsys annuloplasty system (Myocor, MN)

This device has two epicardial pads connected by a flexible cord. It compresses the left ventricle (LV) at the papillary muscles' level as well as the mitral annulus after being placed under echocardiographic guidance. Its efficacy has been analyzed in a randomized trial. Patients with ischemic dilated cardiomyopathy and ≥2+ functional MR undergoing coronary artery bypass grafting (CABG) randomized to either CABG/mitral valve repair or CABG/Coapsys. Intraoperative MR was reduced in 95% of patients, and 84% had MR grade ≤ 1 after implantation [34,35]. At 1-year follow-up, effect on MR grade, MR jet area, and NYHA class were all significantly improved with no reported deaths, device failures, reemergence of grade 3 or 4 MR, heart failure readmission, or valve reoperations [36]. At two years, patients who undergone the Coapsys device had an improved overall survival (87% vs. 77%, P=0.038) and greater freedom from adverse events (76% vs. 63%, P=0.022). The same study's four-year midterm follow-up data in a single randomization center also showed a persistent survival benefit of this device over repair. (74% vs. 50%, P=0.09) [21].

PS³ System (MVRx, CA)

The Percutaneous Septal Sinus Shortening device anchors a cord between the coronary sinus and the atrial septum that can be shortened and decrease the mitral annular septolateral distance. Its EFS study has been conducted on two patients, and MR grade was reduced from 3+ to 1+ with an additional decrease in the mean septal-lateral systolic dimension (31% reduction). No procedural complications were reported [37].

Ancora Device (Ancora Heart, Santa Clara, CA)

Through a transfemoral approach, the self-expanding, movable nitinol anchors are placed on the subannular LV myocardium. The device is gradually tightened to lessen the LV chamber circumference and the mitral annulus size [8]. The safety and early feasibility studies (EFS) of this device are currently enrolling and are expected to be completed in 2024.

An approach that addresses the basic problem of ventricular remodeling may be helpful both by reducing the ventricular size, which leads to improved contractility, and by bringing the bases of the papillary muscles closer which improves the leaflet coaptation. Early clinical trials mentioned above show with promising results that both MR and LV dysfunction may be improved by LV remodeling devices.

Transcatheter Mitral Valve Replacement

Although transcatheter mitral valve replacement (TMVR) may offer some advantages over transcatheter repair by providing a completer and more reproducible MR reduction with less technically demanding procedures, it may also have some consequences. Major challenges specific to TMVR include difficulty obtaining prosthesis stability, potential LV outflow tract obstruction, structural degeneration of the prosthetic valve, and the possibility of greater risk of injury with more catastrophic complications. Accordingly, designing

these devices seems more challenging compared to the transcatheter repair devices.

CardiAQ/EVOQUE (Edwards LifeSciences Inc)

CardiAQ is a trileaflet-bovine non-recapturable and self-expanding valve located on a nitinol frame. Implantion of the device is via the transapical or transfemoral route [38]. The early clinical trial showed a technical success of 92.3% and all-cause 30-day mortality of 53.8%. However, the trial was put on hold after 1 year to reevaluate the device design. The device had an effective anchoring mechanism but also had the possibility of LVOT obstruction due to its large profile. The device was redesigned in 2018 and was renamed EVOQUE. The new system provides a low profile to help to reduce procedural complications. The EFS for this valve is currently recruiting and is expected to be completed in 2024 [39,40].

CardioValve (Cardiovalve, Israel)

The Cardiovalve system involves of 2 nitinol frames (atrial and ventricular), 24 grasping legs, and a bovine pericardium valve. It is implanted through a transfemoral route and comes in 3 different sizes. First in-human cases were performed all with an excellent technical success (100%) with no LVOT obstruction or MR [8,39]. Device EFS study is currently enrolling patients with an estimated study completion date of December 2026 [41].

Intrepid (Medtronic Inc)

The intrepid device contains a dual nitinol self-expanding stent and a tri-leaflet bovine pericardial valve. Its champagne cork-like design is thought to oppose valve migration during high systolic pressures and help to prevent LVOT obstruction [39,42]. The device is delivered transapically. In its first EFS, 50 patients were enrolled in the study. The procedural success was reported as 98% with early mortality of 14%. No deaths were reported after 4 months. The second clinical trial, in which the patients were randomized 1:1 between surgery and this device, is currently recruiting with an estimated completion date of 2028 [43].

Tendyne (Abbott Inc)

The Tendyne system is a double frame, self-expanding porcine pericardial valve. It is delivered through a transapical route and held in the LV apex. It also has an atrial cuff which prevents the valve from entering the ventricle when the tether is under tension and perivalvular leak (PVL). Another advantage of this device is that it can be fully retrieved after surgery, if necessary [44]. Its EFS showed a procedural success rate of 96%. The 30-day mortality rate was 6% and the 1-year survival free of all-cause mortality was 72.4%. At 1 year follow-up, the majority of the patients were NYHA class I/II, and significant improvements in 6-min walk distance and quality-of-life measures were noted [45]. Currently, another trial is ongoing, actively enrolling patients, and is estimated to be completed in 2026 [46].

Sapien M3 (Edwards Lifesciences)

The SAPIEN M3 device is an adaptation of the SAPIEN 3 system that is used for the aortic position. It consists of shape memory and a nitinol stent with a trileaflet bovine pericardial valve. It also has an addition of a polyethylene terephthalate (PET) skirt to minimize paravalvular leakage and an additional shape memory nitinol dock which helps to seal the valve into place.

In the EFS including 15 patients, the device was successfully implanted in 90% of patients. MR was reduced to ≤ trivial in all implanted patients. At 30 days, there was no stroke, myocardial infarction, rehospitalization, left ventricular outflow tract obstruction, device migration, embolization, or conversion to mitral surgery. Only one patient had recurrent regurgitation due to a paravalvular leak. No mortality was noted [47]. The outcomes of the first 35 patients treated were recently presented. All-cause mortality at 30 days was 2.9%, while the procedural success rate was 88.6%. One patient had a stroke at 30 days [48].

Tiara (Neovasc Inc, Canada)

This is a self-expanding trileaflet bovine pericardial valve that is mounted on a nitinol frame. It is implanted through the transapical approach and sits in the asymmetrical mitral annulus. Its large atrial skirt helps better seating of the device and minimizes paravalvular leak. Initial results of its EFS including 71 patients showed a 94% implant success and a 11.3% 30-day mortality rate [8]. A transfemoral version of this device is also currently under development.

FORTIS (Edwards Lifesciences, Irvine, USA)

The FORTIS is a circular cloth-covered trileaflet bovine pericardial valve mounted on a self-expanding nitinol frame. It has a non-recapturable frame consists of an atrial flange and two opposing paddles that fold out at the base. When deployed, surgeons align the paddles to the MV leaflets under TEE guidance. The first-in-human implantation of this device on 13 patients showed an implant success of 76.9% and all-cause 30-day mortality of 38.5%. At 2-year follow-up, all patients but 1 were in NYHA functional class II, and there were no cases of valve malfunction [42]. However, the clinical trial was stopped because of reports of valve thrombosis [49].

Other technologies, in addition to the ones mentioned above, are being developed with fewer cases at the time being. These devices include the HighLife valve (HighLife Medical, Paris, France), the Cephea Valve (Cephea Valve Technologies, San Jose, CA), the AltaValve (4C Medical Technologies Inc, Minnesota, USA), and the NAVI system (NaviGate Cardiac Structures Inc, Lake Forest, USA).

In summary, many devices are under development in the pool of TMVR systems. Technological improvements that lead to better device delivery systems will likely make the transseptal approach more preferable in the near future. Above all, successful development of a TMVR device requires both an understanding of the complex mitral valve mechanics and knowledge of engineering parameters like material design, product development, and fabrication. Thus, to develop a simple and safe device the collaborative effort of the technical and clinical expertise is paramount.

The success of TAVR for the treatment of aortic stenosis has accelerated the progress and development of catheter-based technologies in the industry. There's been also a lot of advancement and exciting emerging technologies in the arena of catheter - based mitral valve treatments. However, it should be kept in mind that TMVR has many more challenges to overcome when compared to TAVR, and yet the trajectory is expected to be slower. Nonetheless, current devices are showing promising results in their trials and indicating that TMVR will be an available therapeutic option in the treatment of high-risk patients with MV disease in the near future. Further development of system designs, imaging techniques, and involvement of clinicians/surgeons in the development of these technologies will potentially expedite the process.

Author contribution

Study conception and design: UK; data collection: RB and UK; analysis and interpretation of results: RB and UK; draft manuscript preparation: UK. All authors reviewed the results and approved the final version of the manuscript.

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acta medica

REVIEW

Effects of anemia and peri-operative medication on wound healing in cleft lip and palate patients

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ABSTRACT

Introduction: Wound healing complications among cleft lip and palate patients pose risks of additional surgeries, reduced scar quality, and increased patient morbidity. This literature review aims to explore the impact of anemia and drug usage associated with respiratory complications, frequently encountered in these patients, on wound healing.

Materials and Method: We conducted an extensive literature search without time constraints using PubMed and Google Scholar databases. The investigation into the effects of anemia and medications on wound healing was divided into three stages. The first stage examined the general effects of anemia and medications on wound healing. The second and final stages assessed wound healing in craniofacial and cleft lip/palate surgeries, respectively.

Results: Preoperative anemia exerts no substantial influence on wound healing unless hemoglobin levels drop below 5 g/dl. No studies reported detrimental effects of glucocorticoids on wound healing. The impact of salbutamol use on wound healing remains controversial, while data regarding ipratropium usage are insufficient.

Conclusion: Severe anemia is the only significant concern for wound healing, necessitating transfusion or improvement in anemia. It appears that short-term use of glucocorticoids after surgery does not have a negative effect on wound healing.It is worth noting that salbutamol and ipratropium usage may have detrimental effects, and while complete avoidance may not be feasible, their potential impact on wound healing should be considered.

Keywords: Anemia, cleft lip, cleft palate, glucocorticoid, ipratropium, salbutamol

INTRODUCTION

A wound is characterized as damage to the normal anatomical structure, initiating a complex cascade of reactions and interactions among cells and mediators. Numerous factors, both local (such as ischemia, infection, foreign bodies, and edema) and systemic (including diabetes mellitus, hypothyroidism, age, tissue perfusion, hypothermia, pain, trauma, and burns), have been identified as influential contributors to the intricate process of wound healing [1].

Wound healing complications in patients with cleft lip and palate present significant challenges, encompassing the potential for additional surgeries, compromised scar quality, and heightened patient morbidity [2]. While extensive research has examined the effects of various diseases and agents on wound healing, it is crucial to recognize that many of these agents and diseases are not routinely encountered by cleft lip and palate patients.

Anemia is a common condition among individuals with cleft lip and palate, with an observed preoperative prevalence of 81%[3]. Among systemic factors, anemia stands out as one that can disrupt tissue perfusion [1]. Iron deficiency anemia, predominantly observed in infants, including those with cleft lip and palate, often necessitates iron supplementation, typically commencing at four months for full-term breastfed infants (elemental iron 1 mg/kg daily, maximum 15 mg), and continues until the infant begins consuming iron-rich complementary foods [4]. Notably, cleft lip and palate patients often undergo surgery during the recommended iron supplementation period [4]. Despite these considerations, a consensus regarding the necessity of erythrocyte replacement in cleft lip and palate patients remains elusive, as does a clear consensus regarding the impact of pre-existing anemia on wound healing in these patients [3].

Furthermore, an investigation extending to the use of glucocorticoids, including "methylprednisolone" and "dexamethasone," as well as β2-Agonist agents such as "Salbutamol," frequently administered as bronchodilators in the intensive care unit for postoperative airway issues, is needed. These medications serve various roles in managing upper and lower airway obstructions (Table 1), and their potential effects on wound healing require careful consideration [5].

The impact of anemia and postoperative medication use is a subject of current interest in the field of wound healing among cleft lip and palate patients. This study is undertaken with the primary goal of investigating the influence of preoperative anemia and postoperative drug administration on the wound healing process in individuals with cleft lip and palate.

MATERIALS and METHOD

Binary combinations of the keywords "cleft lip,""cleft palate," "craniofacial," "wound healing," "steroid," "glucocorticoid," "salbutamol," "dexamethasone," "prednisolone," "ipratropium," and "anemia" were searched without time constraints on the PubMed and Google Scholar databases. Articles in English with an available full text were evaluated. The effects of anemia and medications on wound healing were investigated in three steps. The effects on general wound healing of anemia and medications were examined in the first step. Wound healing of craniofacial and cleft lip/palate surgeries was evaluated in the second and last steps, respectively.

RESULTS

When we analyzed the results, we obtained the following data concerning the effects of anemia and drug use, both of which serve as independent variables.

Anemia

Since the 1940s, the effect of preoperative anemia on wound healing has been discussed in the literature[6]. Some studies have stated that preoperative anemia has no effect on wound healing, while others have reported that anemia has a negative effect on wound healing [7-9].

Despite anemia, the peripheral circulatory system can protect the level of oxygen in the tissue, and wound healing is not affected if the level of oxygen in the tissue is within normal limits [10]. If the level of hematocrit is above 15%, wound healing is specified not to be interrupted [9]. In a study on wound complications in laryngectomized patients, prior radiation therapy, diabetes mellitus, preoperative hypoalbuminemia, anemia, and thrombocytosis were found to be independent etiological factors for wound complications [11].

Table 1. Medication Protocol in Intensive Care Unit for Airway Obstruction

Upper Airway Obstruction	Lower Airway Obstruction	Upper and Lower Airway Obstruction
Dexamethasone 4 x 0.15mg/kg	Methylprednisolone 2 x 1 mg/kg Salbutamol (Three doses every twenty minutes) (Loading dose)	Ipratropium 4 x 250 mcg/2 mL
Methylprednisolone 2 x 1 mg/kg	Salbutamol single dose every hour or 2 hours (Maintenance dose)	

In patients with aplastic anemia, it has been shown that anemia can be kept under control without disrupting wound healing during dental implant application [12]. Cleft palate can be associated with Diamond-Blackfan anemia [13,14]. As a consensus on this issue, preoperative anemia does not affect wound healing unless the level of hemoglobin is below 5 g/dl. In the literature, the importance of malnutrition has been emphasized, which can cause low hemoglobin levels.

Glucocorticoids

Glucocorticoids can easily pass through the membrane and bind to glucocorticoid receptors in the cytoplasm. The receptors are glucocorticoid receptor and mineralocorticoid receptor [15]. When glucocorticoid receptors are stimulated, chaperone proteins are activated, and these proteins affect the transcription of anti-inflammatory and inflammatory proteins in the nucleus. Glucocorticoids reduce the transcription of cytokines, chemokines, enzymes, peptides, inflammatory mediator receptors, and adhesion molecules that stimulate inflammation. Glucocorticoids are pharmacological agents used as drugs in asthma and chronic obstructive pulmonary disease due to their feature of reducing inflammation. In addition to glucocorticoids, β2 agonists are also used in the perioperative period to reduce airway obstruction in patients with cleft lip and palate in intensive care [16].

The effect of the anti-inflammatory feature of glucocorticoids on wound healing has been investigated since the 1960s. In the early period of wound healing, glucocorticoids have been shown to slow the migration of inflammatory cells and fibroblasts, inhibiting collagen formation, capillary regeneration, and epithelial migration [17]. Glucocorticoids are divided into six types according to duration of action and anti-inflammatory properties (Table 2).

The use of methylprednisolone has been shown to reduce TGF-β and IGF levels. Collagen deposition was decreased in wound healing due to the antagonistic effects of glucocorticoids [18].

The level of hydroxyproline was diminished in an animal model study of wound healing by 1 mg/kg dexamethasone. Additionally, this effect of dexamethasone was found to be dose-dependent [19].

In a review of 45 studies involving 5796 patients in the perioperative period, patients who received dexamethasone reported less pain, resulting in decreased use of opioids and analgesics. In the same study, patients who used dexamethasone had fewer follow-ups in the intensive care unit, but their blood glucose levels were higher compared to patients who did not use dexamethasone. Nevertheless, normal wound healing was observed [20]. In tonsillectomy patients, when prednisolone was used to decrease nausea and pain in the postoperative period, no serious complications were detected [21]. Furthermore, a single dose of dexamethasone was found to reduce pain without causing serious complications in adult tonsillectomy patients [22].

The utility of dexamethasone in maxillofacial fracture surgeries did not affect wound healing [23-25]. Corticosteroid applications were also shown not to increase the risk of infection and wound dehiscence in orthognathic surgery and oral interventions [26].

There were two studies in the literature investigating the relationship between cleft lip and palate patients and steroids. Dexamethasone was found to reduce airway problems, fever, and hospitalization in the postoperative period, while the drug did not increase the formation of fistulas in both studies [27,28].

Table 2. Types of glucocorticoids

Glucocorticoids	Duration of action	Anti-inflammatory potency	Equivalent dose
Cortisone	Short (<12 hours)	1	20 mg
Hidrocortisone	Intermediate (12-36 hours)	4	5 mg
Prednisone		4	5 mg
Methylprednisolone		5	4 mg
Dexamethasone	Long (>36 hours)	25	0.75 mg
Bethamethasone		25	0.75 mg

No studies reporting negative effects of glucocorticoids on wound healing were found in the literature[29].

β2-Agonists

When β2 agonists stimulate the membrane receptor, adenylate cyclase is activated. Increasing cAMP reduces the level of intracellular calcium. Muscle relaxation occurs due to decreased levels of calcium, which leads to bronchodilation.

The most commonly used β2 agonists are formoterol, salmeterol, and salbutamol (Table 3). Formoterol and salmeterol are long-acting β2 agonists, while salbutamol is short-acting. Salbutamol is used as a bronchodilator in intensive care units [30]. Salbutamol has been found to be a teratogenic agent and can cause cleft lip and palate deformity [31,32]. The effect of salbutamol on wound healing has been tested in different epithelial studies. There are different opinions in the literature regarding corneal epithelium and alveolar epithelium. In some studies, salbutamol was found to accelerate wound healing, while in others, it was found to impair wound healing. It was determined that salbutamol could slow down epithelial cell migration via the protein phosphatase-2A pathway in cellular research [33]. However, convincing clinical evidence is lacking.

Ipratropium

Ipratropium is used as an acetylcholine antagonist by blocking muscarinic cholinergic receptors. Bronchoconstriction is decreased due to the diminished contraction of smooth muscle by the antimuscarinic effect of ipratropium [34]. No studies were found in the PubMed and Google Scholar databases on the effect of ipratropium bromide, which is used for patients in the intensive care unit.

DISCUSSION

Understanding the impact of medications administered to address perioperative respiratory tract complications, a common occurrence in cleft lip and palate patients, as well as the influence of anemia on wound healing, holds significant relevance for plastic surgeons. This knowledge equips them with the ability to make necessary dosage adjustments or medication changes, enabling the anticipation of potential complications and the provision of informed guidance to patients and their families.

In preoperative assessment of every cleft lip and palate patient, a comprehensive blood count should be routinely conducted. It is noteworthy that mild anemia typically does not exert an adverse influence on surgical outcomes. However, when hemoglobin concentrations fall within the range of 8 to 10 g/dl, it becomes imperative to assess the patients' developmental stages. If there are no underlying developmental concerns, proceeding with the surgical procedure may be deemed appropriate. It is important to acknowledge that there is a dearth of specific data pertaining to the wound healing process in cleft lip and palate patients undergoing surgery, as indicated in -4. Nonetheless, it is essential to underscore that erythrocyte replacement is generally unnecessary unless severe anemia is evident.

Our review of the existing literature did not uncover any clinically proven evidence suggesting that the use of glucocorticoids is detrimental to wound healing. When administered in short-term and low doses to address airway problems in patients, glucocorticoids have not been shown to impair wound healing, as outlined in Table 4. While the available literature remains limited in addressing the potential impact of salbutamol on wound healing, it is prudent to consider that salbutamol may have the potential to disrupt the wound healing process, as summarized in Table-3. Similarly, no studies specifically investigating the effects of salbutamol on wound healing can be found in the existing literature, thus precluding any commentary on its safety. In cases of postoperative airway problems, the recommendation is to

Table 3. Types of β2 agonists

β2 agonists	Selectivity	Receptor binding affinity	Onset of action	Duration bronchodilator effect	Agonist activity	Lipid solubility
Salbutamol	Moderately high	High	Fast	Short	Full	Low
Formoterol	High	High	Fast	Long	Full	Moderate
Salmeterol	Very high	High	Slow	Long	Partial	High

Table 4. The effect of anemia, β2 agonists and ipratropium on wound healing

	Wound Healing	Wound Healing in Craniomaxillofacial Surgery	Wound Healing in Cleft Lip and Palate Surgery
Anemia	If severe, disrupts wound healing	Opposite results - the data is inadequate	No study
Dexamethasone	Disrupts wound healing	With low dosage, no effect	With low dosage, no effect
Methylprednisolone	Disrupts wound healing	With low dosage, no effect	With low dosage, no effect
Salbutamol	Disrupts wound healing	Disrupts wound healing	No study
Ipratropium	No study	No study	No study

preferentially opt for a single dose or to reduce the doses of glucocorticoids.

This discussion underscores the importance of individualized assessment and decision-making in managing cleft lip and palate patients, taking into account their unique medical profiles and specific needs. Further research is warranted to establish a more comprehensive understanding of the effects of medications and anemia on wound healing in this patient population.

CONCLUSION

Severe anemia is the only significant concern for wound healing, necessitating transfusion or improvement in anemia. It appears that short-term use of glucocorticoids after surgery does not have a negative effect on wound healing.It is worth noting that salbutamol and ipratropium usage may have detrimental effects, and while complete avoidance may not be feasible, their potential impact on wound healing should be considered.

Author contribution

Study conception and design: GGÜ, FÖ; data collection: GGU and FDMO; analysis and interpretation of results: GGU and FDMO; draft manuscript preparation: GGU, FDMO and FO. All authors reviewed the results and approved the final version of the manuscript.

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Ethical Approval was not required due to the nature of the study.

Conflict of interest

The authors declare that there is no conflict of interest.

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REVIEW

Return to work for cancer survivors: Importance and challenges

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ABSTRACT

The loss of gainful employment can lead to a diminished quality of life, reduced self-esteem, and financial difficulties for cancer survivors. Understanding the factors influencing their ability to re-enter the workforce is crucial. Providing workplace accommodations, reducing workloads, and fostering support from employers and colleagues can incentivize cancer survivors to return to work. Physicians also play a pivotal role in aiding patients through the return-to-work process, thereby enhancing their overall quality of life. Early evaluation and access to social support systems are essential for cancer survivors. In this comprehensive review, we examine the process of cancer survivors returning to work, the associated factors, and the primary challenges, drawing upon current research.

Keywords: Cancer, return-to-work, survivor, social support, quality of life

INTRODUCTION

Cancer is a global health concern, with significant prevalence in Turkey and worldwide. According to the Global Cancer Observatory (GLOBOCAN) 2020, there were 19.3 million new cancer cases diagnosed in 2020, resulting in nearly 10.0 million cancer-related deaths [1]. GLOBOCAN predicts a 47% increase in cancer cases to 28.4 million by 2040 [1]. Cancer treatment is a protracted and life-altering process, significantly impacting the quality of life and affecting multiple facets of survivors' lives [2]. Early detection and medical interventions have improved cancer survival rates for specific cancer types [3].

A substantial proportion of cancer survivors are under the age of 65, with 35% falling within the 40 to 64 age range, and predominantly part of the working population [4]. The increasing number of cancer survivors, attributed to early diagnosis and enhanced treatment approaches, has underscored the importance of addressing their re-employment, workability, and social reintegration [5,6]. Systematic reviews have shown that 43-93% of cancer survivors aim to return to work within six months to two years post-diagnosis [7]. Additionally, Mehnert et al. conducted a comprehensive review of 64 studies spanning from 2000 to 2009, revealing that 62% (24–94%) of cancer patients had successfully re-entered the workforce during or after primary therapies [8]. Returning to work is a critical determinant of cancer patients' overall quality of life. It not only provides a source of income but also signifies a triumph over the disease and a return to normalcy [9].

However, cancer survivors encounter various challenges when attempting to return to work, including a 37% higher risk of unemployment compared to healthy individuals [10]. Factors such as job discrimination, the difficulty of balancing treatment with full-time employment, and mental or physical limitations contribute significantly to unemployment [11,12]. Moreover, the likelihood of returning to work varies depending on the type of cancer, with survivors of breast, skin, and certain genital cancers demonstrating higher

re-employment rates, while those with lung, gastrointestinal, and hematological cancers face greater difficulties [13].

The initial phase of returning to work is pivotal, but understanding the factors that sustain survivors in their working lives is equally important [14]. A meta-synthesis identifies three categories of factors influencing the return to work: personal factors, the presence of a support system, and occupational factors [15]. While there has been a notable increase in qualitative studies on cancer survivorship and returning to work in recent years, these studies have often focused on specific cancer types or employment issues [15]. This review aims to outline the process and factors influencing cancer survivors' return to work across four main domains.

Why is the return to work important for cancer survivors?

The significance of returning to work for cancer survivors cannot be overstated. Employment enhances their sense of identity, self-esteem, financial stability, and social relationships [16]. Many cancer patients aspire to and can re-enter the workforce after treatment [17]. Returning to work can mitigate social isolation, loss of self-esteem, boredom, and financial distress among cancer survivors [18]. For patients, resuming work signifies complete recovery and a return to normalcy [19,20].

Work provides structure and fosters social connections, contributing positively to cancer survivors' psychological well-being [17]. Since work can help patients regain a sense of normalcy, value, meaning, and reintegration into society, work may comprise a range of positive consequences for the recovery and the psychological well-being of cancer survivors [21] A positive attitude toward work is conducive to work resumption and is a crucial factor in work performance among patients with chronic conditions [22]. Recent empirical studies have highlighted the importance of patients' expectations of recovery as predictors of return to work, irrespective of their diagnosis or treatment [23].

The loss of employment leads to a reduced quality of life, diminished self-esteem, and financial strain [24]. Financial distress has been significantly associated with an increased risk of cancer-related mortality [25]. Efforts to identify barriers to returning to work are essential to ensure the financial stability of patients and their families, who often depend on their income [26]. Consequently, it is imperative to identify at-risk patients promptly and provide them with the support of social security systems [26].

What are the challenges in work life for cancer survivors?

Unfortunately, cancer survivors face a higher risk of unemployment compared to their healthy counterparts [27]. Researches indicate that unemployment risk is associated with extensive surgery, advanced tumor stage, and specific cancer types, including liver, lung, hematological malignancies, brain and central nervous system cancers, gastrointestinal cancers, pancreatic cancer, head and neck cancers, and gynecological cancers [5,8]. Indeed, quantitative studies indicate that cancer survivors experience a range of disadvantages in the labor market, varying by cancer type18.

Factors such as chemotherapy, older age, and lower educational levels have also been linked to unemployment risk [8]. Chemotherapy has consistently emerged as a negative prognostic factor for return to work [28].

Occupationally active cancer survivors often deal with physical and psychosocial challenges in the workplace [29]. Fatigue, a common and debilitating side effect of cancer and its treatment, poses a substantial problem at work and has been identified as a primary impediment to return to work [30-32]. Cognitive limitations, such as poor concentration and memory deficits, are reported as the most problematic post-treatment symptoms by breast cancer survivors [33].

Persistent physical limitations, including difficulties with lifting, fatigue, treatment-induced menopausal symptoms, and cognitive impairments such as poor concentration, memory deficits, attention problems, coping issues, depression, and anxiety, may hinder the workability of cancer survivors [32]. Physical workload, such as heavy lifting, has also been found to negatively impact cancer survivors' employment prospects [34]. Many returning survivors report a loss of self-confidence, difficulty managing symptoms at work, reduced job performance, and compromised career prospects [18].

In one study, 17% of all returning cancer survivors experienced a cancer-related reduction in working hours [13]. Physical impairments may adversely affect work performance, resulting in productivity loss, reduced workability, presenteeism, recurrent sick leave, or long-term work disability [32]. A study by Steiner demonstrated that over half of the sample changed their occupational roles upon returning to work due to cancer-related physical and psychological symptoms [35].

To address these challenges, various solutions have been proposed, including gradual return to work, increased job autonomy, reduced workloads, and enhanced communication between occupational and attending physicians [36,37]. Early intervention by physicians and employers is crucial for improving the quality of life and addressing these issues.

Roles of Physicians in the Return to Work of Cancer Survivors

Physicians play a vital role in facilitating the return-to-work process and enhancing the quality of life for cancer survivors. Physicians should inquire about patients' return to work status and any challenges they may encounter during the process [38]. Fitness assessments for work should be conducted to evaluate workers' capabilities and health risks in their work environment, ensuring that they can perform their tasks without jeopardizing their health [39]. Referral to occupational physicians may be necessary and beneficial in cases where work-related health issues arise. The occupational physician should distinguish between symptoms caused by exposure to work and those due to other origins [40]. Oncologists and psychologists address various distressing issues related to cancer, including depression, diminished self-image, and family functioning, which can have a more significant impact than work-related concerns [41].

Advances in treatment, clinical services, symptom management, rehabilitation, and disability accommodation can improve employment outcomes for cancer survivors [27]. Occupational physicians are well-placed to implement workplace accommodations [42]. Studies have shown that the occupational physician's assessment of return to work can shorten sick leave and increase patient satisfaction [24]. Tailored interventions and rehabilitation programs should be developed to meet individual patient needs [5].

Physical exercise, particularly high-intensity exercise during or after chemotherapy, has been shown to enhance the work participation of cancer survivors by improving return to work rates and working hours [43]. Beyond medical and physical considerations, comprehensive support for return to work should encompass psychological and social assistance [5]. Healthcare professionals must also be informed about the adverse consequences of not returning to work on cancer survivors' overall well-being [44].

The management of return-to-work issues should begin during cancer diagnosis and treatment and involve primary oncologists and workplace physicians, particularly for curable cancers. Collaboration between oncologists and workplace/occupational physicians can provide valuable insights into the employee's health, performance status, risk factors to avoid, potential complications, and workplace conditions, ultimately enhancing return to work and productivity. Workplace doctors, working in conjunction with oncologists, occupational physicians, and occupational safety professionals, should conduct risk assessments and management in the workplace. Clinical guidelines on cancer management, especially for curable cancers, should incorporate detailed advice on returning to work for cancer survivors.

Roles of Employers in the Return to Work of Cancer Survivors

Physical and psychological factors, workplace social dynamics, and support during the return-to-work process are critical considerations for cancer survivors [26]. Some cancer patients face job discrimination, hostility in the workplace, a lack of emotional and practical support from supervisors and occupational health services, and disputes related to employment terms [10]. Studies have shown that social support from supervisors and colleagues positively correlates with the value cancer survivors place on their work [45]. Flexible work arrangements, a potential managerial function, have been associated with a higher likelihood of employment or return to work among cancer survivors [5].

Employers can facilitate the return-to-work process by offering workplace modifications, simplifying job roles, reducing workloads, adjusting work schedules (including eliminating night shifts),

providing medical leave, and granting hourly leave. Additionally, support from colleagues can encourage cancer survivors to return to work [46]. A study showed that a high percentage of employed breast cancer patients returned to work after treatment and that workplace accommodations played an important role in their return [47]. Workplace accommodations and paid sick leave during treatment are crucial for a successful return to work [27].

The creation of favorable conditions for social integration within the workplace, along with equitable employment terms and professional assistance during the resumption of work, significantly enhance the likelihood of a successful return to work [26].

It is imperative to gather data on work-related impairments and challenges faced by cancer survivors across various work sectors and to understand how employers address these issues [8]. Stakeholders should develop strategies for vocational interventions that facilitate the return to work or enable survivors to find more suitable employment, as such interventions are currently lacking [39]. These strategies should include workplace modifications, adjustments to working hours and duties, accommodation for hospital appointments, load reduction, assistance provisions, and personnel changes [18].

What do clinical guidelines recommend?: The IOSH Research Committee

Current clinical guidelines for various cancer types inadequately address the topic of returning to work. The Institution of Occupational Safety and Health (IOSH) Research Committee conducted a systematic literature review and case studies on occupational safety and health considerations for cancer survivors returning to work [48]. The risk assessment process and the implementation of risk reduction measures are critical aspects of ensuring safety and health in the workplace. In the context of returning to work after cancer, the risk assessment process is pivotal in determining the measures that can facilitate the return to work process. Individualized risk assessments have proven effective in integrating individuals back into the workplace. Effective communication among employers, employees, and returning individuals is crucial for a successful return to work for cancer survivors.

The systematic literature review and case studies have identified key themes that should be considered in risk assessments for returning to work after cancer. Regarding safety concerns, research evidence highlights the impact of physical and psychological demands at work and the persistence of fatigue as a risk factor. Fatigue management can be addressed through flexible work schedules and breaks. Cognitive changes, such as poor concentration and memory deficits, also warrant consideration, with job design and breaks serving as potential solutions. Line managers play a crucial role in ensuring that returning individuals are not overwhelmed [48].

The number of cancer survivors returning to work is on the rise, promising an expanding evidence base. Further research is needed to identify barriers and facilitators to remaining in or changing jobs, interventions to enhance return to work and work-life quality, and clinical management guidelines that adequately address return to work issues for clinicians who care for cancer patients.

Given the potential benefits and challenges associated with returning to work for cancer survivors, there is an urgent need to develop new employment-promoting strategies, policies, and improved health and social support programs. These initiatives should support cancer survivors in successfully returning to work and maintaining their productivity.

Author contribution

Study conception and design: YKG and NÇB; data collection: YKG and NÇB; analysis and interpretation of results: YKG and NÇB; draft manuscript preparation: YKG and NÇB; All authors reviewed the results and approved the final version of the manuscript.

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ORIGINAL ARTICLE

De-novo use of generic tacrolimus (Adoport) in renal transplant recipients: A single center experience from Türkiye*

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ABSTRACT

Objective: Renal transplantation is the best treatment for end-stage kidney disease, and tacrolimus has become an important immunosuppressive treatment for kidney transplant patients since it was introduced. After the first generic tacrolimus has been approved by the FDA, studies have begun to compare the effectiveness and safety of generic tacrolimus with the original tacrolimus. When using generic immunosuppressive therapies, it is also necessary to ensure that it provides adequate immunosuppression and does not cause severe toxicity.

This study aims to compare clinical outcomes, including acute rejection, graft loss and adverse reactions, in patients receiving brand tacrolimus (Prograf, Astellas Pharma, U.S.) or generic tacrolimus (Adoport, Sandoz, UK) from the start of kidney transplant therapy.

Study Design: Renal transplant recipients between 1 January 2015-1 March 2020 were screened retrospectively. All patients receiving de novo generic tacrolimus (n:51) and randomly selected 102 control renal transplant recipients receiving original tacrolimus were included in this study.

Materials and Methods: We evaluated and recorded demographic, clinical and laboratory data including age, gender, primary kidney disease, donor type (live or dead), induction and death regimen, tacrolimus dose, tacrolimus through levels, serum creatinine, biopsy-confirmed acute rejection episodes, delayed transplant function, positive BK polyomavirus in the urine, BK polyomavirus-related nephropathy, cytomegalovirus infection in 1-year follow-up.

Results: Most of the patients were male (64.1%) with a mean age of 38.3 years. There was no significant difference in demographic characteristics between the original and generic tacrolimus groups. No differences were found in terms of creatinine levels, total daily dose of tacrolimus and tacrolimus trough levels at discharge and the first year. Additionally, biopsy-confirmed acute rejection in the following year after transplantation, BKPyV positivity in urine, BKPyVAN, CMV viremia and adverse reactions related to tacrolimus were similar between the two groups.

Conclusion: With this study, we aimed to contribute to the literature with our experience on the use of generic tacrolimus from our country. As a result of our study, we noted that generic tacrolimus can be safely preferred for de-novo use with close drug-level monitoring because it is an immunosuppressant agent with a narrow therapeutic index. There is a continuing need for randomized prospective-designed and multi-centric studies with a wide range of patients.

Keywords: Transplantation, generic drugs drugsimmunosuppressants

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INTRODUCTION

Renal transplantation is the best type of treatment modality for end stage kidney disease [1]. Since its discover in 1984 tacrolimus became an important immunosuppressive agent for renal transplant patients. Tacrolimus, also known as FK506, is a macrolide antibiotic that is derived from *streptomyces tsukubaensis*. It binds to "FK-506 binding protein" with the binding ring it contains. This structure prevents the enzyme activity of calcineurin and this inhibition stops T lymphocyte activation and proliferation [2]. Food and Drug Administration (FDA) approved usage of tacrolimus for renal transplantation in 1997 due to the superior results from randomized controlled trials [3-5].

Subsequently, FDA approved the first generic tacrolimus in August 2009 for cost-saving after the patent duration of the brand tacrolimus ended in April 2008. Generic medications require both pharmaceutical equivalence and bioequivalence with the reference formulation. Bioequivalence tests include comparing the mean maximal plasma concentration (Cmax) and the mean area under the maximal plasma concentration-time curve (AUC) of drugs in 18 to 36 healthy volunteers. If the products mean Cmax and mean AUC ratio are between 80-125% of the reference product, it is considered bioequivalent [6]. However regulatory approval do not require generic medications to undergo bioequivalence tests in renal transplant recipients. This is an important issue as data related to bioequivalence cannot be extrapolated from healthy volunteers to renal transplant recipients who have faster clearance of tacrolimus as a result of several factors including low hematocrit and albumin levels, co-administration of corticosteroids, and high rates of disturbed gastrointestinal motility and diabetes [7,8]. A generic tacrolimus is available at less cost in our country since 2015. There are only a few studies that compare the effectiveness and safety of generic tacrolimus and brand tacrolimus in the literature. Although other bioequivalent medications such as antihypertensives or antidiabetics can be monitored with blood pressure measurements or blood glucose levels, no test measures the effect of immunosuppression provided by generic immunosuppressive agents. Keeping the balance between serious toxicity and adequate immunosuppression is essential. Though the generic tacrolimus has been found safe and effective in studies, the skepticism about generic immunosuppressive drugs seems consistent [9-14].

The aim of this study was to compare clinical outcomes that include acute rejection, graft loss and adverse reactions of the patients administered either brand tacrolimus (Prograf, Astellas Pharma, USA) or generic tacrolimus (Adoport, Sandoz, UK) from the outset of renal transplantation day (de-novo use) in our transplant center.

MATERIALS and METHODS

Renal transplant patients that were transplanted in our renal transplantation unit between January 1, 2015 and March 1, 2020 were screened. All patients that were administered *de novo* generic tacrolimus (n:51) and 102 randomly selected control renal transplant recipients that were administered original tacrolimus included in this study.

All of the patients in this study had been received basiliximab (20 mg each intraoperatively and on day 4) or anti-thymocyte globulin ATG (1,5-3 mg/kg) depending on the immunological risk profile. 500 mg of intravenous methylprednisolone had also been given intraoperatively as a component of induction therapy.

Tacrolimus had been started from the 3rd day before the operation with a total dose of 0,1 mg/kg twice daily. In addition to tacrolimus, mycophenolate mofetil/mycophenolate sodium or steroids had been used for the maintenance immunosuppression regimen.

We evaluated and recorded Demographic, clinical and laboratory data including age, sex, primary renal disease, type of donor (living or deceased), induction and maintenance regimen, the dose of tacrolimus, tacrolimus trough levels, serum creatinine, biopsy-confirmed acute rejection (BCAR) episodes during the first year after transplantation, delayed graft function (DGF), BK polyomavirus

(BKPyV) positivity in urine, BKPyV associated nephropathy (BKPyVAN), cytomegalovirus (CMV) infection, adverse reactions that require switching medications in a 1-year follow-up.

BKPyV positivity in urine was defined as urinary BK virus copy number in any urine sample that was >107 copies/L. CMV infection was considered present if the patient was recommended to start antiviral treatment by infectious disease department along with any level of viremia. DGF was defined as the requirement of dialysis during the first 7 days post-transplant.

Statistical analysis

Data were collected and analyzed using IBM SPSS Statistics Version 26.0. Numerical variables are summarized with mean ± standard deviation or median (minimum-maximum) values and categorical variables are presented as numbers and percentages. The chi-square test was used to determine the relationships between categorical variables. Variables with normal distribution were compared by the t-test. $P < 0.05$ was considered statistically significant.

Ethical approval

The study was approved by the local ethics committee of Hacettepe University Medical Faculty and was conducted in accordance with the Declaration of Helsinki. Ethics committee approval date is 15.06.2021, approval number is GO21/535.

RESULTS

A total of 153 patients, 102 in the original tacrolimus group and 51 in the generic tacrolimus group, were included in this study. The demographic characteristics of the patients are presented in Table 1. The mean age of the study population was 38.3±13.0. Majority of the patients were male (98, 64.1%). Renal transplantation had been performed from living donors in 139 (90.8%) of the patients. Preemptive transplantation had been performed in 83 patients. There were no significant differences with regard to the demographic characteristics between the original and generic tacrolimus groups.

2 patients had delayed graft function. One of these patients was transplanted from a cadaveric donor with 9-hour cold ischemia time and one from a living donor. Both of them had biopsy-proven acute cellular rejection within 1 month of post transplantation. Post-transplant rejection and viral complications are presented in Table 2.

Nineteen of patients who used original tacrolimus and 9 of patients who used generic tacrolimus had BCAR in the first year after the transplant. There were no statistically significant differences in acute rejection within post-transplant 1 year between the two groups (p:1). There were no tacrolimus-related side effects that required drug changes in either group in one year follow up period.

Table 1. Demographic characteristics

	Original tacrolimus (n:102)	Generic tacrolimus (n:51)	Total (n:153)	P value
Age (mean)	38.0	38.9	38.3 (± 13.0)	0.7
Sex (F/M)	34/68	21/30	55/98	0.3
Etiology of renal disease				
Hypertensive nephrosclerosis	9	5	14	
Diabetic kidney disease	2	2	4	
Chronic glomerulonephritis	18	15	33	
Congenital urogenital anomalies	12	6	18	
Amyloidosis	5	3	8	
Other renal diseases	17	4	21	
Unknown	39	16	55	
Donor				
Cadaveric	11 (10.8%)	3	14	0.04*
Living	91 (89.2%)	48	139	

Table 2. Post-transplant rejection and viral complications

	Original tacrolimus (n:102)	Generic tacrolimus (n:51)	Total (n:153)	P value
BCAR				1.0
T-cell mediated	14	7	7	
Antibody-mediated	1	1	1	
Mixed	4	1	1	
CMV viremia	15 (14.7%)	11(21.6%)	11(21.6%)	0.36
BK viruria	11 (10.8%)	9 (17.6%)	9 (17.6%)	0.30

*BCAR: biopsy-confirmed acute rejection, CMV: cytomegalovirus

In the first post-transplant year, the BK virus positivity in urine was observed in 11 patients (10.8%) and 9 patients (17.6%) who were using original and generic tacrolimus, respectively.

CMV viral load positivity in blood was observed in 15 patients (14.7%) of the original tacrolimus group and 11 patients (21.6%) of the generic tacrolimus group within 1 year after transplantation.

BK virus positivity in urine and CMV viremia were not significantly different between the two groups (p: 0.30, p: 0.36, respectively). BKPyVAN was not detected in any of the patients.

There was no difference between the two groups in terms of creatinine levels, tacrolimus dose and tacrolimus trough levels at discharge and at first year (Table 3).

DISCUSSION

Since tacrolimus is an immunosuppressive drug with a narrow therapeutic index, it is important that bioequivalent should be stringent similar to the original tacrolimus to provide adequate immunosuppression without developing toxicity. There are many studies from all over the world that showed the safety and effectiveness of several generic tacrolimus in both *de novo* use and also after conversion from original tacrolimus in kidney transplant patients since 2008 [9,11,12,15,16]. We believe that these studies may have different results in different races due to pharmacogenetic diversity and increasing these studies will provide useful data for clinicians dealing with patients using immunosuppressive drugs.

A prospective, multicenter, parallel-group, open-label study in *de novo* kidney transplant patients was published in 2017. The patients in this study were randomly assigned to receive generic tacrolimus (TacHexal) and the original tacrolimus (Prograf). The study was conducted with a total of 73 patients, 35 in generic and 38 in original tacrolimus groups. At the end of the 6-month follow-up, it was shown that drugs were similar in pharmacokinetic terms and there was no difference in terms of BCAR, safety, adverse reactions. However, the authors stated that it may not be valid for other generic drugs [9].

In a study by Robertson et al. in elderly patients, it was stated that the generic tacrolimus they used (Tacni; Teva Pharmaceutical Industries Ltd., Petah Tikva, Israel) was not bioequivalent to the original tacrolimus, and a higher dose should be given to obtain a similar tacrolimus trough level, which may increase the incidence of side effects in the long term [17]. As a result of these studies, it was thought that there may be differences between generic drugs and individual variability may

Table 3. Laboratory results and tacrolimus dose at discharge and at first year

	Original tacrolimus	Generic tacrolimus	P value
Cre (mg/dL) at discharge	1.17±0.39	1.03±0.27	0.09
Cre (mg/dL) at first year after transplant	1.26±0.40	1.20±0.35	0.18
Tacrolimus level at discharge	7.5±3.7	7.6±2.5	0.68
Total dose at discharge (mg)	6.9±2.1	7.5±2.1	0.09
Tacrolimus level at first year	6.7±2.4	6.3±2.2	0.52
Total dose at first year (mg)	3.6±1.6	4.2±1.9	0.07

Cre: creatinine

contribute to these differences. Therefore, studies were continued with different generic tacrolimus preparations.

To our best knowledge, the study from the South West Transplant Center is the first study to compare the clinical outcomes of patients receiving generic tacrolimus with the original tacrolimus. Conner et al. reported in this study that there was no difference in clinical outcomes between the two groups in *de novo* use during the 6-month follow-up period [14].

In a *de novo* study with the same generic tacrolimus as in our study, the 6-month results of a total of 120 kidney transplant patients were evaluated. Renal function, tacrolimus trough concentrations and tacrolimus dose, acute rejection incident, delayed graft function were similar between the two groups as in our study. Additionally, in this study, the patients were evaluated histopathologically with the protocol biopsy performed at the 6th month, and *de novo* DSA evaluation was performed with the single antigen bead assay, and no difference was found in either of them [15].

In a study from our country, evaluating the data about conversion from original tacrolimus to generic tacrolimus in 36 patients, it was stated that generic tacrolimus was safe and effective [18]. Similar to our study, in another study from our country, the data of 145 patients using *de novo* generic tacrolimus were presented, the authors stated that there was no difference in renal function, adverse reaction, BKPyV and CMV viremia, acute rejection at the end of a median of the 31-month follow-up period [19].

We have compared the clinical outcomes within 1-year after transplantation of 102 patients *de novo* receiving original tacrolimus and 51 patients *de novo* receiving generic tacrolimus at a single center. No differences were found in terms of creatinine levels, total daily dose of tacrolimus and tacrolimus trough levels at discharge and at the first year. Additionally, biopsy-confirmed acute rejection in the following year after transplantation, BKPyV positivity in urine, BKPyVAN, CMV viremia and adverse reactions related to tacrolimus were similar between the two groups.

With this study, we aimed to give our experience in the use of generic tacrolimus from our country to the literature. As a result of our study, we stated that generic tacrolimus can be safely preferred in *de novo* use with close drug level monitoring since it is an immunosuppressive agent with a narrow therapeutic index.

The requirement for a multicentric study with a randomized prospective design and large patient number continues.

Author contribution

Study conception and design: NSC, TY; data collection: NSC; analysis and interpretation of results: NSC, TY; draft manuscript preparation: NSC, TY, ŞRY, YE. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee of Hacettepe University (Protocol no. GO 21/535 /15.06.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Eighteen years of the medical scientist training program at Hacettepe University

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ABSTRACT

Objective: Medical Scientist Training Program (MSTP) combining acquisition of both MD and PhD degrees, was implemented in 2003 at Hacettepe University. The purpose of this report is to evaluate the program outcomes by assessing the graduates from the first 18 years of the program.

Materials and Methods: A web survey was conducted with the 37 participants who graduated between 2009-2020. Data were analysed using descriptive statistical methods.

Results: About half of the graduates were found to devote a considerable amount of time to scientific research. Although nine participants do not perform any physician duties, about a quarter of graduates concomitantly pursue scientific as well as clinical activities. This implies that the program's primary goal, to train clinician-scientists in both MD and PhD curriculums have been achieved. 90% of graduates completed their residency in 24 different clinical disciplines. 40% of the graduates have already achieved faculty status at universities in Turkey or abroad. Academic performance indicators of MSTP graduates including the number of publications and citations in leading databases and the number of grants received were notably high.

Conclusion: This study reveals the role of the MSTP at Hacettepe University towards education of highly qualified clinicians with academic and scientific activities.

Keywords: Medical education, MD-PhD, medical scientist training program, students, graduates

INTRODUCTION

Understanding pathophysiological processes at the molecular, cell, and tissue level is essential for the development of novel diagnostic and treatment approaches, that is, the goal of modern biomedical research. Building bridges between basic research results from the laboratory bench and clinical applications at the bedside is necessary to achieve this goal. Researchers with experience in both clinical and fundamental sciences can successfully fill this bridging function owing to their capabilities and talents in both areas [1-3]. To match the growing demand for physician-scientists around the world, Medical-Scientist Training Programs (MSTP), which combine medical and Ph.D. trainings hold promise. The programs are most prevalent in the United States of America and Canada where they originated, but during the past 20 years they have spread across Europe, Australia, and New Zealand as well. The main goal of these programs is to expose medical students to basic scientific research at an early point in their career while maintaining their focus on the clinical curriculum. Students are selected based on their potential to concomitantly pursue a career in basic and clinical sciences, conduct independent research, and significantly contribute to their disciplines in the

future. Expectedly, studies examining the outcomes of primarily the National Institutes of Health (NIH)-funded MSTP programs in the United States since 1964 have revealed that a higher percentage of MSTP graduates obtain academic positions than typical for medical school graduates [4,5].

In Europe, MSTPs were initially introduced in the 1990s in England, Sweden, and Switzerland; however, the number of programs is significantly lower than in North American countries [3]. There are only few articles examining European program outcomes [6,7], unlike abundant reports on North American, Australian, and New Zealand programs [3,8-12].

The MSTP in the Hacettepe University Faculty of Medicine was implemented in 2003, following approval by the Higher Education Council (Yükseköğretim Kurulu, YÖK) of Turkey. A total of 175 students were admitted to the program and 47 graduated earning both MD and PhD degrees by 2022, when this report was prepared. Briefly, the dual program begins with enrollment of selected medical students at the start of their third year of medical school (Phase III) (Figure 1). They are given privileged pre-PhD student status for the first two years (phases III and IV of medical school), which allows them to take PhD courses from various programs that correspond to their interests. After being matched to the core PhD programs at the beginning of the fifth year of medical school (phase V), they complete the courses required by the core PhD program, then pass the proficiency exam, and prepare the subject of their dissertation project. At the end of phase V, they freeze their medical education for 2 years, during which time they work on their dissertation project full time. They resume to the final year of their medical education (internship, phase VI) after this 2-year break to complete their medical education and Ph.D. dissertation study. At the end of the eighth year of medical and sixth year of Ph.D. training, the students first receive their M.D. and then Ph.D. degree after successfully defending their dissertation. The 2-year break was implemented in 2016 to allow them to solely focus on their dissertation projects and to avoid conflicts with other curricular activities including residency programs.

In this first systematic analysis of the program outcomes, we have assessed the professional and academic standing, attitudes and opinions toward the program of our 37 students who graduated within the first 18 years (2020 and before).

MATERIALS and METHODS

Study Design

The study protocol and survey were approved by the Hacettepe University Ethics Board (Approval No. GO 22/1215). Students who graduated

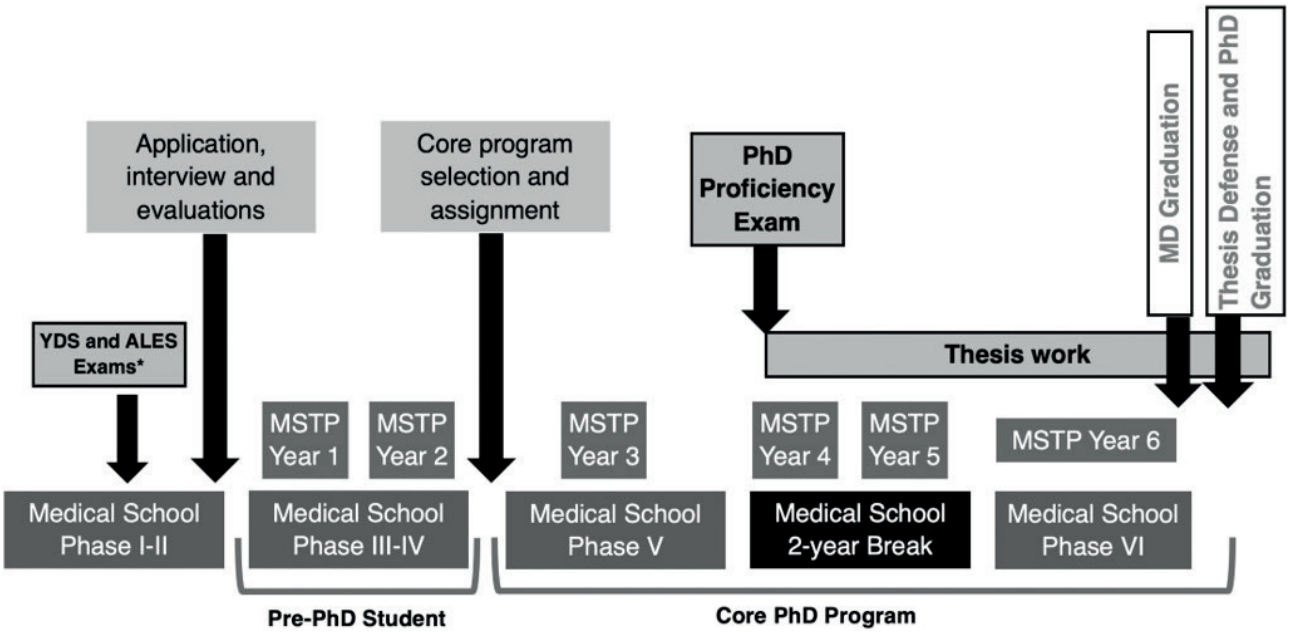

Figure 1. Flowchart of the curriculum for the Hacettepe University Medical Scientist Training Program
YDS: Foreign Language Proficiency Exam (for English), ALES: Academic Personnel and Graduate Education Entrance Exam.

between 2009 and 2020 (corresponding to their admission between 2003 and 2012) were selected from our student database. The survey form (a Google Web form) and study information were sent to the students through email. Two reminder emails were issued to non-responders at intervals of two weeks and the survey responses were electronically saved in Microsoft Excel format, and 25 graduates responded to the survey at the end of the survey response period, out of a total of 37 graduates. The names of 12 non-responders were entered into the Google search bar together with the phrase "Hacettepe", and the institutional web sites of those positions identified from the search results were used to record the current positions and academic activities of the non-responders. Current online professional profile of one of the non-responders could not be reached. Details of the Ph.D. education records of the 37 graduates were acquired from our student database, while their academic performance indexes were obtained from Web of Science. These investigations were conducted during the first quarter of 2022.

Survey Form

40 multiple-choice or short-answer questions about the profession, positions, scholarly output, financial support and grants, and career goals were included on the survey form. Additionally, they were asked to rate five statements on a 5-point Likert scale (1=Totally disagree; 2= Disagree; 3= No idea; 4=Agree; 5=Totally agree) based on their subjective attitudes and opinions about the program. Finally, they were asked two open-ended questions about the aspects of the program that they thought were the most successful and where they thought it might be improved.

Data Analysis

Data were analyzed using SPSS Version 23 for descriptive statistics, and graphs were prepared using GraphPad Prism 7. Data are expressed as median and interquartile range (IQR), unless otherwise indicated. Responses to open-ended questions were grouped into common thematic categories and analyzed.

RESULTS

PhD Education

In 2022, the median age of graduate responders was 37 (30-38). The gender split was 19:18 (M:F). The average number of years from admission to graduation was 8 (Range: 5–15). It should be noted that students who graduated within 5 years of admission did not have a mandatory 2-year break as they had been enrolled before the year 2016. Two students graduated 15 years after admission had initially been expelled from the program but returned with academic amnesty. Tumor Biology and Immunology had the highest number of graduates among all the programs with 10 participants. It was followed by Neuroscience, Medical Biology and Medical Pharmacology programs. Figure 2 shows the distribution of 37 graduates within the core programs.

Post-Graduate Activities

Thirty-three participants (89 %) have already completed or are about to complete their medical residency program. Table 1 shows the distribution of speciality fields. One postdoctoral researcher living in Turkey and three others abroad chose not to pursue residency. Fifteen graduates completed residency as well as postdoctoral fellowship, 4 of whom additionally completed their obligatory medical service (OMS) in Turkey[1]. Notably, 18 except one graduate did their postdoctoral fellowship at universities abroad, whereas residency training preference was split between hospitals in Turkey and abroad. The majority of 19 graduates who performed their OMS chose to continue their career with clinical duties mainly in Turkey, without doing a postdoctoral fellowship,

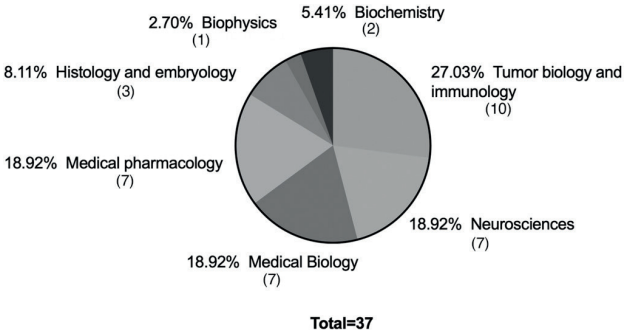

Figure 2. Distribution of graduates within the core programs

1 Medical education, residency and fellowship, each has an obligatory medical service carried out in the field for 1-2 years in Turkey.

Table 1. Distribution of medical residency fields

Department	Number of students (Percent%)
Neurology	3 (9%)
Internal medicine	3 (9%)
Psychiatry	2 (6%)
Obstetrics-Gynecology	2 (6%)
Pulmonology	2 (6%)
Cardiology	2 (6%)
Pathology	2 (6%)
Pediatrics	1 (3%)
Pediatric neurology	1 (3%)
Pediatric metabolism	1 (3%)
Pediatric nephrology	1 (3%)
Pediatric gastroenterology	1 (3%)
Pediatric immunology	1 (3%)
Pediatric pulmonology	1 (3%)
Radiation oncology	1 (3%)
Cardiovascular surgery	1 (3%)
Medical genetics	1 (3%)
Medical biochemistry	1 (3%)
Radiology	1 (3%)
Neurosurgery	1 (3%)
Hematology and oncology	1 (3%)
Otorhinolaryngology	1 (3%)
Dermatology	1 (3%)
Ophthalmology	1 (3%)
TOTAL	33 (100%)

while those who postponed the OMS went abroad as postdocs except one out of 19, suggesting that the decision of performing OMS appears to depend on the decision of where early career postgraduate training will be performed, abroad or Turkey (Figure 3).

As of 2022, 20 of our graduates (54 %) are employed in Turkey. Fourteen of the 16 graduates who are still employed abroad (38 %) are based in the United States, while two are in Europe. Out of 36, only three graduates practice as physicians, two in private healthcare facilities and one in a state hospital. The remaining 33 graduates work at Turkish or international academic institutions. Of these, 10 (30%) have achieved the title of principal investigator, 6 are postdoctoral or staff researchers (18%), while 17 graduates (52 %) are primarily practicing medicine (Figure 4a). One graduate who is known to have done postdoctoral fellowship after residency in Turkey, has been eventually lost

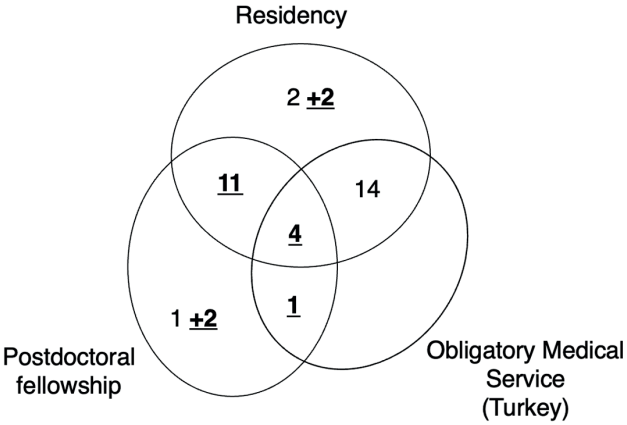

Figure 3. Venn diagram showing number of participants who completed their residency, postdoctoral fellowship or obligatory medical service. Regular typeset numbers indicate all positions held in Turkey, whereas bold and underlined numbers indicate either residency or research fellowship completed abroad.

to follow up and her current academic position or location is unknown. It took a median of 6 years (Range: 1–10 years) for graduates to acquire their first independent position as principal investigator. Seven graduates returned to Hacettepe University as academicians, and 5 more are employed as faculty members by other Turkish universities, bringing the total number graduates who achieved faculty positions to 12, as of 2022.

Eleven out of 36 graduates (31 %) devote at least 70% of their working hours to scientific research

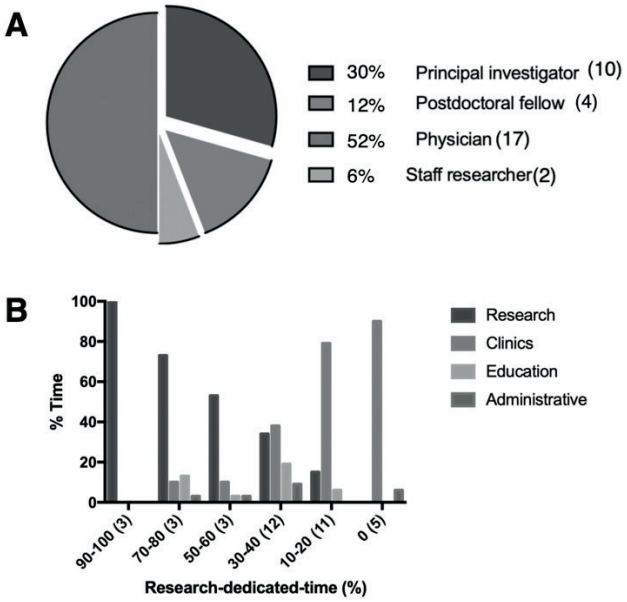

Figure 4. (A) Distribution of employment categories of graduates working at academic institutions (B) Distribution of clinical, educational and administrative activities relative to the time devoted to scientific research.

and educational activities. Nine of these eleven do not have clinical responsibilities. The remaining 16 graduates have at least 70% (or 44%) of their working hours dedicated to clinical activities. Five of these 16 do not engage in any scientific research. On the other hand, 9 graduates (29%) reported that they divided their time equally between clinical work and scientific research. Figure 4b displays the distribution of the time devoted to research and other activities.

Academic Performance

Twenty graduates (56%) published at least one article prior to receiving their PhD and 19 graduates (53%) had their dissertation published by 2022. The median number of SCI-indexed publications for our graduates is 15 (IQR: 15.4). They have a median of 5 (IQR: 7) first-author publications, and 10 graduates have published at least one article as the last author. Five graduates have H-indices above 10, and the median H-index is 4 (IQR: 5). The median number of citations is 64 (IQR: 274). Fourteen graduates (39 %) have received honors from at least one international academic organization, and two graduates (5 %) have authorized patents.

Participants graduating in 2016 or earlier (i.e., at least 5 years have passed since graduation) were more likely to have grants as principal investigators if they hold research-focused positions or equally divided their research and clinical employment. Seventeen graduates (47%) have ongoing or completed research grants (national or international). Six of these principal investigators devote almost all their time to scientific research. Four of the 16 graduates who devote less than 30% of their working time to research also have active or completed grants. Participants who graduated in 2016 or before are more likely to hold grants as principal investigators, particularly if they have research-focused or balanced research-clinical employment positions (Figure 5).

Currently, six participants are serving as PhD student advisors. Five of these six participants devote practically all their working hours to research, and one equally splits his time between clinical and research responsibilities.

Attitudes and Opinions Toward the Program

Among the 25 responder 96% agree that the MSTP has significantly affected their professional careers and contributed to the creation of Turkey's new generation of young scientists in medicine (Table 2). Fourteen (14/25) of these graduates feel to have profited from the curriculum by developing their skills and perspectives in scientific research. Four participants see the creation of an opportunity to work at distinguished scientific centers overseas as the main advantage of the program. For five of the participants, it provided a chance to network and be introduced to eminent scientists on a local, national, and international scale.

Two of our graduates do not advise a new medical student to enroll in the MSTP. One of them saw uncertainties in the future of the program as the main issue, while the other graduate has expressed concern about the lack of a workable and practical plan. It is interesting to note that both graduates concur that the program was successful in steering their careers and that it significantly contributed

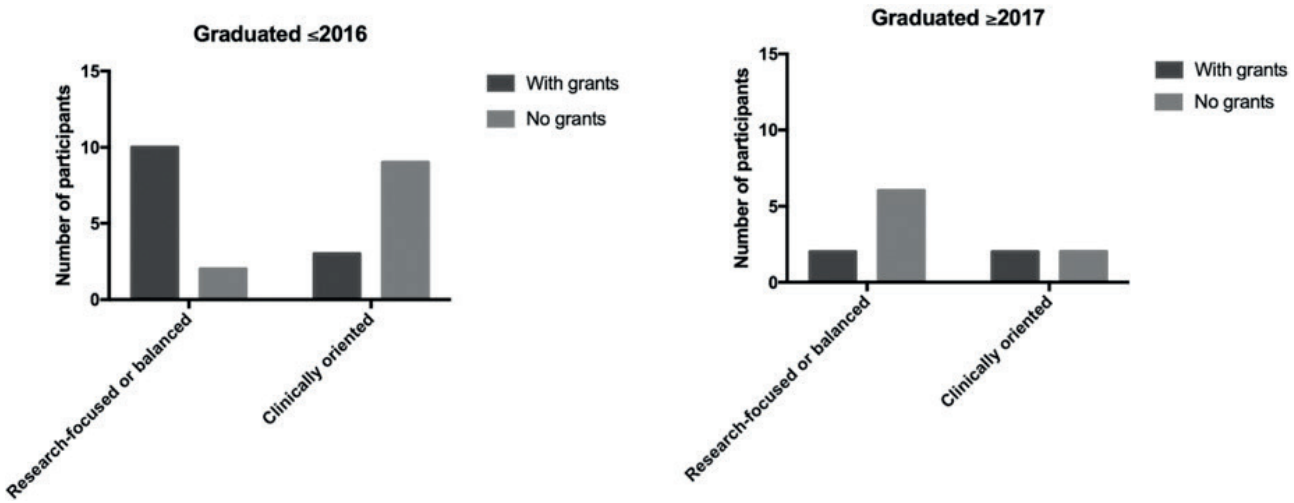

Figure 5. Grant ownership as principal investigators with respect to post-graduation and research-devoted time.

to the education of scientists in Turkey, suggesting that they expected a more structured roadmap to be offered for the program.

Fourteen respondents believe that they need to spare more time to engage in scientific research but do not have enough time to do so (Table 2). Six of these graduates are employed in clinically focused positions, while five of them have more balanced time for research and clinical duties.

The conflict between clinical internship and doctoral course programs, is the most frequent criticism about the program voiced by 9 participants who had been enrolled before 2016, i.e. before the 2-year break was implemented. The other primary complaints included the insufficient financial support for research projects, scholarships, and international activities (5 participants) and the limited time allotted for research and dissertation studies (5 participants).

DISCUSSION

The first 18 years of Turkey's first MSTP are reported in this study together with the distribution of alumni output and current professional status of the graduates. The program's primary goal, to train clinician-scientists who have mastered both MD and PhD curriculum seems to have been largely achieved since at least half of the program graduates devote a sizable amount of time to scientific research, and especially since nearly a quarter of them conduct both scientific and clinical activities simultaneously. Altogether, 32 (86%) of the 37 graduates are involved in scientific research to some extent, and more importantly, they wish to increase the time they devote to scientific research. The amount of time devoted to research and clinical activities varies considerably, similar to previous reports from MSTP graduates in the USA [5]. Therefore, like earlier surveys, this study also suggests that incentives should be considered for nearly half of the graduates who cannot devote enough time to advance their experience in scientific research. Although it is concerning that only 50% of doctoral theses written by graduates have been published (the publication process for theses that are ready to be published may still be in progress), the requirement that the PhD thesis be published before the thesis defense has recently been added to the graduation requirements, which may soon significantly increase the rate of published theses.

The majority of graduates have also completed their residency training in diverse specialty fields, comparable to graduates from MSTP programs abroad [5,13]. Compared to the regular graduate program, MSTP motivates students to stay in academia at a high rate, as evidenced by the fact that almost 40% of the graduates hold faculty status at Turkish and international institutions. However, the data from MD PhD candidates who were not elected due to the quota limits could have been a better comparator to separately investigate the impact of the intuitive motivation of the student with the motivation MD PhDs earned during training. Yet, the program's recognition and its contribution to the number of qualified researchers in our country are particularly evident in the fact that 12 of our graduates hold academic positions in various Turkish universities. Our graduates take six years on average to obtain their first independent academic position, which is comparable to graduates of similar programs in the United States [13]. The outstanding academic performance indicators of

Table 2. Some subjective attitudes of graduates towards the MSTP

	Strongly agree	Agree	No idea	Disagree	Strongly disagree
My professional career has been significantly influenced by the MD-PhD Integrated program.	17 (%68)	7 (%28)	0 (%0)	1 (%4)	0 (%0)
I would recommend a student who is just starting medical school to join the MD-PhD program.	15 (%60)	4 (%16)	4 (%16)	2 (%8)	0 (%0)
I'd like to spend more of my working hours on scientific research, but I can't seem to find the time.	11 (%44)	3 (%12)	4 (%16)	2 (%8)	5 (%20)
I believe that Turkey's MD-PhD program helps to train scientists in the field of medicine.	22 (%88)	2 (%8)	1 (%4)	0 (%0)	0 (%0)
My financial situation would have been better if I hadn't started this program.	3 (%12)	3 (%12)	10 (%40)	2 (%8)	7 (%28)

MSTP graduates, such as the number of citations and the number of research grants obtained, are also in line with those of the North American MSTP programs [2,14].

CONCLUSION

In conclusion, this study reveals the role of the Hacettepe University Faculty of Medicine's MSTP in educating highly qualified academicians and medical scientists in Turkey. We believe that this contribution will be strenghthened even further when more MD-PhD graduates are promoted to the top academic administration posts in Turkey and have greater access to research funding. The Higher Education Council of Turkey have recently approved the establishment of new MD and PhD programs ina some of Turkey's leading medical schools. Together these encouraging developments may lay down the foundations of world class scientific discoveries in Turkey within the next decades. We conclude that the MSTP has a significant potential to increase interest in science among medical students at an early stage of their career and to recruit increasing number of clinician scientists in medicine.

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Author contribution

Study conception and design: ŞEE, AÖ and TD; data collection: ŞEE, AÖ; analysis and interpretation of results: ŞEE, AÖ, AE and TD; draft manuscript preparation: ŞEE, AÖ, AE and TD. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Hacettepe University (Protocol No: GO 22/1215).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Clinical results of patients with variceal bleeding and risk analysis of scoring systems

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ABSTRACT

Introduction: Gastroesophageal varices are a common complication of chronic liver disease and the associated portal hypertension. Gastroesophageal variceal bleeding is the most important cause of mortality in cirrhotic patients, and the risk of developing varices and bleeding significantly increases when hepatic venous pressure gradient (HVPG) exceeds 10-12 mmHg.

Aim: In this study, we aimed to determine the most useful scoring system to assess patients with gastric and esophageal variceal bleeding to guide treatment according to the type of varices, to predict the risk of rebleeding and mortality, and to determine the relationship between types of varices, comorbidities, and mortality.

Results: We retrospectively analyzed the files of 566 patients who presented to the Emergency Internal Medicine Department with gastrointestinal hemorrhage. Among these, we recruited 117 patients who were diagnosed with varices. Hematemesis and melena were significantly more common in patients with esophageal variceal bleeding compared to patients with gastric variceal bleeding (p=0.025 and p=0.036, respectively) Among the analyzed scoring systems, the Child-Pugh score most successfully predicted mortality with the highest AUC value (AUC: 0.851, 95% CI: 0.770-0.932, p<0.0001)

Conclusion: Assessment with scoring systems upon admission is useful for risk classification and prediction of mortality risk. In this context, the Child-Pugh score can be used to assess acute variceal hemorrhages.

Keywords: variceal bleeding, risk analysis, Child-pugh score

INTRODUCTION

Gastroesophageal varices are a common complication of chronic liver disease and the associated portal hypertension. The incidence of gastroesophageal varices correlates with the severity of chronic liver disease and they occur in approximately 50% of all patients, with an annual risk of approximately 8%. Gastroesophageal varices predominantly result from increased resistance to portal flow secondary to regenerative nodules and fibrosis, intrahepatic vasoconstriction, splanchnic vasodilation, and increased portal flow [1]. Gastroesophageal variceal bleeding is the most important cause of mortality in cirrhotic patients, and the risk of developing varices and bleeding significantly increases when hepatic venous pressure gradient (HVPG) exceeds 10-12 mmHg [2]. The most important risk factors for variceal bleeding are the size of the varices and having decompensated disease, and the annual risk of hemorrhage is approximately 15% per year [3]. The literature reports variable results regarding the diagnostic value of the numerous scoring systems that have been developed to predict mortality due to variceal bleeding in cirrhotic patients, and there currently is no consensus.

In cirrhotic patients, scoring systems are crucial to predict prognosis in order to reduce the risk of varices and variceal bleeding, to determine the appropriate intervention and follow-up method for variceal bleeding, and to reduce the risk of rebleeding to improve survival and quality of life. In this study, we aimed to determine the most useful scoring system to assess patients with gastric and esophageal variceal bleeding to guide treatment according to the type of varices, to predict the risk of rebleeding and mortality, and to determine the relationship between types of varices, comorbidities, and mortality.

MATERIALS and METHODS

We retrospectively analyzed the files of 566 patients who presented to the Emergency Internal Medicine Department with gastrointestinal hemorrhage between October 2020-october 2021. Among these, we recruited 117 patients who were diagnosed with varices. All patients underwent gastroscopy within twenty-four hours. Patients presenting with gastrointestinal bleeding were treated with proton pump inhibitors, and all patients with variceal bleeding were treated with somatostatin analogues. The treatment was reviewed and revised daily. The patients were aged between 19 and 89 years. We excluded patients who were aged below 18 years and patients who were diagnosed and started treatment in a different center and were then referred to our hospital. The data obtained from hospital HBYS

the Hospital Information Management Systems (HIMS) notes. Age, sex, symptoms indicating bleeding, concomitant diseases, endoscopic and/or surgical treatments, and follow-up results were recorded from the patients' files. The Rockall score takes into account age, presence of shock, comorbidities, diagnosis, and the type of lesion that is the cause of the recent bleeding after endoscopy [4]. The Glasgow-Blatchford score is calculated using blood urea nitrogen level, hemoglobin, pulse rate per minute, systolic blood pressure, melena, hepatic disease, syncope, and/or cardiac failure and does not require endoscopic data [5]. AIMS65 is based on the criteria of pre-endoscopy serum albumin and international normalized ratio (INR) levels, altered mental status, age, and systolic blood pressure [6]. The MELD-Na score is a combination of serum sodium (Na) levels and the MELD score, which is calculated based on serum bilirubin, creatinine, and INR levels, and it aims to predict the prognosis of cirrhotic patients [7]. The Child-Pugh classification is used to determine the severity of cirrhosis based on the extent of hepatic encephalopathy, ascites, and serum bilirubin, albumin, and INR levels [8]. Assessment scores were calculated during the patients' hospital stays. We analyzed the diagnostic value of the applied scoring systems. The principles of the Helsinki Declaration were followed throughout the research. Mortality observed during hospitalization was noted.

This study was confirmed by the local ethics board (Number: E1-21-2032) on 20.10.2021, and no written informed consent form was obtained from patients.

Statistical analysis

Data were analyzed using SPSS 25.0. Categorical data were expressed as numbers and percentages,

and continuous data as mean ± standard deviation and median (minimum and maximum, interquartile range). According to endoscopy findings, patients were classified into two groups according to esophageal and gastric varices. The Kolmogorov-Smirnov test was used to analyze whether patient ages were normally distributed for each group. Patient ages did not show normal distribution. Therefore, Pearson's chi-square test was used for the analysis of categorical variables. Receiver operating characteristic (ROC) curves were used to assess and compare the diagnostic value of each scoring system. Subsequently, the area under the curve (AUC), sensitivity, and specificity were calculated. Values of $p<0.05$ were accepted as statistically significant.

RESULTS

The average ages of patients with esophageal and gastric varices were 62.96±14.66 years and 56.52±14.18 years, respectively (Table 1). There were 64 male (54.71%) and 53 female (45.29%) patients. Nine (7.69%) patients were discharged within 24 hours, whereas 42 (35.89%) were admitted to the ward and 66 (56.41%) were admitted to the intensive care unit. Endoscopic procedure could not be performed in the acute period due to clinical instability in 23 (19.65%) of the patients who had variceal bleeding, 2 (1.71%) were treated with argon plasma coagulation, 1 (0.85%) with hemoclip, 59 (71.79%) with band ligation, and 7 (5.98%) with sclerotherapy. Seventy-three (62.40%) patients required endoscopic re-intervention. While 30 (25.64%) patients did not require a blood transfusion, 27 (23.08%) were transfused with 1 unit and 60 (51.28%) with more than 1 unit of blood products. Hematemesis and melena were significantly more common in patients with esophageal variceal bleeding compared to patients with gastric variceal bleeding ($p=0.025$ and $p=0.036$, respectively) (Table 2). In terms of comorbidities, chronic kidney disease was significantly less common in patients with gastric varices ($p=0.036$) (Table 3). In addition, appropriate antibiotic therapy was applied to all patients during their hospitalization.

Among the patients who underwent endoscopic treatment for esophageal varices, 2 (2.17%) underwent transarterial embolization (TAE) and 2 (2.17%) surgery, and among patients with gastric varices, 2 (9.52%) underwent TAE and 1 (4.76%) surgery. Thirty-two (33.34%) patients with esophageal varices and 9 (42.86%) patients with gastric varices developed variceal rebleeding. In long-term follow-up, recurrent variceal bleeding was significantly more common in patients with gastric varices ($p=0.003$). Nineteen (19.79%) patients with esophageal varices and 4 (19.05%) patients with gastric varices died during follow-up (Table 4).

Among the analyzed scoring systems, the Child-Pugh score most successfully predicted mortality with the highest AUC value (AUC: 0.851, 95% CI: 0.770-0.932, $p<0.0001$) (Table 5) (Figure 1).

Table 1. Age distribution

Endoscopic Findings		n	Min.	Median	IQR	Max
Age	Esophageal varices	96	19	65	18	89
	Gastric varices	21	26	61	21	76

Table 2. The incidence of symptoms indicating bleeding

		Endoscopic Findings		p
		Esophageal varices (N: 96)	Gastric varices (N:21)	
Hematemesis	N	77	12	0.025
	%	80,21%	57,14%	
Melena	N	54	17	0.036
	%	56,25%	80,95%	
Hematochezia	N	16	1	0.161
	%	16,67%	4,76%	

Table 3. Other diseases accompanying variceal bleeding

		Endoscopic Findings		p
		Esophageal varices	Gastric varices	
Heart failure	N	5	0	0.285
	%	5,21%	0,00%	
Arrhythmia	N	7	0	0.202
	%	7,29%	0,00%	
Coronary artery disease	N	17	4	0.885
	%	17,71%	19,05%	
Chronic kidney disease	N	3	3	0.036
	%	3,13%	14,29%	
Chronic liver disease	N	87	18	0.502
		0,90625	85,71%	

TAE: Transarterial embolization.

Table 4. Follow-up results of patients who underwent endoscopic treatment

			Endoscopic Findings		p
			Esophageal varices	Gastric varices	
Surgical	TAE	N	2	2	0.177
		%	2,08%	9,52%	
	Surgical	N	2	1	
		%	2,08%	4,76%	
Rebleeding		N	32	9	0.407
		%	33,33%	42,86%	
Long-Term Rebleeding		N	17	10	0.003
		%	17,71%	47,62%	
Mortality		N	19	4	0.938
		%	19,79%	19,05%	

Table 5. AUC values indicating how scoring methods predict mortality

Test Result Variable(s)	Area Under the Curve (AUC)	p	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
Full Rockall Score	0.749	<0.001	0.639	0.860
Blatchford Score	0.782	<0.001	0.675	0.888
Child-Pugh Score	0.851	<0.001	0.770	0.932
MELD-Na Score	0.765	<0.001	0.653	0.877
AIMS65 Score	0.757	<0.001	0.638	0.877

DISCUSSION

Gastrointestinal varices are associated with portal hypertension and chronic liver disease and can potentially cause life-threatening hemorrhage [9]. In the setting of portal hypertension, the incidence of esophageal varices is higher compared to gastric varices. One study reported gastric varices in 25.10% and esophageal varices in 57% of cirrhotic patients [10]. Similarly, our patients predominantly had esophageal varices.

In our study, hematemesis and melena at admission were more common in patients with esophageal varices than in patients with gastric varices. One study reported that hematemesis was associated with increased mortality among cirrhotic patients [11]. The literature reports a higher risk of initial bleeding and long-term rebleeding for esophageal varices [12], similarly to our results.

Without proper treatment, the risk of rebleeding for esophageal varices is about 60%; thus, emergency intervention and appropriate treatment are vital

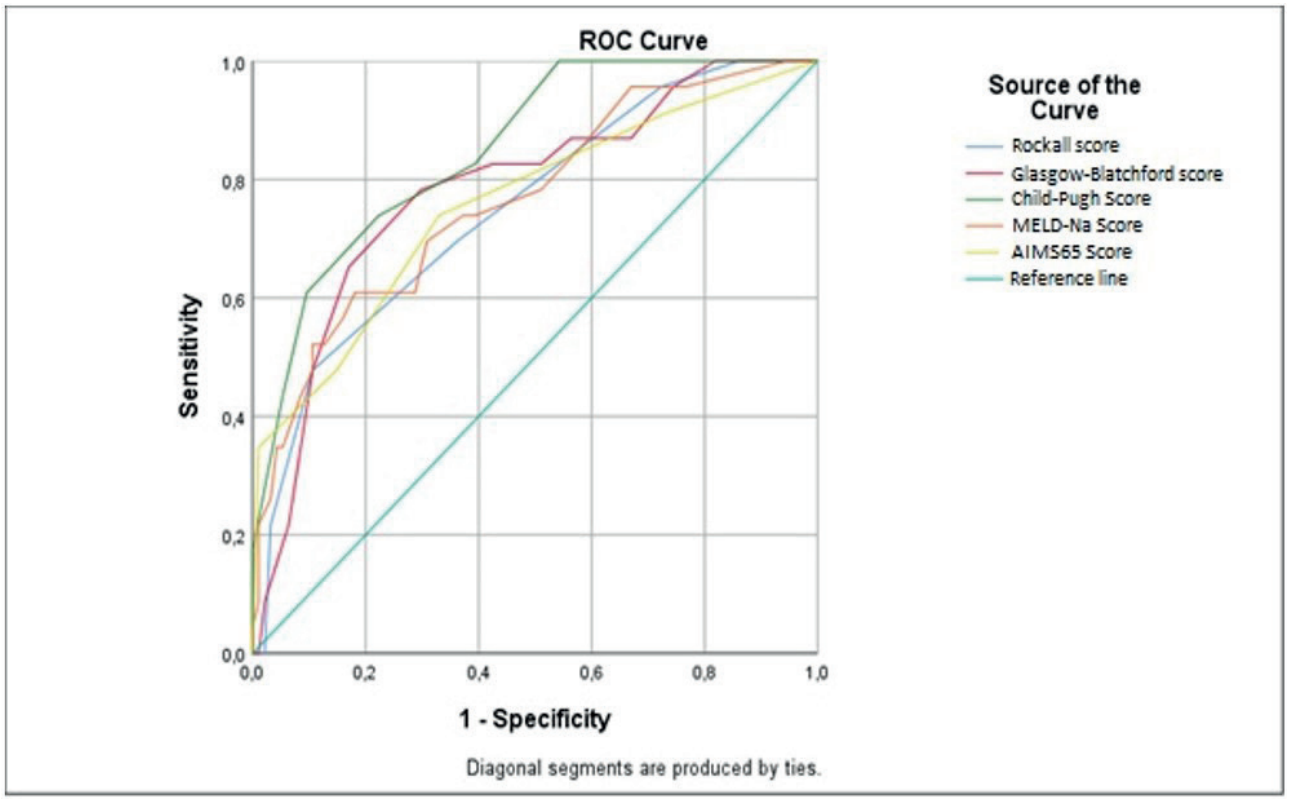

Figure 1. ROC curves indicating how scoring methods predict mortality

when managing variceal bleeding. Apart from endoscopic treatment, the initial interventions should include adequate volume replacement, achieving hemodynamic stability with blood product transfusions if needed, and administering vasoactive drugs to reduce portal blood pressure [13]. For some patients, this treatment approach is sufficient and endoscopic treatment will not be needed. These treatments are applied to patients who cannot undergo emergency endoscopic intervention. Similarly, the rate of patients who could not undergo an emergency endoscopic treatment was 23 (19.65%) in our study. These patients were followed up with vasoactive drug therapy in the acute period. Hemodynamic stability was tried to be achieved. As gastroesophageal varices can potentially cause massive bleeding, a significant number of patients require transfusion of one or more units of blood products, as was the case in our study.

If variceal bleeding is suspected, endoscopic intervention is required and should be performed within the first 12 hours [14]. Delayed endoscopic interventions are associated with a higher mortality risk [15]. Endoscopic band ligation has been demonstrated to be one of the most effective treatments of variceal hemorrhage and to reduce the incidence of rebleeding compared to sclerotherapy [16]. In our study, endoscopic band ligation was the predominant endoscopic intervention and was applied to more than half of all patients with variceal bleeding.

Although gastric varices are rarer and carry a lower risk of initial bleeding compared to esophageal varices, they are more likely to rebleed [17]. Gastric varices tend to be deeper and larger in size; therefore, endoscopic band ligation is less likely to be successful in patients with gastric varices [18,19]. Compared to esophageal varices, gastric varices are more likely to rebleed after band ligation due to exposure to gastric acids and pepsin and gastric peristalsis [20,21]. Consistently, the rebleeding rate was 42.86% vs. 33.34% for gastric versus esophageal varices in our study. Gastric varices carry an increased risk of gastrorenal shunt and therefore an increased risk of migration of the sclerosing substance into the systemic circulation; consequently, sclerotherapy is not an effective or safe approach for the treatment of gastric varices [22]. In our study, the predominant endoscopic treatment method was band ligation, and gastric varicose patients were more likely to require

surgical intervention and TAE due to rebleeding compared to patients with esophageal varices, consistently with the literature.

Gastric varices are observed in approximately 5-33% of cirrhotic patients and are associated with a lower risk of bleeding but higher mortality rates [23]. In our study, the mortality rate was 19.79% for esophageal varices and 19.04% for gastric varices. The fact that the mortality rate was lower in patients with gastric varices compared to esophageal varices in our study may be attributed to the small number of patients with gastric varices.

Large spontaneous gastrorenal shunts are more common in gastric varices than in esophageal veins, which allows for a comparatively lower portal pressure [22,24]. This phenomenon may explain the lower prevalence of chronic kidney disease in the gastric varices group compared to the esophageal varices group.

Predicting the risk of mortality due to variceal bleeding in cirrhotic patients helps guide clinicians in patient management, where patients with a high risk of mortality are followed and treated more closely in the intensive care setting. Numerous scoring systems have been developed to predict mortality in the setting of cirrhosis. It is well known that esophageal variceal bleeding is the most important cause of mortality in cirrhotic patients. One study used the AIMS65, MELD, APACHE II, and Child-Pugh scores to predict mortality in cases of acute variceal hemorrhage in cirrhotic patients and showed that the AIMS65 score had the highest sensitivity and specificity [25]. A different study reported that the AIMS65 and Rockall scores were superior to the other assessed scoring systems in predicting mortality [26]. In our study, the AIMS65 and Rockall scoring systems were less reliable compared to other scoring systems. This discrepancy may be attributed to our sample size and differences in the distribution of disease stages. One study reported that esophageal varices were correlated with the MELD score in cirrhotic patients [27]. A different study compared the Glasgow-Blatchford score, Child-Pugh score, and MELD score in predicting 1- and 6-week mortality in patients with esophageal variceal bleeding. Glasgow-Blatchford scoring was found to be superior to other scores in predicting 1-week mortality, whereas the MELD score was superior in predicting 6-week mortality [28]. One study demonstrated that the Glasgow-Blatchford score was superior in predicting the need for transfusion and additional interventions in patients with esophageal variceal bleeding [26]. Another study indicated that the MELD and Child-Pugh scores were the most valuable in determining 6-week mortality in cirrhotic patients with gastroesophageal varices bleeding [29]. Similarly, we found that the Child-Pugh score was the most valuable scoring system in predicting mortality due to variceal bleeding, followed by the Glasgow-Blatchford and MELD-Na scores. The Child-Pugh score can reliably predict the prognosis of cirrhotic patients together with endoscopic criteria including varices size, red wale sign (an endoscopic sign suggestive of recent hemorrhage), and recent variceal bleedings [3]. In reference to this information, the Child-Pugh score is an important tool for risk classification, treatment, and follow-up of cirrhotic patients with varices.

The limitations of this study were that, since it was evaluated retrospectively, we could not obtain information about long-term mortality and rebleeding rates. Therefore, prospective studies are needed on the relationship between the examined scores and long-term mortality.

CONCLUSION

Esophageal varices are at higher risk for bleeding whereas gastric variceal bleedings are at higher risk for rebleeding, mortality, and secondary intervention after endoscopy. Pre- and post-bleeding management of these patients is of vital importance for prognosis. Assessment with scoring systems upon admission is useful for risk classification and prediction of mortality risk. In this context, the Child-Pugh score can be used to assess acute variceal hemorrhages. Our study is noteworthy for shedding light on differences in the approach to treatment and follow-up in patients with portal hypertension with gastric versus esophageal varices.

Author contribution

Study conception and design: OE, EG, and NYÇ; data collection: EG, RE, BK, MB, NYÇ and OE; analysis and interpretation of results: NYÇ, SK and EG; draft manuscript preparation: NYÇ, EG, OE and SK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

This study was confirmed by the local ethics Ankara City Hospital Ethical 1 board (Number: E1-21-2032) on 20.10.2021.

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acta medica

ORIGINAL ARTICLE

Investigation of the anticancer, antimigration and antiangiogenesis effects of an oxadiazole derivative in two- and three-dimensional cultured Ishikawa and Huvec cells; *in vitro* and *in silico* studies

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ABSTRACT

Introduction: There are various methods used in cases of metastatic endometrial cancer. However, the prognosis is generally poor. Therefore, new anticancer agents are expected to exhibit the potential to prevent metastasis, as well as their effects on the viability of cancer cells. In in vitro conditions, the Ishikawa cell line represents endometrial adenocarcinoma and the Huvec cell line represents human umbilical vein endothelial cells. Oxadiazole derivatives may be promising new agents for endometrial cancer treatments by exhibiting anticancer and antiangiogenic properties.

Objective: The aim of this study was to examine the anticancer, antimigration and antiangiogenic effects of 5-[(4-Phenylpiperazine-1-yl)methyl]-1,3,4-oxadiazol-2-thiol (FP-Oxa) on co-cultured Ishikawa and Huvec cells.

Materials and Methods: In silico molecular docking, ADME and toxicity analyzes were performed for FP-Oxa. The effect on the viability of two- and three-dimensional mono- and co-culture models created with HUVEC and Ishikawa cells was evaluated by MTT analysis and IC_{50} values were calculated. The effect on the migration of two-dimensional cultured cells was determined by wound healing assay. Changes in VEGF expression in three-dimensional co-culture models were evaluated by immunofluorescence staining.

Results: As a result of in silico analyses, it was determined that FP-Oxa was within the oral bioavailability limits, exhibited class 4 toxicity, and had inhibition potential by binding to VEGR2. While FP-Oxa clearly inhibited migration in two-dimensionally cultured Ishikawa cells, it did not show the same level of success in Huvec and co-cultured cells. It was effective in reducing VEGF expression in three-dimensional co-cultures.

Conclusion: FP-Oxa may have various therapeutic effects on endometrial adenocarcinoma cells. It will be important to conduct biological activity studies in three-dimensional models that mimic the tumor structure created with different types of cancer cells.

Keywords: Endometrial carcinoma, molecular docking, multicellular spheroids, anticancer, VEGF

INTRODUCTION

Endometrial cancer (EC) is one of the most frequently diagnosed gynecological tumors worldwide [1]. Women with deep myometrial invasion, high-risk histological types such as serous or clear cell, or in the third stage of the disease are at increased risk for recurrence and/or metastasis [2]. Paclitaxel plus carboplatin therapy is the standard first-line chemotherapy for advanced, recurrent, and metastatic endometrial carcinoma [3]. Until recently, only 2 different treatments were specifically approved for use in metastatic cases, making it necessary to continue studies on the development of new anticancer agents in the chemotherapeutic treatment of endometrial cancer [4].

The blood vessel network that develops in the tumor microenvironment not only mediates the nutrition and oxygen supply of cancer cells, but also enables them to come into contact with the lymphatic network, causing metastasis [5]. It is known that vascular endothelial growth factor (VEGF) directly affects endothelial cells and changes cell migration, division and gene expression profiles [6]. Previous studies have shown that VEGF types are associated with the progression of endometrial cancer levels at different stages [7]. It has also been concluded that drugs such as Lenvatinib [8], Nintedanib [9], Brivanib [10] have the potential to prevent tumor progression by inhibiting the VEGF-VEGFR cascade. Human umbilical vein endothelial cells (Huvec) express VEGF-A, -B, -C and VEGF receptors R1, R2, and R3 [11]. In our study, co-culture models were established with endometrial adenocarcinoma cells (Ishikawa) and human umbilical vein endothelial cells (Huvec) to mimic the effects of VEGF, which promotes the progression of endometrial cancer *in vivo*.

Traditional two-dimensional (2D) cell culture models cannot represent the tumor microenvironment because they do not have a three-dimensional cell network and extracellular matrix found in the tumor structure [12]. Studies have shown that gene expression in three-dimensional cultures (3D) is closer to clinical expression profiles than those seen in 2D cultures [13]. Furthermore, it is thought that the different responses of 2D cultured cells and *in vivo* tumor cells to radiotherapy and chemotherapy may be a direct result of variation in spatial organization and cell-cell contacts [14].

The 1,3,4-oxadiazole ring, which is a widely used pharmacophore, has attracted attention of many studies in recent years due to its metabolic profiles and ability to form hydrogen bonds with the receptor site. 1,3,4-oxadiazole are bioisosteres of amides and esters that, due to the azole group (-N=C-O) in the oxadiazole core, increase lipophilicity affecting the drug's ability to reach the target by transmembrane diffusion, and may contribute to important pharmacokinetic properties [15]. 1,3,4-oxadiazoles demonsrate various biological activities such as anti-inflammatory, hypoglycemic, antianxiety, antidepressant, antiproliferative, antifungal, antibacterial and antitubercular [16]. Among them, different mono and di-substituted 1,3,4-oxadiazole exhibit potent antitumor activities against different cancer cell cultures with selective target [17].

In this study, it was aimed to investigate the biological activities of the compound, an oxadiazole derivative previously synthesized by our group [18] and which DNA gyrase activity studies (10.12 ± 0.14 μM) were performed, in 2D and 3D cell culture models. Drug target determination (drugability), absorption, distribution, metabolism, and excretion (ADME), toxicity, and molecular docking analysis of the compound we named 5-[(4-Phenylpiperazine-1-yl)methyl]-1,3,4-oxadiazol-2-thiol (FP-Oxa) were examined. The effect of FP-Oxa on Huvec and Ishikawa has been demonstrated in co-cultures developed to mimic the tumor microenvironment. Additionally, the effects on VEGF expressions in 3D culture models and changes in migration of 2D cultured cells were also analyzed.

MATERIALS and METHODS

In silico druglikeness analysis

The pharmacokinetics (ADME) properties, druglikeness, medicinal chemistry, and toxicity calculations were done by online web tools. The used web servers are that SwissADME [19], ProTox-II [20], SwissTargetPrediction [21], Boiled-Egg Method [22].

Toxicity analysis

The web-based Pro Tox II (https://tox-new.charite.de/protox_II/) free software was used for toxicity assessment and was calculated by entering the smile notation of the compound.

Molecular docking studies, ligand preparation

SDF files of ligands were generated using DataWarrior. Briefly, SMILES codes are used to generate conformers with setting as following: Random, Low Energy Bias, Torsions based on crystallographic database, energy minimization based on MMFF94s+ Force field. SD file version 3 with 3D atom coordinates has been used. The SDF files of known and potent VEGFR2 inhibitors (IC_{50}s of 12 nM-40 nM) were also generated and used for comparison purposes (Table 1).

Docking parameters and protein preparation

Human VEGFR2 ligand-binding domain in complex with benzimidazole-urea inhibitor (PDB ID: 2OH4) has been used to for docking studies. Briefly, crystal structure of protein was downloaded in pdb format from https://www.rcsb.org. Receptor structures were checked for incorrect charges and missing atoms and were optimized by removing water molecules, ions, and small molecules. A grid box with 26°Ax28°Ax26°A around the ATP binding pocket has been generated using AutoDockTools 1.5.6. Automated docking of ligands was performed with PaDelADV with as we have done previously [23].

Cell culture

Huvec (Human umbilical vein endothelial cell line-ATCC/CRL-1730) cells were cultured in DMEM F12K (21127022, Gibco) medium containing 10% FBS (FB-1001/500, Biosera), 1% streptomycin / penicillin (XC-A4110/100, Biosera) and 0.2% EGF solution. Ishikawa (endometrial cancer cell line-MERCK/99040201-1VL) cells were cultured in MEM (LM-E1149/500, Biosera) medium containing 10% FBS, 1% streptomycin/penicillin, 1% non-essential amino acid (XC-E1154/100, Biosera). Both cell lines were incubated at 37°C in a humidified atmosphere with 5% CO_2.

2D mono-culture and co-culture of huvec and ishikawa cells, MTT cell viability assay

Huvec and Ishikawa cells were seeded $1x10^4$ cells per well in 96 well-plate for monoculture model. For co-culture model, $5x10^3$: $5x10^3$ Huvec and Ishikawa cells were seeded per well in 96 well-plate. It was left to grow in an incubator with 5% CO_2 at 37°C. Huvec and Ishikawa cells were seeded for two-dimensional co-culture applications. In each well of an 96-well chamber, the medium of both cell lines was mixed in a ratio of 1: 1. After the cells become confluent, the 96-well microculture plates were treated with various concentrations (4-3-2-1-0.5 µM in DMSO) of FP-Oxa. Cells treated with %0.1 TritonX-100 (Millipore, 108603) were used as the positive control group, and cells not treated with FP-Oxa formed the negative control group. After 24 hours of incubation, medium FP-Oxa mixtures were aspirated. The wells were

Table 1. SMILES and information of molecules used in the docking study

Name	SMILES	Information
Ki20227	CC(C1=NC=CS1)NC(=O)NC2=C(C=C(C=C2)OC3=C4C=C(C(=CC4=NC=C3)OC)OC)OC	Ki20227 is an orally active and highly selective inhibitor of vascular endothelial growth factor receptor-2 (KDR/VEGFR-2, IC_{50}: 12 nM)
SKLB1002	CC1=NN=C(S1)SC2=NC=NC3=CC(=C(C=C32)OC)OC	SKLB1002 is a potent and ATP-competitive VEGFR2 inhibitor with IC_{50} of 32 nM.
CS-2660 (JNJ-38158471)	CCNC(=O)NC1=C(C=C(C=C1)OC2=NC=NC(=C2/C=N/OC)N)Cl	CS-2660 (JNJ-38158471) is a well tolerated, orally available, highly selective VEGFR-2 inhibitor with IC_{50} of 40 nM while it has no significant activity (>1 microM) against VEGFR-1 and VEGFR-3.
Taxifolin (Dihydroquercetin)	C1=CC(=C(C=C1[C@@H]2[C@H](C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	Taxifolin, type I inhibitor for VEGFR-2 kinase, is a flavonoid in many plants such as Taxus chinensis, Siberian larch, Cedrus deodara and so on.
Nintedanib (BIBF 1120)	CN1CCN(CC1)CC(=O)N(C)C2=CC=C(C=C2)N=C(C3=CC=CC=C3)C4=C(NC5=C4C=CC(=C5)C(=O)OC)O	Nintedanib (BIBF 1120, Intedanib, Vargatef, Ofev) is a potent triple angiokinase inhibitor for VEGFR1/2/3 with IC_{50} of 34 nM/13 nM/13 nM in cell-free assays.
FP-Oxa	Sc3nnc(CN1CCN(CC1)c2ccccc2)o3	This molecule has been developed in this study.

washed with PBS so as not to damage the cells. 200 microliters of MTT (Vybrant® MTT Cell Proliferation Assay Kit) solution, diluted 1:20 in medium, was added to each well. MTT solutions were aspirated after 3 hours of incubation. Formazan salts were dissolved by adding 200 microliters of DMSO to each well. Measurements were made with a 570 nm SpectraMax i3 microplate reader device. The IC_{50} value was detected by a dose response inhibition panel graph at GraphPad Prism 6.0 software.

Preparation of 3D mono-culture and 3D co-culture, MTT cell viability assay

Each well of 96-well plates (VWR-734-2327, Nest) should be coated to prepare a non-adhesive surface. 3% agarose (BP160-100, Fisher Scientific) was prepared for the coating process. Huvec and Ishikawa cells were seeded in 96 well-plate coated with agarose for 3D mono-culture applications. For the mono-culture model, Huvec and Ishikawa cells were seeded at $1x10^4$ cells per well. For the coculture model, $5x10^3$: $5x10^3$ Huvec and Ishikawa cells were seeded per well. It was left to grow in an incubator with 5% CO_2 at 37 °C. Following the formation of spheroid forms, as we demonstrated in our previous study [24], 96-well microculture plates were treated with various concentrations (4-3-2-1-0.5 µM in DMSO) of FP-Oxa. Cells treated with % 0.1 TritonX-100 (Millipore, 108603) were used as the positive control group, and cells not treated with FP-Oxa formed the negative control group. After 24 hours of incubation, medium FP-Oxa mixtures were aspirated. The wells were washed with PBS so as not to damage the cells. 200 microliters of MTT (Vybrant® MTT Cell Proliferation Assay Kit) solution, diluted 1:20 in medium, was added to each well. MTT solutions were aspirated after 3 hours of incubation. Formazan salts were dissolved by adding 200 microliters of DMSO to each well. Measurements were made with a 570 nm SpectraMax i3 microplate reader device. The IC_{50} value was detected by a dose response inhibition panel graph at GraphPad Prism 6.0 software.

Demonstration of VEGF expressions in 3D co-cultures by immunofluorescent staining

The spheroids were treated for 24 hours with the determined IC_{50} values of the compound FP-Oxa. Spheroids not exposed to FP-Oxa were used as the control group. The spheroids were then transferred to an 8-well chamber (80841, Ibidi). For fixation, it was incubated for 20 minutes with 4% PFA (P6148, Sigma-Aldrich) diluted in 1X PBS (LM-S2041/500, Biosera) at room temperature. After aspirating 4% PFA, it was washed 3 times with 1X PBS for 5 minutes. Permeabilized with 0.1% Triton™ X-100 for 15 minutes. It was blocked with 2% BSA for 45 minutes at room temperature. The blocking solution was aspirated, washed 3 times with 1X PBS for 5 minutes. Spheroids were labeled with VEGF Monoclonal Antibody (JH121) (MA5-13182) at a 1:100 dilution in 0.1% BSA by incubation for 24 hours at +4°C. After the primary antibody was aspirated, it was washed 3 times with 1X PBS for 5 minutes. Secondary antibody diluted 1:2000 was added to the wells and incubated for 1 hour at room temperature. At the end of the incubation period, washing was done 3 times for 5 minutes with 1X PBS. The slide was covered with a coverslip solution containing DAPI (00-4959-52, Invitrogen). Three digital sections of equal thickness were taken and imaged from the spheroids using the Z Stack method via a Zeiss LSM 800 laser scanning confocal microscope. Measurements of VEGF densities of cells in all sections were analyzed and recorded with Zeiss Zen 2 Blue version. Expression levels were analyzed and compared semi-quantitatively.

Migration (Wound Healing) assay

As has been done in previous studies, Culture-Insert 2 Well in µ-Dish 35 mm (ibidi, Cat. No 80206) was used for a wound healing and migration experiment 3×10^5 cells were added in a 70 microliter medium each in two wells [25]. These were incubated at 37°C and 5 % CO_2 as usual. After appropriate cell attachment (24 hours), the Culture-Insert 2 Well was gently removed using sterile tweezers. IC_{50} value of the compound FP-Oxa was added to the experimental group cells in 2 mL doses. The migration rate in both experimental and control group cells was displayed at 0. and 24. hours. Wound closure rates were measured and recorded with the ImageJ software program.

Statistical analysis

MTT tests were performed in triplicate and data was represented as mean ± standard deviation (SD). Statistical significance was measured using analysis of variance (ANOVA one-way) and multiple comparisons test. Migration and immunofluorescence staining analyzes were evaluated with the Unpaired t test. Values were

processed using GraphPad Prism 5.0 software (San Diego, USA). $p < 0.05$ values were considered statistically significant.

RESULTS

Chemistry

In order to obtain the target compound (Fp-Oxa) in the study, starting from the phenylpiperazine compound (1), the ester compound (2) was obtained by reaction with ethyl bromoacetate, followed by nucleophilic addition reaction with hydrazine hydrate to give the hydrazide compound (3). Then, the target compound was synthesised by ring closure reaction of the hydrazide compound with CS_2 in the presence of KOH (Figure 1). FT-IR (υ_{max}, cm^{-1}): 3078.22 (ar-H), 2954.64 (aliphatic CH), 1633.61 (C=N). MALDI-TOF/MS: calculated: 276.104481; found: 278.078952 ($[M+2]^+$) (Figure S1, Figure S2).

Drug target identifcation (Druggability) and ADME analysis

SwissADME

Examining the ADME results range limits of the prediction algorithms, such as molecular weight, lipophilicity, polarity, saturation, number of rotatable bonds, H-bond acceptors and donors, AlogP, for drug applicability, it was seen that the synthesized compound was in the applicability zone and was therefore found to be safe (Table 2, Figure 2). All results can be accessible in Figure S3.

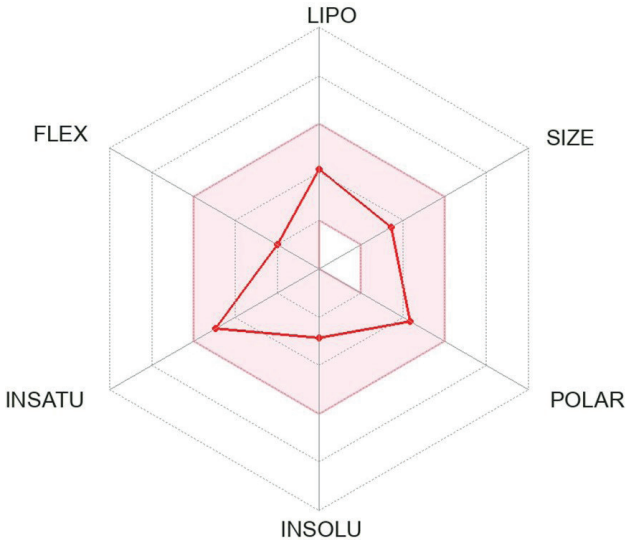

Figure 2. The colored zone is the suitable physicochemical space for oral bioavailability: LIPO (Lipophility): -0.7 < XLOGP3 < +5.0, SIZE : 150 g/mol < MW < 500 g/mol, POLAR (Polarity) : 20 $Å^2$ < TPSA < 130 $Å^2$, INSOLU (Insolubility) : -6.0 < Log S (ESOL) < 0, INSATU (Insaturation) : 0.25 < Fraction Csp3 < 1, FLEX (Flexibility) : 0 < Num. rotatable bonds < 9

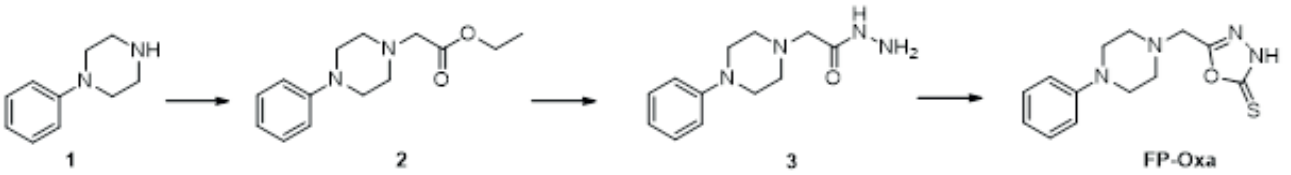

Figure 1. Synthetic schema for the target compound

Table 2. Limit values of drug applicability domains according to the SwissADME and the places of the target compound. The green color indicates that the compound obey the rules and is in domain. The red color indicates that the compound does not permeate into BBB and is not an inhibitor of CYP2C19

Molecular weight (g/mol)	TPSA (Å)	H-bond acceptors	H-bond donors	Lipophilicity (Log Po/w)	Water Solubility (Log S)	LogKp (skin permeation)	Druglikeness	Medicinal chemistry	Pharmacokinetics
276.36	84.20	4	0	1.59	-2.85	-6.78	Lipinski	PAINS	GI abs.[b]
							Ghose	Leadlikeness	BBB perm.[c]
							Weber	Syn. availability[a] (2.33)	P-gp subs.[d]
							Egan		CYP1A2 inhibitor
							Muegge		CYP2C19 inhibitor

[a] Synthetic availability. [b] Gastrointestinal absorption. [c] Blood Brain Barrier. [d] P-glycoprotein substrate.

Table 3. The calculated toxicity report by Pro Tox-II web-server of the compound

Classification	Target	Prediction	Probability
Organ Toxicity	Hepatotoxicity	Inactive	0.61
Toxicity End Points	Carcinogenicity	Active	0.60
Toxicity End Points	Immunotoxicity	Inactive	0.99
Toxicity End Points	Mutagenicity	Inactive	0.63
Tox21- Nuclear Receptor Signalling Pathways	Aryl Hydrocarbon Receptor	Inactive	0.76
Tox21- Nuclear Receptor Signalling Pathways	Androgen Receptor	Inactive	0.91
Tox21- Nuclear Receptor Signalling Pathways	Androgen Receptor Ligand Binding Domain	Inactive	0.95
Tox21- Nuclear Receptor Signalling Pathways	Aromatase	Inactive	0.80
Tox21- Nuclear Receptor Signalling Pathways	Estrogen Receptor Alpha	Inactive	0.83
Tox21- Nuclear Receptor Signalling Pathways	Estrogen Receptor Ligand Binding Domain	Inactive	0.95
Tox21- Nuclear Receptor Signalling Pathways	Peroxisome Proliferator Activated Receptor Gamma	Inactive	0.92
Tox21- Stress Response Pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element	Inactive	0.85
Tox21- Stress Response Pathways	Heat Shock Factor Response Element	Inactive	0.85
Tox21- Stress Response Pathways	Mitochondrial Membrane Potential	Inactive	0.74
Tox21- Stress Response Pathways	Phosphoprotein Tumor Supressor p53	Inactive	0.88
Tox21- Stress Response Pathways	ATPase family AAA domain-containing protein 5	Inactive	0.92

Pro Tox-II

When the toxicity evaluation was examined, it was determined that the target compound showed toxicity within the limits determined for carcinogenicity and had a positive borderline toxicity value for hepatotoxicity and mutagenicity Table 3 and Figure S4 (Toxicity radar). Additionally, the compound is classified as toxicity class 4, where it is considered harmful if swallowed (300< LD50 ≤ 2000) (Figure 3).

SwissTargetPrediction

The target estimation of the chosen compounds was examined using the SwissTargetPrediction platform with the following investigations 15 of the outcomes depicted as a pie-chart (Figure S5). The compound was predicted as 6.7% Cytochrome P450 and 13.3% Family CG protein coupled-receptor.

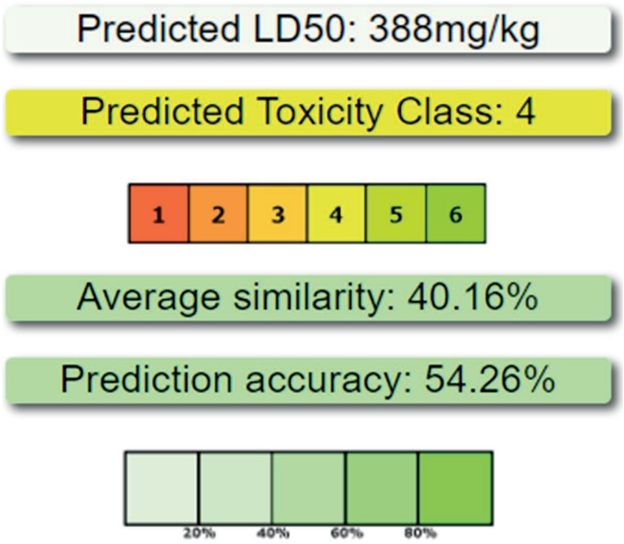

Figure 3. Toxicity classification of the compound

Boiled-egg

The BOILED-Egg profile which lets for intuitive consideration of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in the function of the position of the molecules in the WLOGP-vs-TPSA referential was screened for the target compound. The white area is for the high probability of passive absorption by the gastrointestinal tract, while the yellow area is for the high probability of brain penetration. Also, the marks are colored in blue if predicted as actively effluxed by P-gp (PGP+) and in red if estimated as non-substrate of P-gp (PGP-). It was concluded that FP-Oxa was estimated well-absorbed but not accessing the brain, and it was also subject to active efflux (blue dot) (Figure S6).

Molecular Docking Studies

The 3D structure of VEGFR2 was downloaded from the protein database (PDB ID: 2OH4). SDF files of known VEGFR2 inhibitors and molecules including Ki20227, SKLB1002, CS-2660 (JNJ-38158471), Taxifolin (Dihydroquercetin) and Nintedanib (BIBF 1120) were prepared using DataWarrior. VGEFR2 inhibitors with IC_{50} values ranging from 12M to 40 nM were selected. After removing water and

small molecules from the construct, a grid box was created around the ATP binding pocket using AutoDockTools (Figure 4A). Automatic coupling of compounds was performed with PaDelADV. It was found that FP-Oxa showed higher binding affinity than the average of five known VEGFR2 inhibitors or three of the known VEFR2 inhibitors (SKLB1002, Taxifolin, Ki202227) (Figure 4B). FP-Oxa also docked well in the ATP binding pocket with a binding affinity of -7.2 kcal/mol, as observed in molecular docking studies (Figure 4C).

2D mono-culture and co-culture, MTT cell viability assay

2D cultured cells were treated with different doses of FP-Oxa (4-3-2-1-0.5 µM) for 24 hour. IC_{50} values determined in Ishikawa, Huvec and coculture cells at the end of the 24th hour are shown in Table 4. In all experimental models, a decrease in cell viability was observed in parallel with the increase in dose. In addition, analysis of cell viability percentages in doses was performed and calculated separately for each dose. Comparing the percentages of viability in Ishikawa and coculture cells, all drug dose groups, and untreated negative control cells,a highly significant difference was found with a value of ****: $p < 0.0001$ (Figure 5).

Table 4. IC_{50} values of 2D cells cultured with FP-Oxa for 24 hours

Cell Culture (2D)	IC_{50} (µM)	SEMa
Ishikawa	1.648	± 0.280
Huvec	1.591	± 0.254
Co-Culture	2.064	± 0.284

a: Standard Error Mean

3D mono-culture and co-culture, MTT cell viability assay

2D cultured cells were treated with different doses of FP-Oxa (4-3-2-1-0.5 µM) for 24 hour. IC_{50} values determined in Ishikawa, Huvec and coculture cells at the end of the 24th hour are shown in Table 5. Deviations were noted for the 1 µM dose group in 3D cultured Huvec cells and the 2 µM dose group

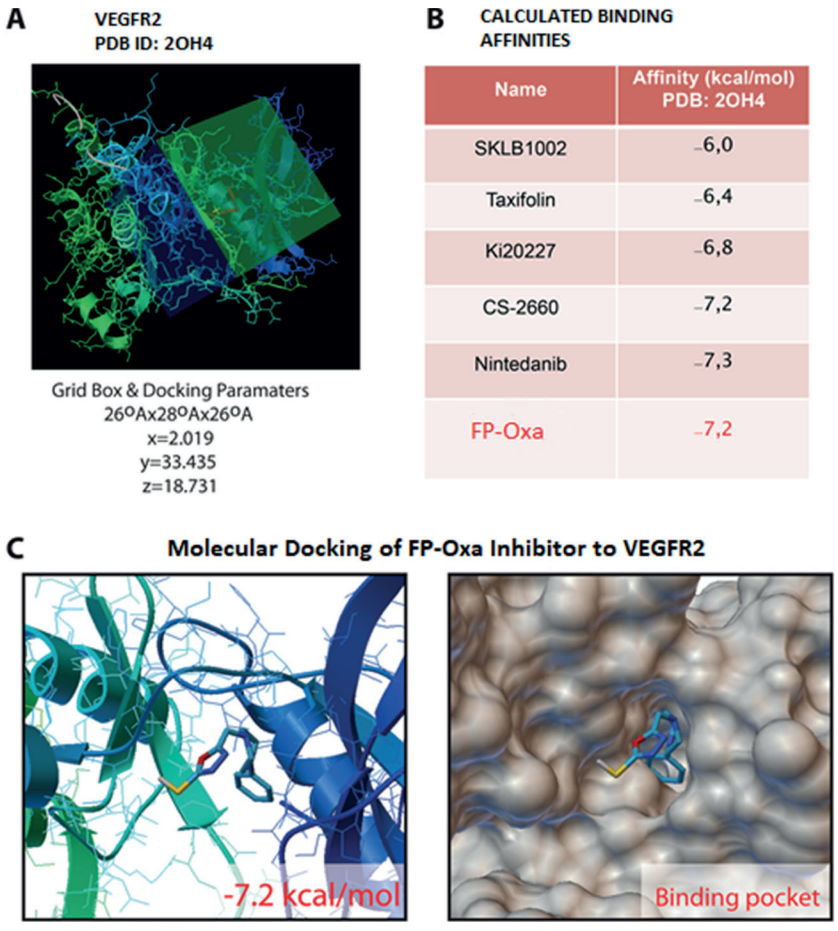

Figure 4. Molecular Docking of Compounds to VEGFR2 and Calculated Binding Affinities. A) VEGFR2 (PDB ID: 2OH4) 3D view and docking parameters. B) Calculated binding affinities via AutodockVina and PaDelAdv. C) Molecular Docking Pose of FP-Oxa Inhibitor to VEGFR2

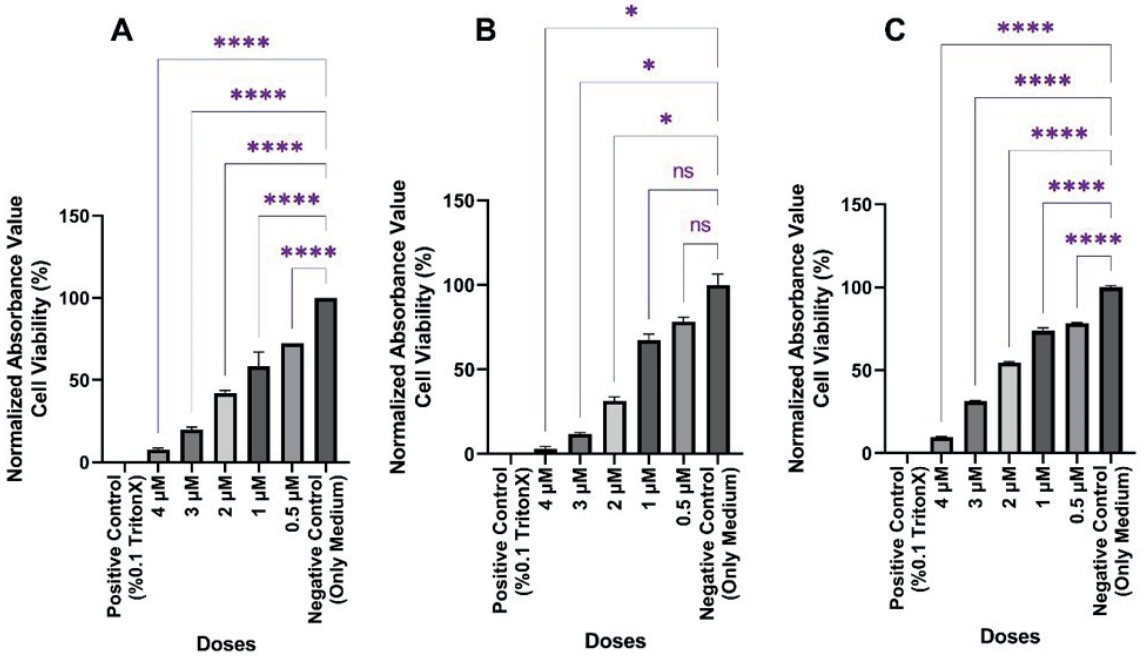

Figure 5. Dose-dependent percent viability plots of Ishikawa 2D culture (A), Huvec 2D culture (B), and Ishikawa, Huvec 2D Co-Culture (C) (****: Extremely significant, ***: Extremely significant, **: Very significant, *: Significant, ns: Not significant)

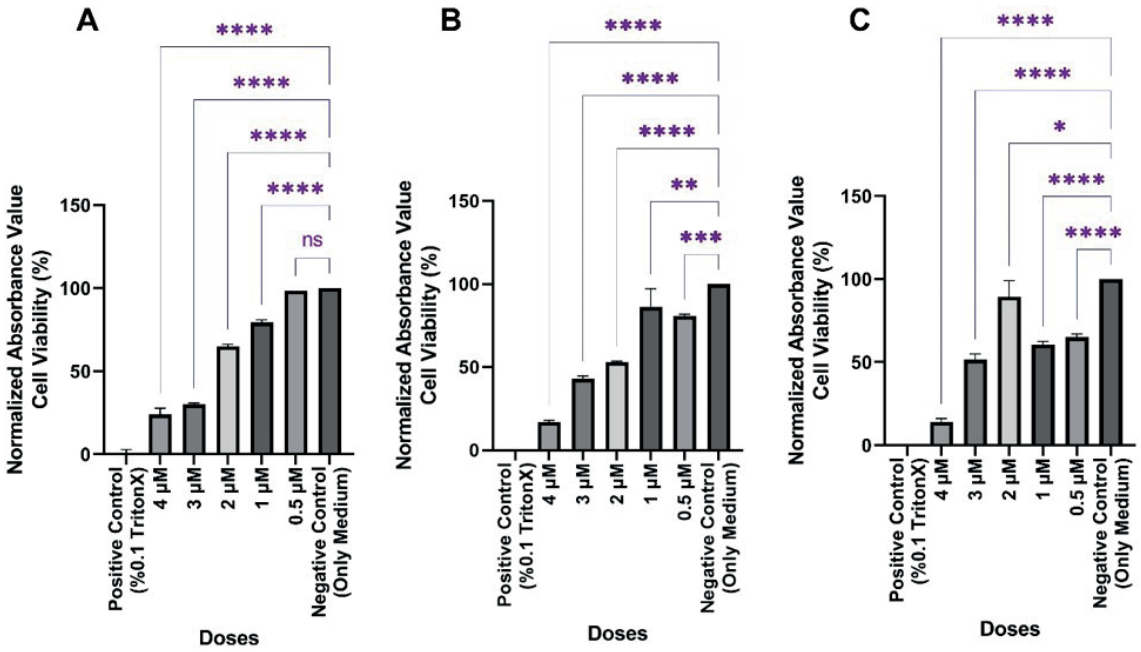

Figure 6. Dose-dependent percent viability plots of Ishikawa 3D culture (A), Huvec 3D culture (B), and Ishikawa, Huvec 3D Co-Culture (C) (****: Extremely significant, ***: Extremely significant, **: Very significant, *: Significant, ns: Not significant)

in 3D co-culture. Except for these dose groups, a decrease in cell viability was detected in all other dose groups due to dose increase (Figure 6).

VEGF expressions in 3D co-cultures

Images of the second sections corresponding to the middle part of the spheroids were added from the 3

Table 5. IC_{50} values of 3D cells cultured with FP-Oxa for 24 hours

Cell Culture (3D)	IC_{50} (µM)	SEM[a]
Ishikawa	2.384	± 0.437
Huvec	2.339	± 0.416
Co-Culture	2.755	± 0.793

a: Standard Error Mean

sections taken with the Z stack method (Figure 7A). Control 3D co-cultures and experimental group 3D co-cultures exposed to the compound FP-Oxa were compared for their integrated VEGF concentrations. It was observed that VEGF expression was decreased in the experimental group models with a significantly significant difference (**: p=0.0053) (Figure 7B).

Migration (Wound Healing) assay

2D cultured Ishikawa cells, Huvec cells, and co-cultures were treated with FP-Oxa for 24 h using predetermined IC_{50} doses. Cell groups not exposed to the FP-Oxa compound were used as controls. At the end of the 24th hour, all wound areas in the control group cells were closed (data not shown). There was no significant difference in the migration of Ishikawa cells when the proportion of wound areas closed at the end of 24 hours was compared with the control groups, while a very significant difference was observed in the Huvec cells (****: p< 0.0001) and Co-Culture groups (****: p< 0.0001) was recorded (Figure 8 A,B).

DISCUSSION

Drug-likeness contains structural, physicochemical, biochemical, pharmacokinetic, and toxicological parameters. On the other hand, ADME describes the absorption, distribution, metabolism, and excretion of a drug candidate compound. These two important processes specify the pharmacokinetic properties. Toxicity, efficacy, and inadequate pharmacokinetic properties are the reasons why compounds have not been able to be studied further or to market in drug discovery studies. Therefore, identifying these properties at the beginning of the study is an essential step for the ultimate clinical success of the compound. In our study, pharmacokinetic and toxicity evaluations were performed before the *in vitro* effects of the compound were demonstrated [26].

Bioavailability radar provides an at-a-glance understanding of the drug-likeness of the compound. The fact that the drug candidate compound is located within the borders of the colored region within the radar indicates that the compound is in the physicochemical area suitable for oral bioavailability. It was determined that the compound we used in our study and named FP-Oxa was within the appropriate limits for oral bioavailability (Figure 2). Toxicity assessment is very important in drug development before clinical trials are performed due to the fact that it is related to human health and the future of drug candidates. Due to the disadvantages of *in vitro* and *in vivo* toxicity studies, such as long duration, expensive and animal welfare, in silico toxicity calculations have been enhanced and become significant [27]. According to the results of the toxicity analysis, the compound was classified as toxicity class 4, which is considered harmful if ingested, with a prediction

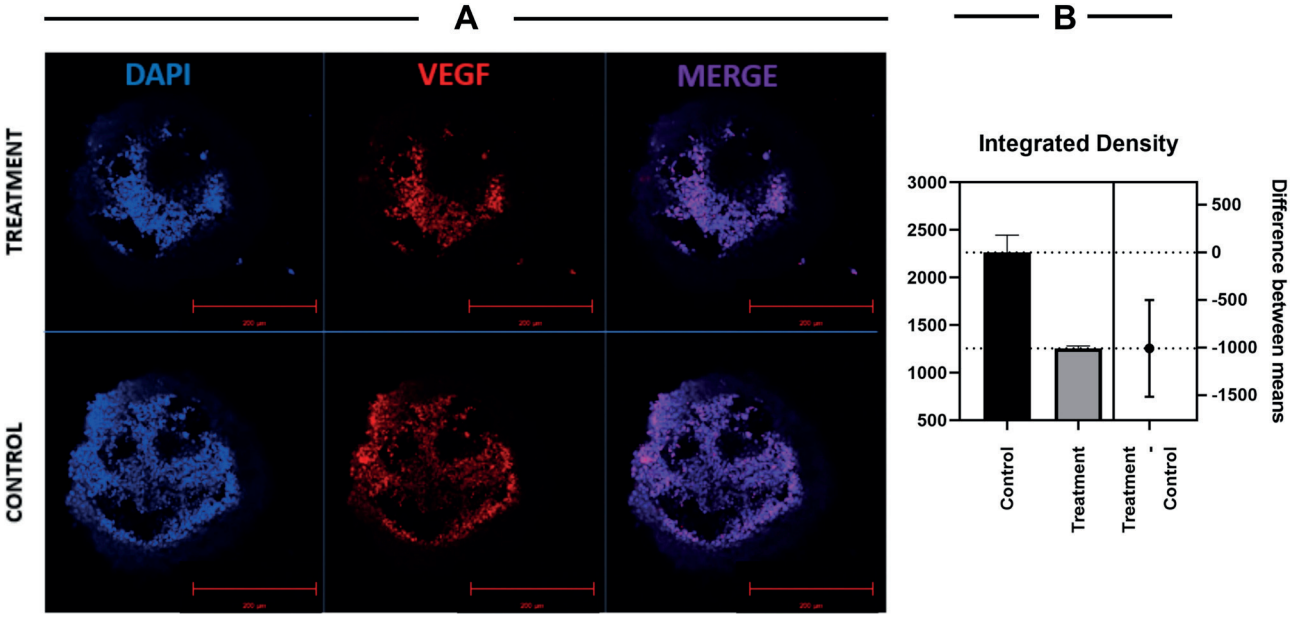

Figure 7. Confocal microscopic images of VEGF expressions of the 3D Co-Culture control and 3D Co-Culture treatment groups (A), VEGF expression rates integrated density from the control and treatment groups (B) (**: p=0,0053)

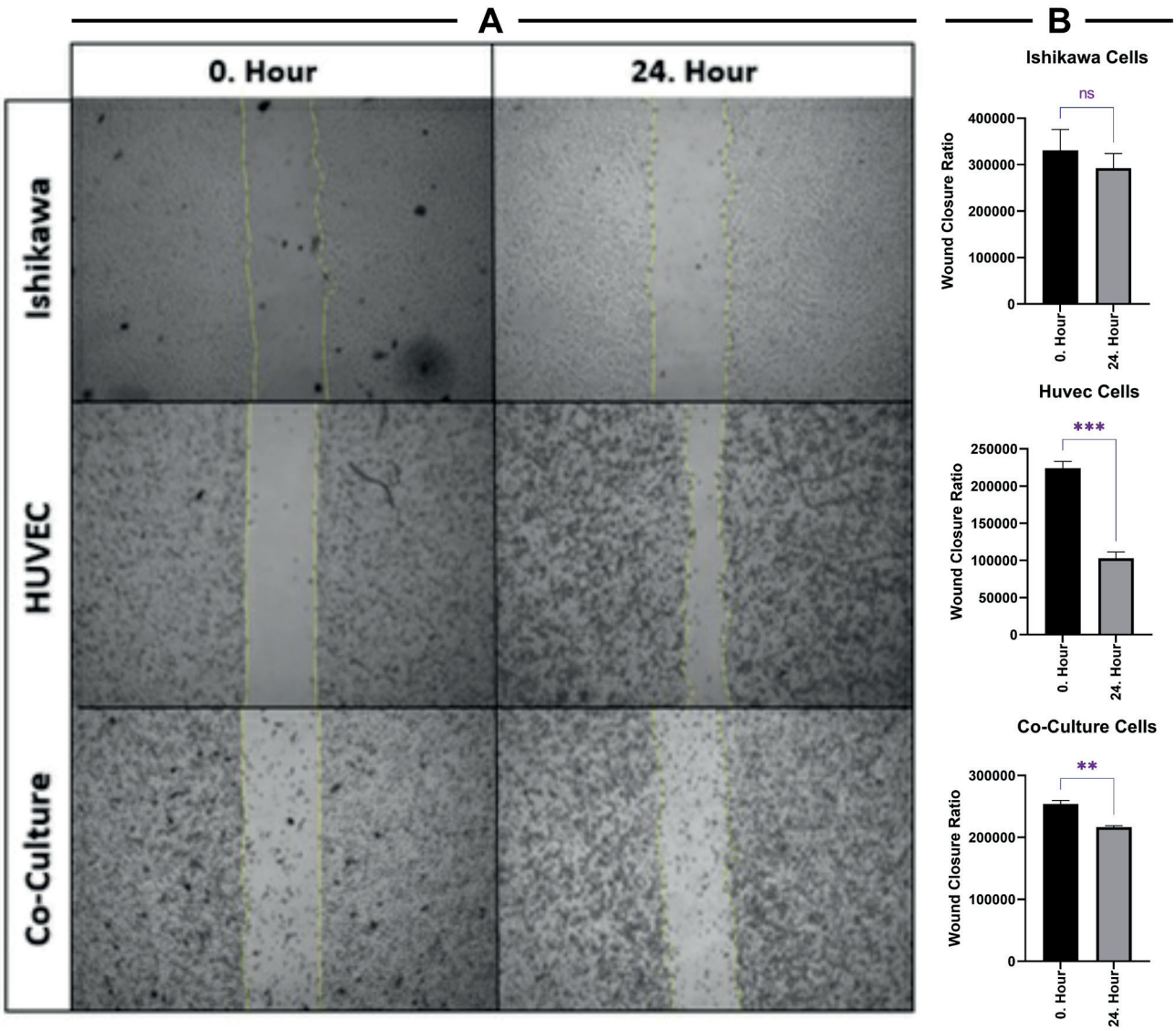

Figure 8. Wound healing areas in the treatment groups at 0. and 24. hours (A), Wound Closure rates at 0. and 24 Hours determined by field measurement (B)

accuracy rate of 54.26%. We think that the reason that may cause toxicity may be due to the presence of the -SH group in the structure of the compound (Figure 3). Additionally, it should not be overlooked that the toxicity results and class detected belong to rodents [28].

Molecular docking is a computational technique that predicts the binding affinity of ligands to receptor proteins [29]. By molecular docking calculations, the binding affinities of compounds to various receptors can be predicted, revealing their inhibition potential [30]. As a result of our molecular docking studies, it was determined that FP-Oxa has VEGFR2 inhibitor potential. When compared to the binding degrees of five compounds previously shown to be VEGFR2 inhibitors, it is promising that FP-Oxa has the best binding degree after Nintedanib (Figure 4). The affinity of the benzimidazole-urea inhibitor (PubChem CID 9935852) in the crystal structure (2OH4) of VEGFR2 was also determined. Our findings indicate a notable higher affinity (-10.6 kcal/mol) for VEGFR2, suggesting a more favorable fit into the inhibition pocket, particularly given the co-crystallization of this molecule with the target protein.

Cytochrome P450 (CYP) enzyme inhibition is the main mechanism in metabolism-based drug-drug interactions, and many chemotherapeutic drugs can cause drug interactions by inhibiting or inducing the CYP enzyme system [31]. CYPs have important roles in the metabolism of drugs that reduce cancer growth. Therefore, inhibitors of CYP

enzymes may potentially act as anticancer agents [32]. As a result of in silico prediction experiments, 6.7% binding of FP-Oxa to CYP can be considered as one of the reasons for switching to *in vitro* experiments (Figure S5).

The ability of anticancer drugs to pass through the blood-brain barrier is taken into account depending on the type of cancer. They are expected to exhibit this ability in tumors related to brain tissue [33]. However, it is not safe for the compound to be used in the treatment of endometrial cancer tumor to exhibit the ability to pass through the blood-brain barrier. In our study, the lack of ability of FP-Oxa to pass through the blood-brain barrier after boiled egg experiments is a promising result.

3D cultures are known to have more drug resistance than 2D cultures [34]. In our study, we found higher IC_{50} values in 3D culture models than in 2D culture models, which supports the literature (Table 4, Table 5).

Just like *in vivo* tumors, hypoxic areas deprived of nutrients and oxygen develop in the centers of tumor model spheroids [35]. This hypoxic state attracts VEGF-expressing endothelial cells towards cancer cells to provide adequate nutrients and oxygen, contributing to tumor progression and metastasis [36]. For these reasons, to ensure that VEGF was present in the medium, Ishikawa cells were co-cultured with VEGF-expressing Huvec cells as 3D spheroid models. Consistent with the molecular docking results, FP-Oxa was successful in significantly suppressing VEGF expression in 3D co-cultures compared with the control group (Figure 7).

As a result of the migration experiments we performed in conventional 2D cultures, FP-Oxa clearly suppressed the migration of Ishikawa cells cultured alone. FP-Oxa was not equally successful in Huvec cells cultured alone and in Huvec-Ishikawa co-cultures. At the end of the 24th hour, it was determined that a certain degree of closure had occurred in the wound areas in the Huvec and co-culture groups (Figure 8). However, it should not be overlooked that while the wound areas were completely closed at the end of the 24th hour in the control group cells, they were not completely closed in the Huvec and co-cultures. In the wound healing experiment, Ishikawa, Huvec and Co-culture cells were each exposed to their respective predetermined IC_{50} doses. This may therefore lead to the paradox that suppression of migration in Ishikawa cells may result from the death of the cells. However, the clear continuation of cell migration in the Huvec and Co-culture groups exposed to IC_{50} doses eliminates this contradiction. If we argue that the reason for migration suppression is due to cell death, we think that we should encounter the same situation in Huvec and Co-culture groups.

This study shows how an oxadiazole derivative, which we call FP-Oxa, affects viability, migration, and angiogenesis in models co-cultured with Ishikawa cells and Huvec cells. In this respect, encouraging research on the effect of FP-Oxa on other types of cancer may contribute to the field. However, the results regarding VEGF expression were revealed semi-quantitatively. This situation can be considered as a limitation of the study. In future studies, it will be important to determine the amount of VEGF expression quantitatively at the mRNA level, to reveal the molecular pathway through which the cell death mechanism continues, and to investigate the biological activities of FP-Oxa in different types of cancer.

Author contribution

Study conception and design: MB, AM, and FK; data collection: MB, BE, and MK; analysis and interpretation of results: MB, AM, MK and IK; draft manuscript preparation: MB, FK, BE and IK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Since the study is a cell culture study performed on commercially purchased cell lines, it is not subject to ethical approval.

Funding

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Conflict of interest

The authors declare that there is no conflict of interest.

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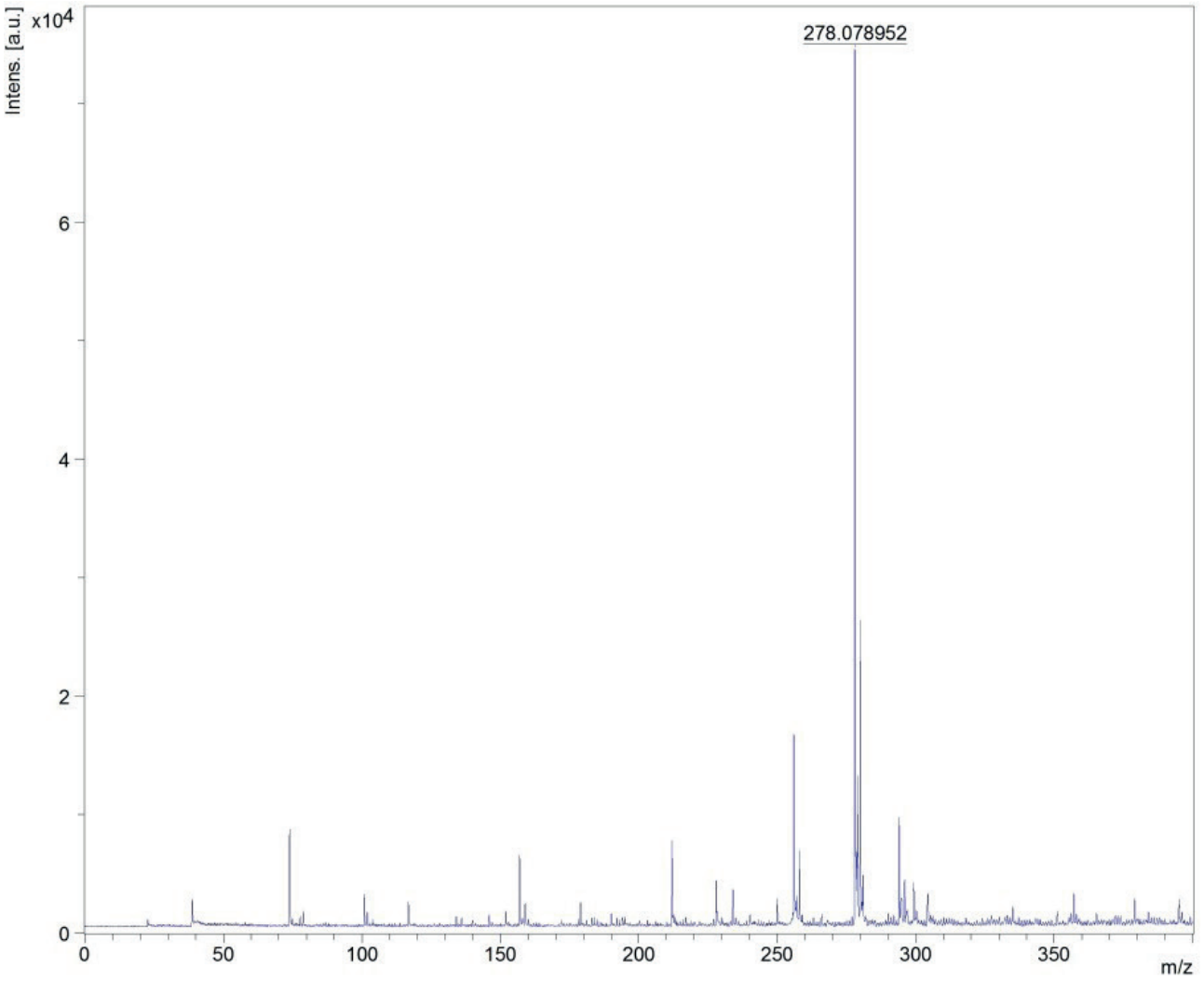
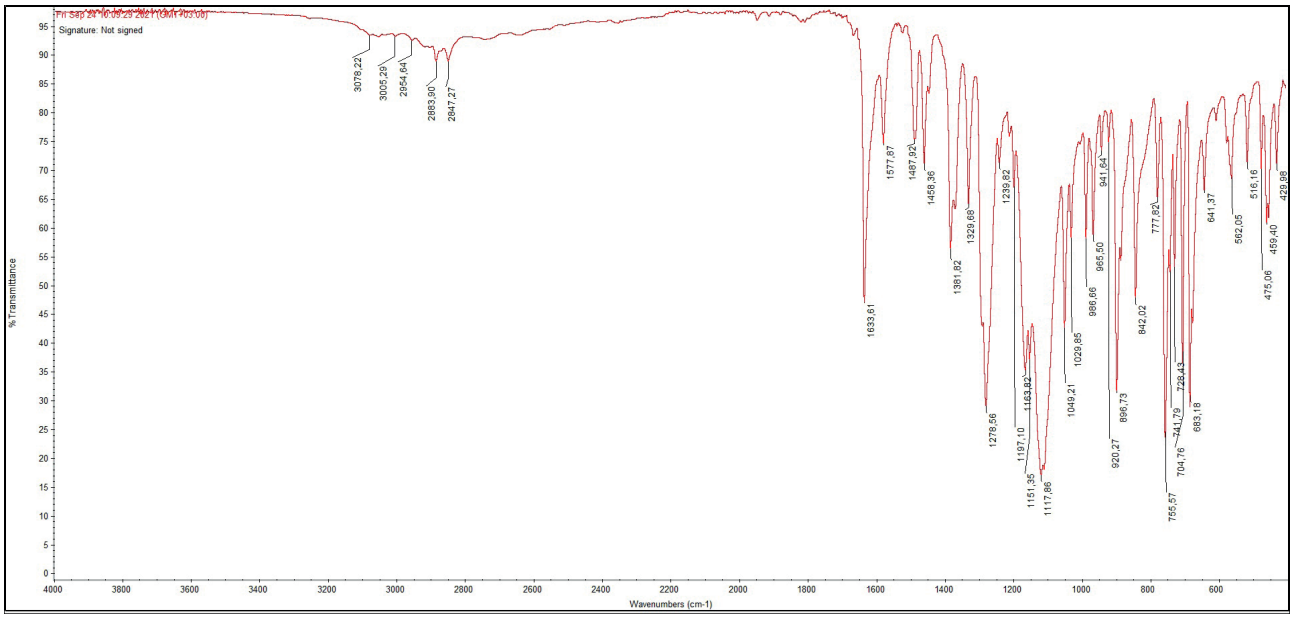


Figure S1. Title



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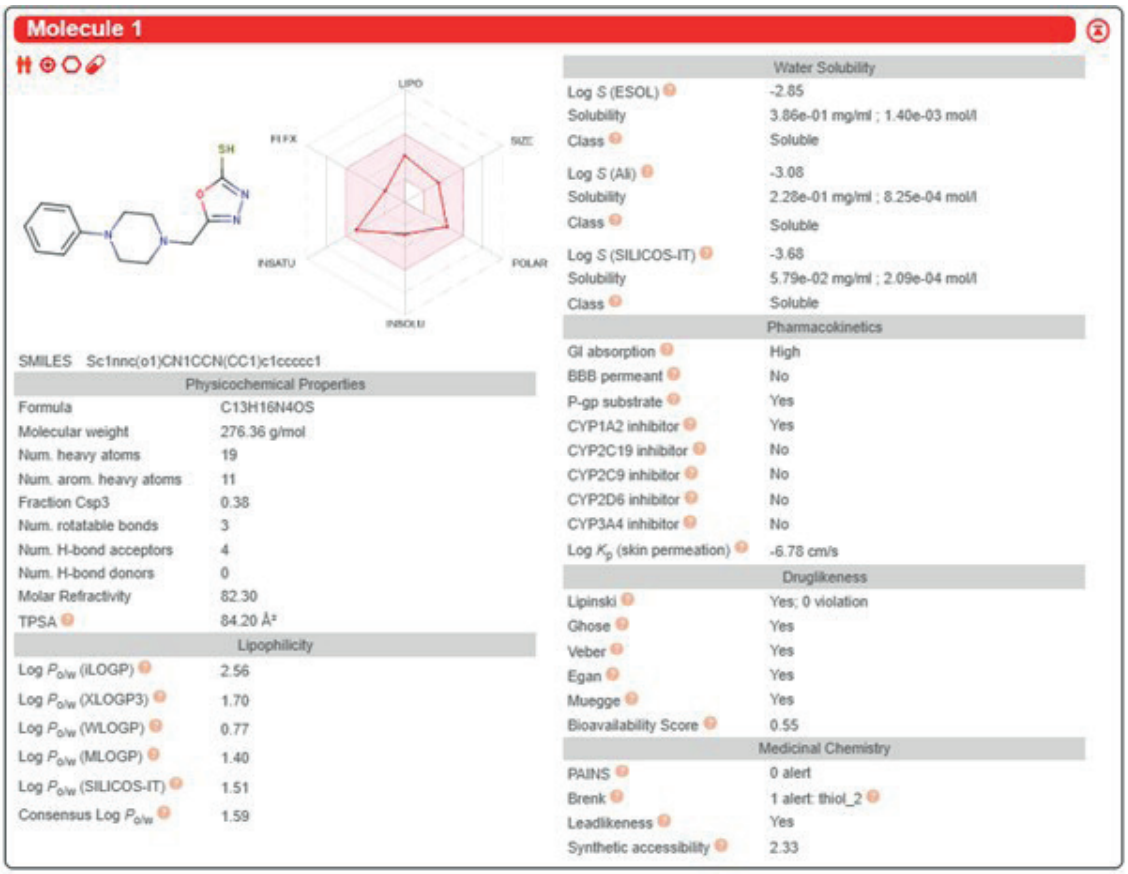

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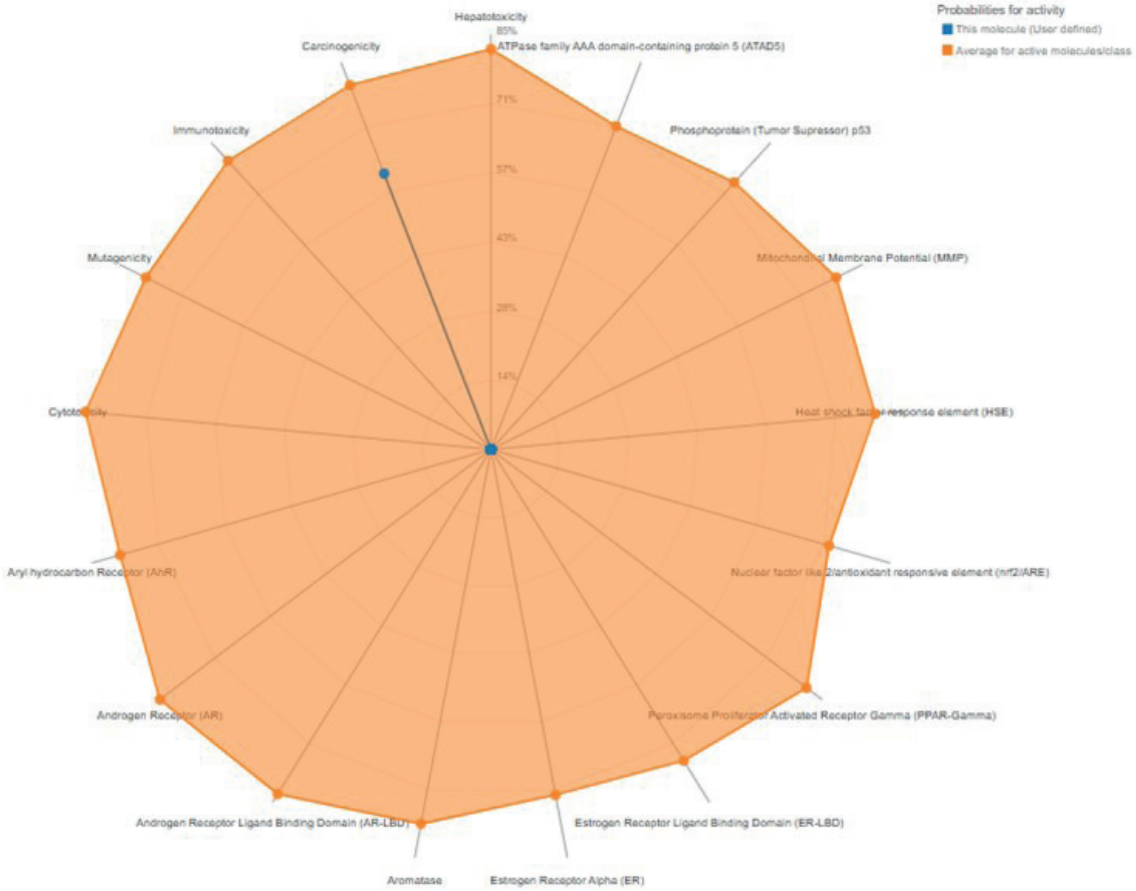

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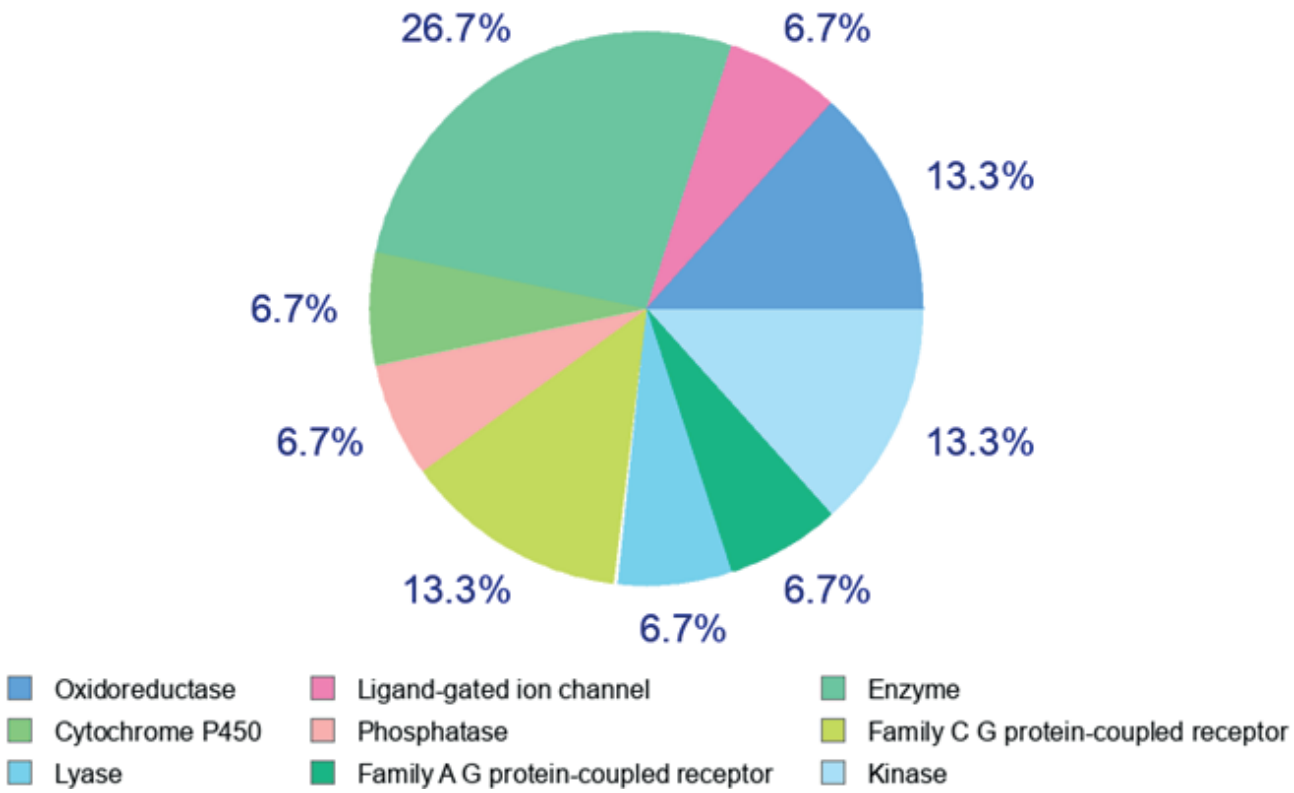

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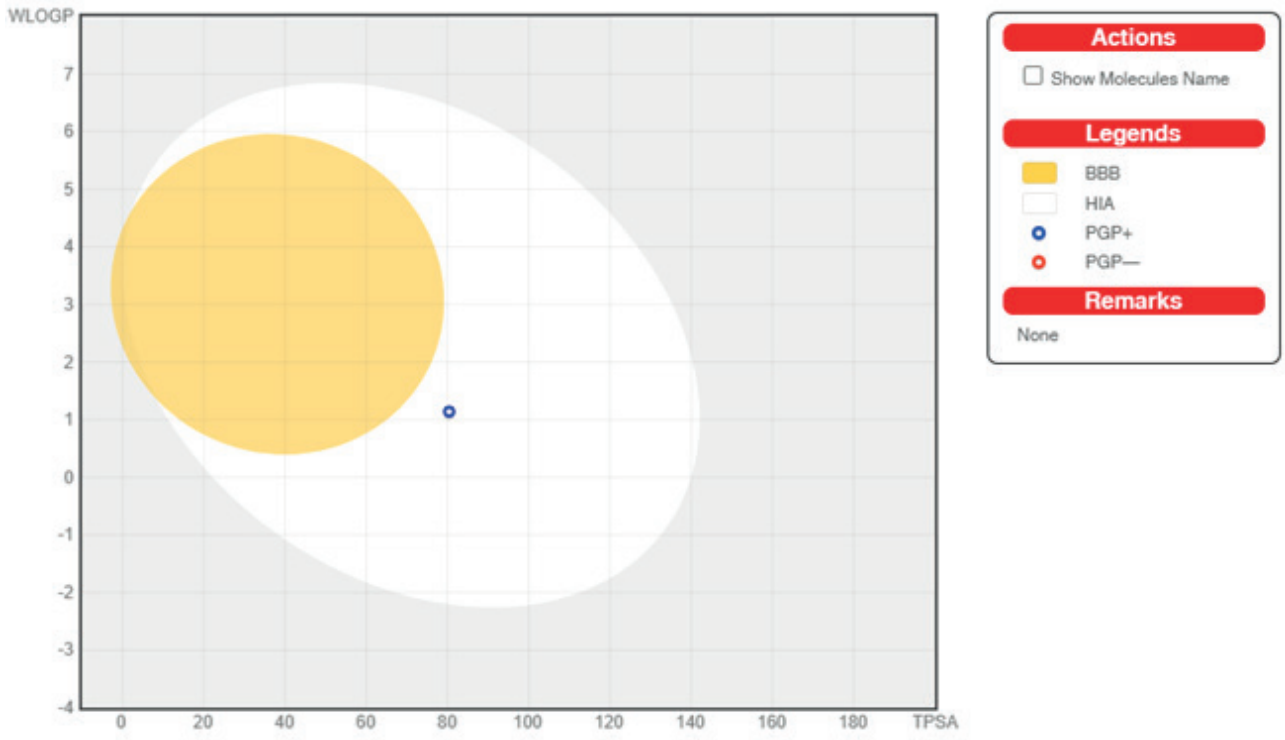

Figure S6. Title

acta medica

ORIGINAL ARTICLE

The role of PEDF, VEGF, and Ki-67 in tissue invasion and tumor angiogenesis of medullary thyroid carcinoma

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ABSTRACT

Objective: This study investigated the role of pigment epithelium-derived factor (PEDF), vascular endothelial growth factor (VEGF), nuclear factor kappa B (NF-kB), and Ki-67 proliferation index (PI) factors in Medullary thyroid carcinoma (MTC) tissue progression and their relationship with tumor angiogenesis. Immunohistochemical (IHC) analyses of PEDF expression in various human tumor samples and healthy tissues have shown that high levels of PEDF expression are associated with a positive prognosis, and low levels of PEDF suggest a poorer prognosis. With the completion of further studies showing the antitumor effects of PEDF on different types of cancer, PEDF's potential as a therapeutic agent has become increasingly promising. To the best of our knowledge, the role of PEDF in tumor angiogenesis in MTC has not been previously investigated.

Material and Methods: Thirty-seven tissue samples were retrospectively analyzed and sent/removed for pathology after total or subtotal thyroidectomy and archived between 01.01.2000 and 31.12.2015 in the Pathology Department of Hacettepe University. Relationships between categorical variables are analyzed using Pearson Chi-squared or Fisher's Exact tests.The correlation between two numerical variables is analyzed using Spearman's Rho correlation coefficient, and a comparison of numerical variables between two groups is made using the Mann-Whitney U test.

Results: The clinicopathological features of the patients were evaluated as sporadic or hereditary, tumor node metastasis (TNM) staging, single or multifocal focus, recurrence or regional or distant metastasis, progression-free survival time (PFS), and appropriate pathology tissue samples were determined as PEDF, VEGF, NF-kB, Ki-67 staining was compared. As a result of this comparison, a moderate negative correlation was found between VEGF expression level and PFS (rho = - 0,407, p = 0,019). No statistically significant correlation was found between single or multifocal groups and Ki-67 proliferation index (PI) expression levels (p = 0.070), but this is a small p value, a variable that is likely to find significant differences in studies with larger sample groups.The relationship between PEDF, VEGF, NF- kB, Ki-67 PI factors and tumor angiogenesis as reflected by microvessel density (MVD) was also investigated. To this end, we correlated the expression levels of PEDF, VEGF, NF-kB, and Ki-67 staining of suitable pathology tissue samples of the patients with the results obtained from the staining with CD 31. As a result of this comparison, no statistically significant relationship was found.

Conclusions: We demonstrated and described for the first time PEDF expression patterns in MTC tumor tissues. A direct relationship between PEDF expression level and angiogenesis, angiogenetic and clinicopathological factors does not seem to exist, at least as it pertains to the group studied. Further studies are required to fully elucidate mechanisms for tumor angiogenesis and invasion in MTC.

Keywords: Medullary thyroid carcinoma (MTC), Pigment epithelium-derived factor (PEDF), Vascular endothelial growth factor (VEGF), Nuclear factor kappa B (NF-kB), Ki-67 proliferation index, Microvessel density (MVD), angiogenesis, invasion.

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a malignant tumor of parafollicular C cells arising from the neural crest. MTC accounts for a significant portion of thyroid cancer morbidity and mortality. At presentation, the rates of regional and distant metastases are as high as 35% and 13%, respectively, and there has been no trend towards earlier diagnosis or improvement in overall survival in recent years (1).

Pigment epithelium-derived factor (PEDF) is a glycoprotein belonging to the serpin family. The gene encoding PEDF, also known as serpin peptidase inhibitor, clade F, member 1 (SERPINF1), is located on chromosome 17p13.3, contains eight exons and seven introns, and codes for a glycoprotein of molecular weight 50 kDa consisting of 418 amino acids (2). PEDF was initially identified as a neuroprotective and antiangiogenic factor secreted by the human fetal retina pigment epithelium. PEDF expression has been reported in various organs and tissues such as the brain, spinal cord, eyes, lungs, heart, liver, uterus, ovary, prostate, pancreas, bone, and plasma (3). Immunohistochemical analyses of PEDF expression in various human tumor samples and healthy tissues have shown that high levels of PEDF expression are associated with a positive prognosis, and low levels of PEDF suggest a poorer prognosis. With the completion of further studies showing the antitumor effects of PEDF on different types of cancer, PEDF's potential as a therapeutic agent has become increasingly promising (4). To the best of our knowledge, the role of PEDF in tumor angiogenesis in MTC has not been previously investigated.

CD 31, also known as platelet endothelial cell adhesion molecule (PECAM-1), is highly expressed on the surface of endothelial cells and is used to detect vessel density in tumor tissue (5).

A high microvessel density (MVD) number is a marker of tumors that are more prone to metastasis and is a sign of poor prognosis (6)

The family and receptors of vascular endothelial growth factor (VEGF) create the most important signaling pathways in tumor angiogenesis. Recognition of the VEGF pathway as a key regulator of angiogenesis has led to the development of various VEGF-targeted agents used in cancer treatment today, including agents that prevent the binding of VEGF-A to its receptors, antibodies that directly block vascular endothelial growth factor receptor-2 (VEGFR-2), and small molecules that inhibit kinase activity (7).

Nuclear factor kappa B (NF-kB) is a family of transcription factors that play critical roles in inflammation, immunity, cell proliferation and apoptosis. Inactive NF-kB is found in most cell types in the cytoplasm and can be activated by various extracellular stimuli such as proinflammatory cytokines, bacterial lipopolysaccharides, viral RNA and DNA via activation of membrane and cytoplasmic receptors. Several experimental studies have shown that the NF-kB transcription factor plays a role in the development or progression of human cancers (9).

Ki-67 is an antigen associated with cell proliferation expressed in all cell cycle stages except G0 (10). Ki-67 has been studied in many types of cancer, including cervical, lung, breast, and thyroid. It has been reported that Ki-67 is an independent prognostic factor in thyroid cancer patients (11).

This study investigated the role of PEDF, VEGF, NF-kB, and Ki-67 proliferation index (PI) factors in the progression of MTC tissue and their relationship with tumor angiogenesis. The study evaluated the correlation between PEDF, VEGF, NF-kB, and Ki-67 proliferation index (PI) factors and Microvessel density (MVD) concerning tumor angiogenesis by analyzing the expression levels of PEDF, VEGF, NF-kB, and Ki-67 in appropriate pathology tissue samples obtained from staining.

MATERIAL AND METHODS

Patients and clinicopathological data collection

Thirty-seven tissue samples were retrospectively analyzed and sent/removed for pathology after total or subtotal thyroidectomy and archived between 01.01.2000 and 31.12.2015 in the Pathology Department of Hacettepe University. TNM (tumor node metastasis) staging; pathological tumor, lymph node, metastasis (pTNM) criteria for clinicopathological tumor staging adopted by the International Union for Cancer Control (UICC) and the American Joint Cancer Committee

(AJCC), tumor size and presence or absence of extrathyroidal invasion, local and based on regional lymph node metastases and distant metastases (12). The patients included in the study were evaluated as recurrence or metastasis cases based on the radiological methods used in the postoperative follow-up of the patients.

Immunohistochemical analysis

Formalin fixed, paraffin-embedded thyroid tissue samples diagnosed with MTC were retrieved from the archive and tissue microarray (TMA) was prepared. Three mm diameter punch biopsy needles were used to transfer tumor tissues for creating (TMA) blocks. Then 3-micrometer sections were made.

The antibody clones were Anti-PEDF (1:25, rabbit polyclonal antibody – Immunohistochemical (IHC) - orb339611 product code, Biorbyt brand), Anti-VEGF (1:25, mouse monoclonal antibody - IHC - sc 7269 product code, Santa Cruz brand), Anti-NF-kB p65 (1:25, rabbit monoclonal antibody – IHC - sc 8008 product code, Santa Cruz brand), Anti-Ki-67 (1:300, mouse monoclonal antibody - IHC - DIA-670-P1 product code, Optistain brand), Anti-CD31 (1:100, mouse monoclonal antibody - IHC - MS -353-S product code, Thermo Scientific brand).

For immunostaining with antibodies including anti-PEDF, anti-VEGF and anti-NF-kB p65; the slides were deparaffinized by xylene 2 x 10' in an oven at 60 degrees after a night. The slides were pre-treated in ER1 (Epitope Retrieval Solution 1) for 10 minutes at 100 degrees. A blocking solution of 6% Hydrogen peroxide and 80% Methanol was used for 20 minutes. The slides were incubated with antibodies for 1 hour. The secondary antibody was applied for 30 minutes, streptavidin peroxidase for 30 minutes, and DAB for 2 minutes. The tissues were treated with hematoxylin for 5 seconds before being passed through alcohol and xylene and closed. For immunostaining with antibodies including Ki-67 and CD31; the slides were deparaffinized by xylene 2 x 10' in an oven at 60 degrees after a night. The slides were pre-treated in ER2 (Epitope Retrieval Solution 2) for 20 minutes at 100 degrees. A blocking solution of 6% Hydrogen peroxide and 80% Methanol was used for 20 minutes. The slides were incubated with antibodies for 1 hour. The samples were treated with post-primary for 9 minutes, polymer for 9 minutes, and DAB for 7 minutes, then left in hematoxylin for 8 minutes before being passed through alcohol and xylene and closed. The immunostained slides were evaluated by one pathologist without previous knowledge of clinical features.

Statistical analysis

In the study, descriptive statistics for numerical variables are given as mean±standard deviation or median (minimum-maximum) values, and for categorical variables as numbers and percentages. Whether the numerical variables were normally distributed or not was examined with the Shapiro Wilk test. Relationships between categorical variables are analyzed using Pearson Chi-squared or Fisher's Exact tests. The correlation between two numerical variables is analyzed using Spearman's Rho correlation coefficient, and a comparison of numerical variables between two groups is made using the Mann-Whitney U test. A comparison of numerical variables between more than two groups is made using the Kruskal-Wallis test. In the study, $p<0.05$ is considered statistically significant. Analyses were conducted in IBM SPSS Statistics 23.0 program.

RESULTS

Thirty-seven tissue samples were retrospectively analyzed and sent/removed for pathology after total or subtotal thyroidectomy and archived between 01.01.2000 and 31.12.2015 in the Pathology Department of Hacettepe University. Of the 37 patients, 30 (81.1%) received a diagnosis of sporadic MTC, and 7 (18.9%) received a diagnosis of hereditary MTC. As to the staging of the patients, 19 (51.4%) were diagnosed in stage I, 4 (10.8%) in stage II, 3 (8.1%) in stage III, and 11 (29.7%) in stage IV. Development of MTC was found to be a single focus in 26 (70.3%) of the 37 patients and multifocal in 11 (29.7%). In the post-surgical follow-up of the 37 patients, 21 (56.8%) had no recurrence or regional or distant metastasis, 12 (32.4%) had a recurrence or regional or distant metastasis, and data on recurrence or regional or distant metastasis could not be obtained for 4 (10.8%) patients. The median progression-free survival (PFS) time was 31 months for 33 (89.2%) patients. Data on PFS time could not be obtained for 4 (10.8%) patients (Table 1).

Table 1. Clinicopathological characteristics of patients (n=37)

Feature	n %
Sporadic or Inherited	
Sporadic	30(81,1)
Inherited	7(18,9)
TNM stage	
Stage I	19(51,4)
Stage II	4(10,8)
Stage III	3(8,1)
Stage IV	11(29,7)
Multifocality	
Single focus	26(70,3)
Multiple foci	11(29,7)
Recurrence, regional, or distant metastasis	
No	21(56,8)
Yes	12(32,4)
Loss	4(10,8)
PFS (median, month)	31
Loss	4(10,8)
PEDF	
Negative (< 5%)	6(16,2)
Weak positive (5-30%)	7 (18,9)
Moderately positive (30–60%)	14 (37,8)
Strongly positive (60 < %)	10 (27)
VEGF	
Negative (< %5)	20 (54,1)
Weak positive (%5-30)	12 (32,4)
Moderately positive (%30–60)	1 (2.7)
Strongly positive (%60 <)	4 (10.8)
NF-kB p65	
Negative (< 5%)	20 (54,1)
Weak positive (5-30%)	12 (32,4)
Moderately positive (30–60%)	1 (2.7)
Strongly positive (60 < %)	4 (10.8)
Ki-67	
< 3 %	33(89,2)
3 < %	4(10,8)
CD31 (median number of vessels)	11,3

İmmunohistochemical scoring

The PEDF score was evaluated for tumor cells. The expression levels of PEDF in tumor cells were semi-quantitatively categorized into four groups: negative (0 points), <5% positive cells (Figure 1A); weakly positive (1 point), 5-30% positive cells (Figure 1B); moderately intense positive (2 points), 30-60% positive cells (Figure 1C); and strongly intense positive (3 points), >60% positive cells (Figure 1D).

VEGF score; the tumor cells were evaluated. The levels of VEGF expression in tumor cells were semi-quantitatively categorized into four groups: negative (0 points), <%5 positive cells (Figure 2A); weakly positive (1 point), %5-30 positive cells (Figure 2B); moderately intense positive (2 points), %30-60 positive cells (Figure 2C); strongly intense positive (3 points), %60 < positive cells (Figure 2D).

NF-Kb p65 score; tumor cells were evaluated. The expression levels of NF-Kb p65 in tumor cells were semi-quantitatively categorized into four groups: negative (0 points), <%5 positive cells (Figure 3A); weakly positive (1 point), %5-30 positive cells (Figure 3B); moderately intense positive (2 points), %30-60 positive cells (Figure 3C); strongly intense positive (3 points), %60< positive cells (Figure 3D).

The Ki-67 PI score is evaluated for tumor cells. To calculate the Ki-67 PI, a count of 1000 cells is performed and then expressed as a percentage. The Ki-67 PI score is categorized as <%3 - 1 point (Figure 4A), %3 < - 2 points (Figure 4B).

The CD 31 score was evaluated for tumor cells by selecting the three areas with the highest microvascular concentration (vascular hot spots) stained with CD 31 in the tumor cell area, avoiding lymphocytic infiltration or fibrotic areas. The number of microvessels in the three areas with the highest microvascular concentration was determined, and the determined number of microvessels was added up and divided by 3 to calculate the average number of microvessels (Figure 5).

Evaluation of the correlation between clinical and pathological characteristics and PEDF expression levels of patients.

In 30 (81.1%) of the patients, sporadic MTC was diagnosed, and in 7 (18.9%) of the patients, hereditary MTC was diagnosed. According to the IHC evaluation results, 5 (16.7%) patients from the sporadic patient group showed negative, 6 (20%) showed weak positive, 11 (36.7%) showed moderate positive, and 8 (6.27%) showed strong positive staining. In the hereditary patient group, 1 (14.3%) patient showed negative, 1 (14.3%) showed weak positive, 3 (42.9%) showed moderate positive, and 2 (28.6%) showed strong positive staining. There was no statistically significant relationship between PEDF expression levels and sporadic or hereditary group of patients (p=1,000) (Table 2).

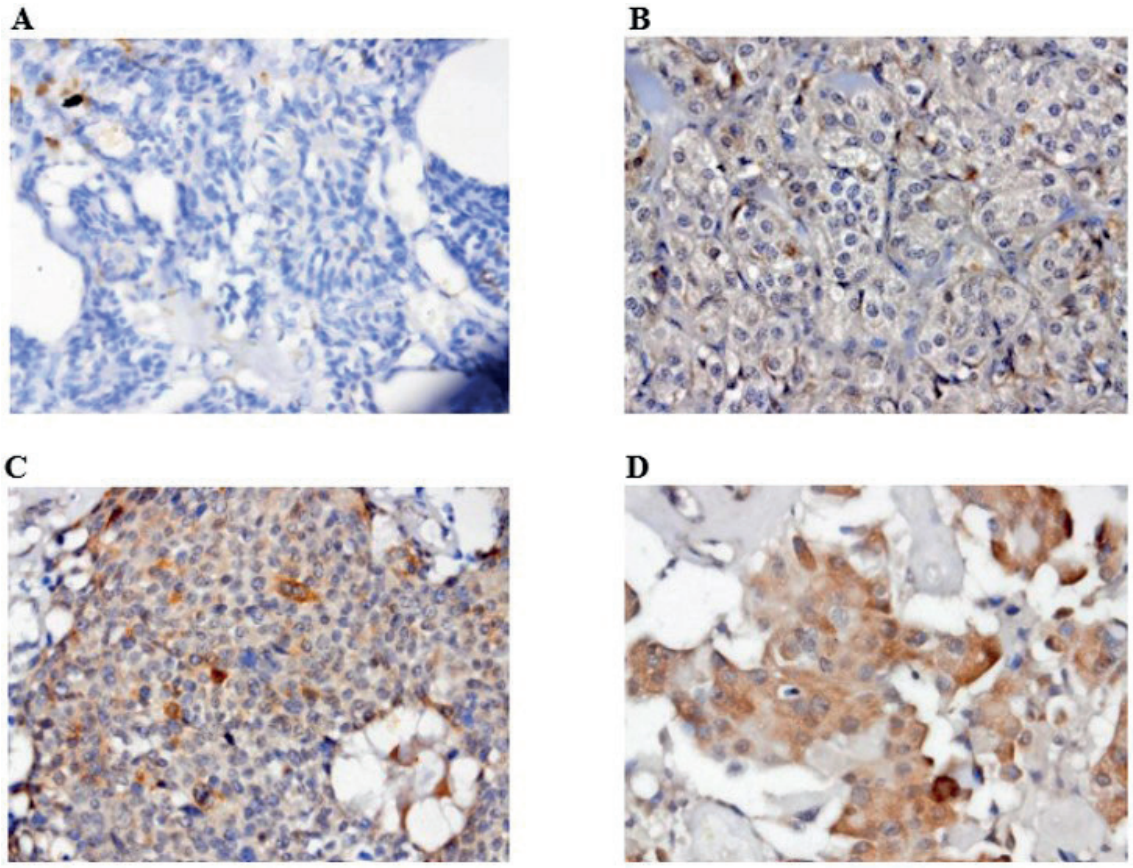

Figure 1. The microscopic image of PEDF evaluated immunohistochemically.
1A – Score 0, 1B – Score 1, 1C – Score 2, 1D – Score 3

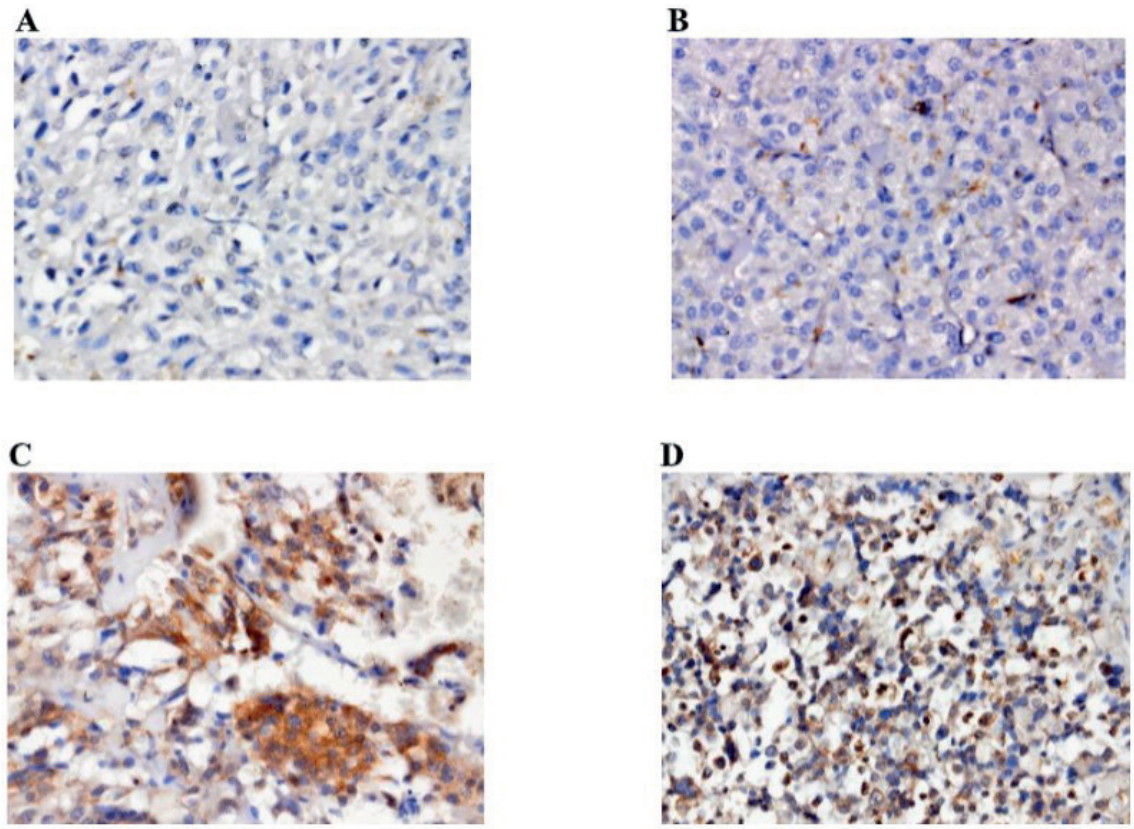

Figure 2. The microscopic image of VEGF evaluated immunohistochemically.
2A – Score 0, 2B – Score 1, 2C – Score 2 and 2D – Score 3

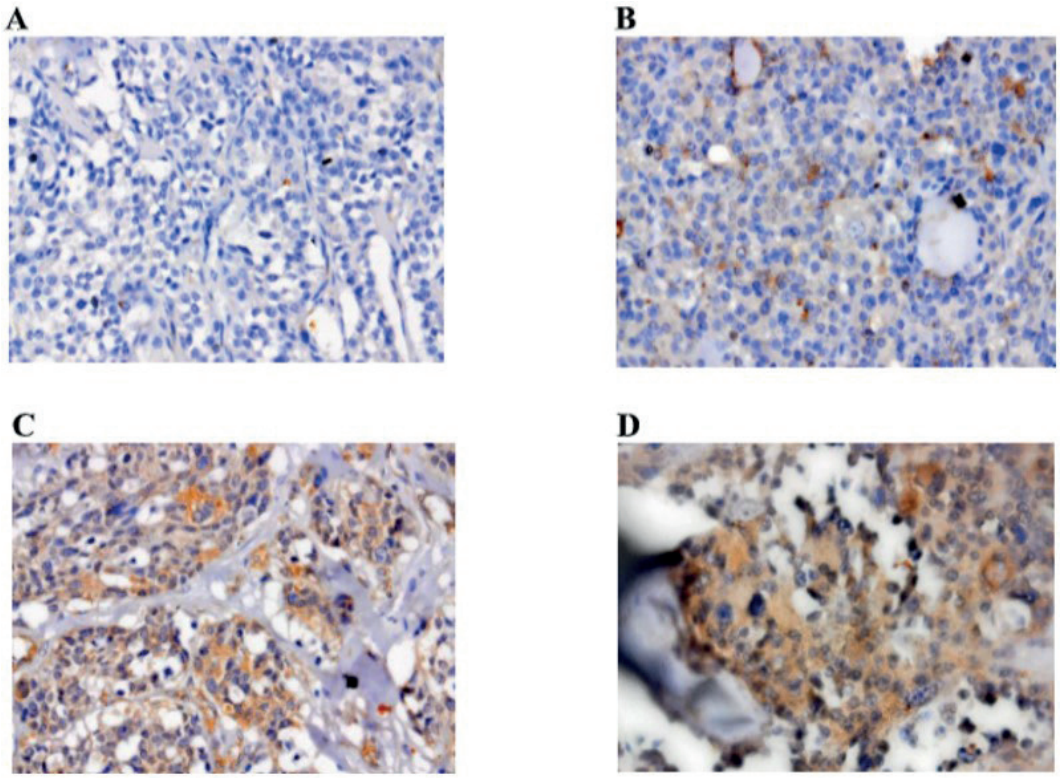

Figure 3. The microscopic image of NF-kB evaluated immunohistochemically.
3A – Score 0, 3B – Score 1, 3C – Score 2 ve 3D – Score 3

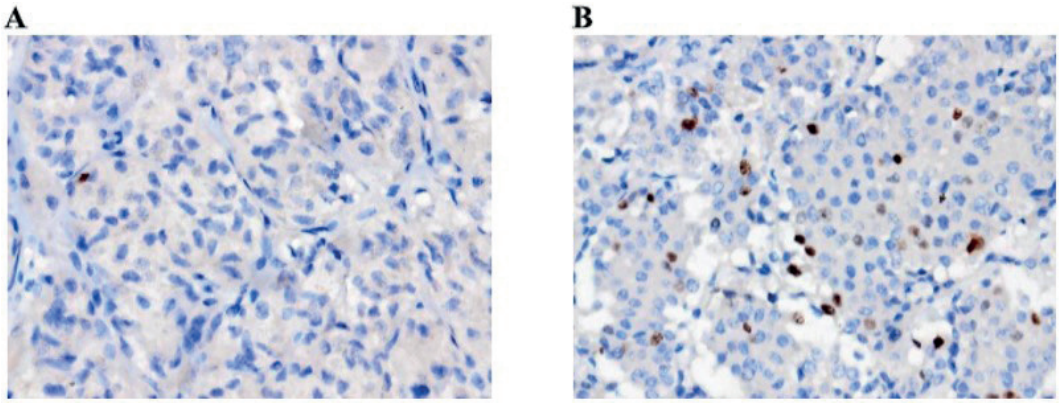

Figure 4. The microscopic image of the Kİ-67 proliferation index evaluated immunohisto chemically.
4A – Score 0, 4B – Score 1

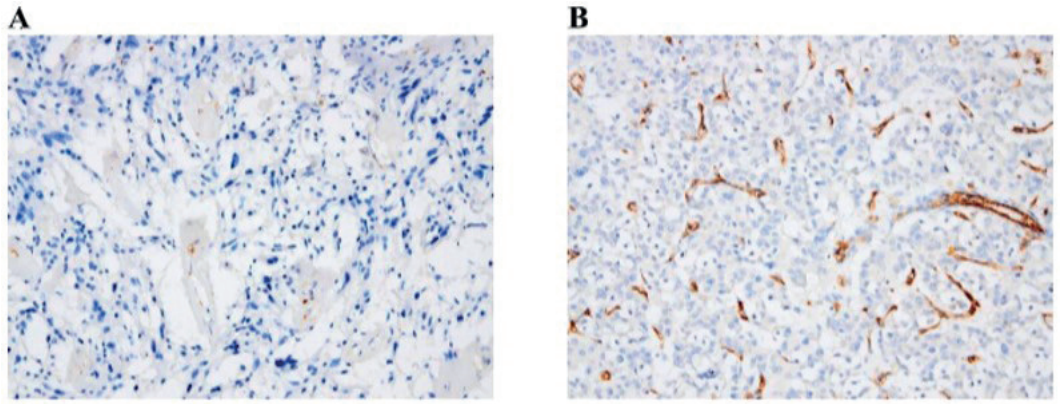

Figure 5. Immunohistochemically evaluated microscopic image of the microvascular area stained with CD-31, with 5A - weak and 5B - strong staining.

According to the TNM staging, 19 (51.4%) patients were diagnosed in stage I, 4 (10.8%) in stage II, 3 (8.1%) in stage III, and 11 (29.7%) in stage IV. Based on the IHC evaluation, 4 (21.1%) of the patients in stage I showed negative, 5 (26.3%) weak positive, 5 (26.3%) moderate positive, and 5 (26.3%) strong positive staining. In stage II, 0 (0%) patients showed negative, 0 (0%) weak positive, 4 (100%) moderate positive, and 0 (0%) strong positive staining. In stage III, 0 (0%) patients showed negative, 1 (33.3%) weak positive, 2 (66.7%) moderate positive, and 0 (0%) strong positive staining. In stage IV, 2 (18.2%) patients showed negative, 1 (9.1%) weak positive, 3 (27.3%) moderate positive, and 5 (45.5%) strong positive staining. No statistically significant relationship existed between the TNM stage groups and PEDF expression levels (p=0.283) (Table 2).

Twenty-six (70.3%) patients had a single focus of MTC, while 11 (29.7%) had a multifocal focus. The IHC evaluation revealed that 5 (19.2%) of the single-focus patients had negative, 6 (23.1%) weakly positive, 8 (30.8%) moderately positive, and 7 (26.9%) strongly positive staining. The multifocal focus patients had 1 (9.1%) negative, 1 (9.1%) weakly positive, 6 (54.5%) moderately positive, and 3 (27.3%) strongly positive staining. No statistically significant correlation existed between single or multifocal focus groups and PEDF expression levels (p=0.544) (Table 2).

In the postoperative follow-up, 21 (56.8%) patients had no recurrence or regional or distant metastasis, while 12 (32.4%) patients had a recurrence or regional or distant metastasis. Data on recurrence or regional or distant metastasis could not be obtained for 4 (10.8%) patients. In the IHC evaluation results, 4 (19.1%) of the patients without recurrence or metastasis showed negative staining, 4 (19%) showed weak positive staining, 9 (42.9%) showed moderate positive staining, and 4 (19%) showed strong positive staining. In the group of patients with recurrence or metastasis, 2 (16.7%) showed negative staining, 1 (8.3%) showed weak positive staining, 4 (33.3%) showed moderate positive staining, and 5 (41.7%) showed strong positive staining. No statistically significant relationship was found between the recurrence or metastasis status and PEDF expression levels (p=0.592) (Table 2).

Evaluation of the correlation between clinical and pathological characteristics and VEGF expression levels of patients.

The results of the IHC assessment showed that in the sporadic patient group, 15 (50%) had negative, 10 (33.3%) had weakly positive, 1 (3.3%) had moderately positive, and 4 (13.3%) had strongly positive staining. In the hereditary patient group, 5 (71.4%) had negative, 2 (28.6%) had weakly positive, 0 (0%) had moderately positive, and 0 (0%) had strongly positive staining. No statistically significant relationship was found between VEGF expression levels and whether the patients were sporadic or hereditary (p=0.772) (Table 2).

The results of the IHC assessment in the TNM stage groups showed that in stage I, 13 (68.4%) had negative, 4 (21.1%) had weakly positive, 0 (0%) had moderately positive, and 2 (10.5%) had strongly positive staining. In stage II, 1 (25%) had negative, 2 (50%) had weakly positive, 0 (0%) had moderately positive, and 1 (25%) had strongly positive staining. In stage III, 2 (66.7%) had negative, 1 (33.3%) had weakly positive, 0 (0%) had moderately positive, and 0 (0%) had strongly positive staining. In stage IV, 4 (36.4%) had negative, 5 (45.5%) had weakly positive, 1 (9.1%) had moderately positive, and 1 (9.1%) had strongly positive staining. No statistically significant relationship was found between TNM stage groups and VEGF expression levels (p=0.464) (Table 2).

The results of the IHC assessment in single-focus MTC patients showed that 13 (50%) were negative, 9 (34.6%) were weakly positive, 1 (3.8%) were moderately positive, and 3 (11.5%) were strongly positive. In multifocal focus MTC patients, 7 (63.6%) were negative, 3 (27.3%) were weakly positive, 0 (0%) were moderately positive, and 1 (9.1%) was strongly positive. No statistically significant relationship was found between single or multifocal focus groups and VEGF expression levels (p=0.910) (Table 2).

The results of the IHC assessment in recurrent or metastasis-free patients showed that 12 (57.1%) were negative, 7 (33.3%) were weakly positive, 0 (0%) were moderately positive, and 2 (9.5%) were strongly positive. In recurrent or metastasis patients, 5 (41.7%) were negative, 5 (41.7%) were weakly positive, 1 (8.3%) was moderately positive, and 1 (8.3%) was strongly positive. No statistically significant relationship was found between recurrent or metastasis and VEGF expression levels (p=0.595) (Table 2).

Table 2. Evaluation of the correlation between clinical and pathological characteristics of the patients and the expression levels of PEDF, VEGF, NF-kB p65, Ki-67 proliferation index

Features	Sporadic/İnherited		TNM stage				Multifocality		Recurrence or metastasis	
PEDF staining intensity	Sporadic	İnherited	stage1	stage2	stage3	stage4	Single focus	Multiple foci	No	Yes
Negative N(%)	5(16.7)	1(14.3)	4(21.1)	0(0)	0(0)	2(18.2)	5(19.2)	1(9.1)	4(19.1)	2(16.7)
Weakly positive N (%)	6(20)	1(14.3)	5(26.3)	0(0)	1(33.3)	1(9.1)	6 (23.1)	1(9.1)	4(19)	1(8.3)
Moderately positive N (%)	11(36.7)	3(42.9)	5(26.3)	4(100)	2(66.7)	3(27.3)	8(30.8)	6(54.5)	9(42.9)	4(33.3)
Strongly positive N (%)	8(6.27)	2(28.6)	5(26.3)	0(0)	0(0)	5(45.5)	7(26.9)	3(27.3)	4(19)	5(41.7)
p value	1.000		0.283				0.544		0.592	
VEGF staining intensity	Sporadic	İnherited	stage1	stage2	stage3	stage4	Single focus	Multiple foci	No	Yes
Negative N(%)	15(50.0)	5(71.4)	13(68.4)	1(25)	2(66.7)	4(36.4)	13(50)	7(63.6)	12(57.1)	5(41.7)
Weakly positive N (%)	10(33.3)	2(28.6)	4(21.1)	2(50.0)	1(33.3)	5(45.5)	9(34.6)	3(27.3)	7(33.3)	5(41.7)
Moderately positive N (%)	1(3.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(9.1)	1(3.8)	0(0.0)	0(0.0)	1(8.3)
Strongly positive N (%)	4(13.3)	0(0.0)	2(10.5)	1(25)	0(0.0)	1(9.1)	3(11.5)	1(9.1)	2(9.5)	1(8.3)
p value	0.772		0.464				0.910		0.595	
NF-Kb staining intensity	Sporadic	İnherited	stage1	stage2	stage3	stage4	Single focus	Multiple foci	No	Yes
Negative N(%)	7(23.3)	3(42.9)	5(26.3)	2(50.0)	0(0.0)	3(27.3)	6(23.1)	4(36.4)	7(33.3)	3(25)
Weakly positive N (%)	15(50)	3(42.9)	9(47.4)	2(50.0)	2(66.7)	5(45.5)	11(42.3)	7(63.6)	9(42.9)	7(58.3)
Moderately positive N (%)	5(16.7)	1(14.3)	2(10.5)	0(0.0)	1(33.3)	3(27.3)	6(23.1)	0(0.0)	3(14.3)	2(16.7)
Strongly positive N (%)	3(10)	0(0.0)	3(15.8)	0(0.0)	0(0.0)	0(0.0)	3(11.5)	0(0.0)	2(9.5)	0(0.0)
p value	0.856		0.775				0.223		0.799	
Ki-67 staining intensity	Sporadic	İnherited	stage1	stage2	stage3	stage4	Single focus	Multiple foci	No	Yes
< 3 %	26(86.7)	7(100)	18(94.7)	4(100)	3(100)	8(72.7)	26(96.2)	8(72.7)	20(95.2)	10(83.3)
3 < %	4(13.3)	0(0.0)	1(5.3)	0(0.0)	0(0.0)	3(27.3)	1(3.8)	3(27.3)	1(4.8)	2(16.7)
p value	0.570		0.316				0.070		0.538	

Evaluation of the correlation between the clinical and pathological characteristics of the patients and the expression levels of NF-kB p65.

The results of the IHC evaluation showed that of the sporadic patients, 7 (23.3%) were negative, 15 (50%) were weakly positive, 5 (16.7%) were moderately positive, and 3 (10%) were strongly positive. Of the hereditary patients, 3 (42.9%) were negative, 3 (42.9%) were weakly positive, 1 (14.3%) was moderately positive, and 0 (0%) were strongly positive. There was no statistically significant relationship between the sporadic or hereditary group and the expression levels of NF-kB (p=0.856) (Table 2).

In the TNM stage groups, of the patients in stage I, 5 (26.3%) were negative, 9 (47.4%) were weakly positive, 2 (10.5%) were moderately positive, and 3 (15.8%) were strongly positive. Of the patients in stage II, 2 (50%) were negative, 2 (50%) were weakly positive, 0 (0%) were moderately positive, and 0 (0%) were strongly positive. Of the patients in stage III, 0 (0%) were negative, 2 (66.7%) were weakly positive, 1 (33.3%) were moderately positive, and 0 (0%) were strongly positive. Of the patients in stage

IV, 3 (27.3%) were negative, 5 (45.5%) were weakly positive, 3 (27.3%) were moderately positive, and 0 (0%) were strongly positive. There was no statistically significant relationship between the TNM stage groups and the expression levels of NF-kB (p=0.775) (Table 2).

Of the patients with single-focus MTC, 6 (23.1%) were negative, 11 (42.3%) were weakly positive, 6 (23.1%) were moderately positive, and 3 (11.5%) were strongly positive. Of the patients with multifocal focus MTC, 4 (36.4%) were negative, 7 (63.6%) were weakly positive, 0 (0%) were moderately positive, and 0 (0%) were strongly positive. There was no statistically significant relationship between the single or multifocal focus groups and the expression levels of NF-kB (p=0.223) (Table 2).

Of the patients without recurrence or metastasis, 7 (33.3%) were negative, 9 (42.9%) were weakly positive, 3 (14.3%) were moderately positive, and 2 (9.5%) were strongly positive. Of the patients with recurrence or metastasis, 3 (25.0%) were negative, 7 (58.3%) were weakly positive, 2 (16.7%) were moderately positive, and 0 (0%) were strongly positive. There was no statistically significant relationship between the absence or presence of recurrence or metastasis and the expression levels of NF-kB (p=0.799) (Table 2).

Evaluation of the correlation between clinical and pathological characteristics of the patients and the expression levels of the Ki-67 proliferation index

The results of the IHC evaluation showed that in the sporadic patient group, 26 (86.7%) patients had a Ki-67 PI of <3%, and 4 (13.3%) patients had 3%<. In the hereditary patient group, 7 (100%) patients had a Ki-67 PI of <3%, and 0 (0.0%) patients had 3%<. There was no statistically significant relationship between Ki-67 PI expression levels and sporadic or hereditary groups (p=0.570) (Table 2).

In the TNM stage groups, 18 (94.7%) of stage I patients were evaluated as <3%, and 1 (5.3%) as 3%<. All patients in stage II were evaluated as <3%, and no patients were evaluated as 3%<. All patients in stage III were evaluated as <3%, and no patients were evaluated as 3%<. In stage IV, 8 (72.7%) patients were evaluated as <3%, and 3 (27.3%) as 3%<. There was no statistically significant relationship between TNM stage groups and Ki-67 PI expression levels (p=0.316) (Table 2).

In single-focus MTC patients, 25 (96.2%) were evaluated as <3%, and 1 (3.8%) as 3%<. In multifocal focus MTC patients, 8 (72.7%) were evaluated as <3%, and 3 (27.3%) as 3%<. As a result of evaluating the correlation between single or multifocal focus groups and Ki-67 PI expression levels, no statistically significant correlation was found (p = 0.070), but this is a small p value, a variable that is likely to find significant differences in studies with larger sample groups (Table 2).

In recurrent or metastasis-free patients, 20 (95.2%) were evaluated as <3%, and 1 (4.8%) as 3%<. In recurrent or metastatic patients, 10 (83.3%) were evaluated as <3%, and 2 (16.7%) as 3%<. No statistically significant relationship existed between recurrent or metastatic and non-recurrent or metastatic groups and Ki-67 proliferation index expression levels (p=0.538) (Table 2).

The evaluation of the correlation between the expression levels of PEDF, VEGF, and NF-kB p65 and PFS in the patients.

The results of the analysis between PEDF expression levels (0, 1, 2, 3) and PFS showed no statistically significant relationship (p=0.193) (Table 3). The analysis results between VEGF expression levels (0, 1, 2, 3) and PFS showed a negative, moderate correlation with a Spearman correlation coefficient value of rho = -0.407, p=0.019, and a statistically significant relationship was found (Table 3). No statistically significant relationship was found between NF-kB p65 expression levels (0, 1, 2, 3) and PFS (p=0.835) (Table 3).

Table 3. Correlation between expression levels of PEDF, VEGF, NF-kB P65 and PFS in patients (Spearman's Rho)

	Correlation with PFS*	p-value
PEDF 0, 1, 2, 3	- 0,232	0,193
VEGF 0, 1, 2, 3	- 0,407	0,019
NF-kB 0, 1, 2, 3	- 0,038	0,835

(*Spearman rho correlation coefficient)
PEDF staining intensity: 0 – negative, 1 – weak positive, 2 – moderately positive, 3 – strongly positive;
VEGF staining intensity: 0 – negative, 1 – weak positive, 2 – moderately positive, 3 – strongly positive;
NF-kB staining intensity: 0 – negative, 1 – weak positive, 2 – moderately positive, 3 – strongly positive;

Evaluation of the correlation between the Ki-67 proliferation index expression levels and PFS in patients.

Ki-67 variable is divided into two groups as %3 < and <%3. These two groups were compared with the Mann-Whitney U test according to PFS values. No statistical difference was found between the groups (p = 0.837). Data on four patients were not available for statistical analysis (Table 4).

Evaluation of the comparison of PEDF expression levels of the patients with CD 31

PEDF variable expression levels are divided into four groups: 0, 1, 2, 3. These four groups were compared with the Kruskal Wallis test according to their CD 31 values. No statistical difference was found between groups (p = 0.435) (Table 5).

Evaluation of the comparison between the VEGF expression levels of patients and CD 31.

VEGF variable expression levels are divided into four groups: 0, 1, 2, 3. These four groups were compared with the Kruskal Wallis test according to their CD 31 values. No statistical difference was found between groups (p = 0.565). However, since there was only one patient in the 2 (moderately positive) category according to VEGF staining intensity, it was not appropriate to provide the Kruskal Wallis test comparison result (Table 5).

Evaluation of comparison of NF-kB p65 expression levels with CD 31 in patients.

NF-kB variable expression levels are divided into four groups: 0, 1, 2, 3. These four groups were compared with the Kruskal Wallis test according to their CD 31 values. No statistical difference was found between groups (p = 0.413) (Table 5).

Evaluation of comparing patients' Ki-67 proliferation index levels with CD 31.

Ki-67 variable is divided into two groups: <3% and <3%. These two groups were compared with the Mann-Whitney U test according to their CD 31 values. No statistical difference was found between the groups (p = 0.688) (Table 6).

Table 4. Correlation between Ki-67 proliferation index expression levels and PFS in patients

	Ki-67 %	Number of patients (n=33)	p-value
PFS (month)	< %3	30	0,837
	%3 <	3	

Table 6. Comparison of patient's Ki-67 proliferation index levels with CD 31

Ki-67 (%LI)	Number of patients (n=37)	p-value
< %3	33	0,688
%3 <	4	

Table 5. Comparison of PEDF, VEGF, NF-Kb expression levels with CD-31 in patients

PEDF staining intensity	Number of Patients (N = 37)	Median value	Minimum value	Maximum value	P value
0	6	9.3300	4.33	22.66	0.435
1	7	12.3300	7.66	23.66	
2	14	9.6650	4.00	14.00	
3	10	11.4950	4.66	20.00	
VEGF staining intensity	Number of Patients (N = 36)	Median value	Minimum value	Maximum value	
0	20	11.3300	4.33	23.66	
1	12	11.3300	4.33	22.66	0.565
3	4	8.6650	4.00	12.66	
NF-kB staining intensity	Number of Patients (N = 37)	Median value	Minimum value	Maximum value	
0	10	7.6650	4.00	22.66	0.413
1	18	11.6600	4.33	23.66	
2	6	13.1600	5.66	17.66	
3	3	11.3300	6.00	17.00	

PEDF staining intensity: 0 – negative, 1 – weak positive, 2 – moderately positive, 3 – strongly positive;
VEGF staining intensity: 0 – negative, 1 – weak positive, 2 – moderately positive, 3 – strongly positive;
NF-kB staining intensity: 0 – negative, 1 – weak positive, 2 – moderately positive, 3 – strongly positive;

DISCUSSION

Our study investigated the role of PEDF, VEGF, NF-kB, and Ki-67 PI factors in the progression of MTC tissue and their relationship with tumor angiogenesis. We evaluated the clinical and pathological characteristics of 37 patients with MTC. To evaluate the role of PEDF, VEGF, NF-kB, and Ki-67 PI factors in the progression of MTC tissue, we compared the relationship between the expression levels obtained from PEDF, VEGF, NF-kB, and Ki-67 staining of appropriate pathology tissue samples and clinical and pathological data such as sporadic or hereditary, TNM stage, single or multifocal focus, recurrent or regional or distant metastasis, and PFS. A moderate negative correlation was found between VEGF expression levels and PFS, and a close correlation was found between Ki-67 PI expression levels and single or multifocal focus groups.

Although PEDF, VEGF, NF-kB and Ki-67 PI factors were expressed in MTC tumor tissue in the study, a direct relationship could not be demonstrated between the expression levels of PEDF, VEGF, NF-kB and Ki-67 PI factors and tumor angiogenesis.

It has been shown that PEDF plays an important role in tumor angiogenesis, growth, and dissemination. PEDF is one of the strongest natural endogenous inhibitors of angiogenesis (13). Many previous studies have shown that PEDF blocks the proliferation and dissemination of nasopharyngeal carcinoma (14), pancreatic cancer (15), glioma (16), melanoma (17) and breast cancer (18) cells. PEDF has also been significantly associated with the progression and metastasis of hepatocellular carcinoma (19). Recently, Tang et al. reported that PEDF supports the growth of esophageal cancer cells (20). Regarding papillary thyroid carcinomas (PTC), Yichen et al. have investigated the role of PEDF and evaluated the relationship between PEDF and PTC tumor angiogenesis concerning PTC. Their study showed a significant correlation between PEDF expression levels and clinicopathological characteristics such as lymph node metastasis (LNM), extrathyroidal invasion, high TNM stage, the presence of BRAFV600E mutation and tumor size in PTC.

Furthermore, the evaluation of the relationship between PEDF and PTC tumor angiogenesis showed that PEDF might have an antiangiogenic role by affecting the hypoxia-inducible factor 1a (HIF1a)-VEGF pathway (21).

Our study is the first study in the literature to evaluate the role of PEDF expression levels in MTC tissue progression and its relationship with tumor angiogenesis, and although there is PEDF expression in MTC tumor tissue, no direct relationship was found between MTC tissue progression and tumor angiogenesis.

In this regard, it seems that PEDF does not play a direct role in modifying angiogenesis in MTC, unlike PTC, but this observation requires further verification with larger number of patients.

As to the thyroid cancers, Vieira et al. found that VEGF expression was significantly more widespread in papillary thyroid carcinomas (79%) compared to follicular thyroid carcinomas (50%) or indifferent thyroid carcinomas (37%), using immunohistochemical analysis. Kılıçarslan et al. found stronger expression in papillary thyroid carcinomas than in normal thyroid tissues (22). In our study, the role of VEGF expression levels in the progression of MTC tissue was evaluated, and a statistically negative, moderate relationship was found between VEGF expression levels and PFS; however, no statistical relationship was found with other clinicopathological features of the tumor such as sporadic or hereditary, TNM staging, recurrent or regional or distant metastasis, single or multifocal focus, and tumor angiogenesis.

Activation of NF-kB in tumors can be caused by both a response to classic inflammatory stimuli such as infectious and physical or chemical agents and as a result of oncogene activation. A typical example of the latter is the rearranged during transfection (RET) oncogene, which is present in various cancer types, including thyroid cancer. Activating mutations of the RET gene are responsible for medullary thyroid carcinomas (23). Interestingly, activating mutations of the RET proto-oncogene leads to structural activation of NF-kB, which is important for RET-mediated carcinogenesis. Therefore, blocking NF-kB signaling could represent a new therapeutic strategy for thyroid carcinoma, especially for advanced disease (24). However, our study found no relationship between NF-kB expression levels and the progression of MTC tissue or tumor angiogenesis.

Carr et al. showed that anaplastic tumors have higher Ki-67 indices than well-differentiated thyroid tumors in thyroid tumors, while Erickson et al. showed that Hurthle cell carcinomas of the thyroid have higher Ki-67 indices than benign Hurthle cell adenomas. Tisel et al. showed that in metastatic primary MTC tumors, significantly higher Ki-67 indices were found than in primary tumors without metastasis (25). Some authors claim that the Ki-67 PI may not always match the clinical features of the tumor and that sometimes tumors with low expression of Ki-67 may be more aggressive than tumors with higher expression of Ki-67 (26). Our study found a significant correlation between Ki-67 PI levels and single or multifocal foci in MTK tissue progression. However, no relationship was found between the other characteristics of the tumor included in the study, such as sporadic or hereditary, TNM staging, recurrent or regional or distant metastasis, PFS and tumor angiogenesis and Ki-67 PI levels.

In conclusion, although there exists various degrees of PEDF expression in MTC tumor tissues, we could not find a direct relationship between PEDF expression level and tumor angiogenesis, tissue progression and poor prognostic clinicopathological parameters. The results suggest that PEDF seems not to directly modify the tumor angiogenesis observed in MTC, contrary to what has been observed in PTC. Further studies are clearly required to further elucidate mechanisms for tumor angiogenesis and invasion in MTC.

Author contribution

The study was designed by AG and GK. Data collection was done by GK, OK, AA, SB, and CS. Analysis and interpretation of the results were made by JK, AG and GK. The study was compiled into an article by AG and GK.

Ethical approval

The study was approved by Hacettepe University Non-İnterventional Clinical Research Ethics Committee (Protocol no: GO 20/887; approval no: 2020/16-74 /date 06.10.2020).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Effects of occupational exposure on the hematologic parameters among welders

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ABSTRACT

Introduction: The effect of prolonged exposure to welding fumes on blood levels remains an unresolved question. In our study, we aimed to determine the effects of metal fume exposure on various blood parameters in welders.

Materials and Methods: The study was cross-sectional. It included all male welding workers admitted to a health institution, a reference hospital for occupational diseases, for 2021. It was conducted with 254 individuals. The variables examined included age, duration of employment, smoking habit, body mass index, hemoglobin levels, erythrocyte, leukocyte and platelet counts, aspartate aminotransferase, alanine aminotransferase, free prostate-specific antigen, creatinine and manganese levels, and erythrocyte sedimentation rates.

Results: In our study, 40.6% of the welders were between 21-30 years old. 65.7% of the participants have been welding for more than five years. According to body-mass index values, 44.1% of the participants were pre-obese, and 16.9% were obese. 63.8% of the participants were smokers. In 35.8% of participants, hyperglycemia was present, and polycythemia was present in 27.2%. When blood parameters were analyzed, fasting blood glucose, aspartate transaminase, alanine transaminase, and sedimentation rate were lower in those who worked less than five years than those who worked five years or more. The difference was statistically significant. In blood test results, leukocyte, glucose, aspartate transaminase, alanine transaminase, and sedimentation rate values in welders were correlated with total working time.

Conclusion: Welders in our study had a high prevalence of smoking, overweight, and obesity. Working time is correlated with liver enzyme levels and fasting blood glucose values. For healthier workers, employers should fulfill their responsibilities for occupational health and safety.

Keywords: Welding, biological monitoring, occupational exposure, health surveillance

INTRODUCTION

Welding is a widely used industrial process in which high temperatures join metals, producing potentially hazardous metal fumes [1]. Approximately 11 million people worldwide work as welders, and 110 million are exposed to welding fumes [2]. The composition of welding fumes, classified as group 1 carcinogen by the International Agency for Research on Cancer, varies depending on the type of welding performed. Although it is affected by many factors such as welding method, electrode type, filler metal, fluxes, protective gases, and base metals, it always contains various metal particles [3].

Metal fume exposure in welders is associated with many health problems. Welders face a heightened risk of chronic exposure, particularly to airborne manganese found in welding materials. However, the impact of prolonged exposure to manganese or welding fumes on blood levels remains an unresolved question [4]. Studies have shown that exposure to metal fumes can lead to hyperglycemia, may be associated with an increase in the prevalence of diabetes, and that occupations associated with high levels of metal exposure, such as welding, are at risk for diabetes [5-7]. Obesity is a common risk factor for diabetes. Since reducing body weight will lead to the prevention, control, and regression of diabetes, it is essential to examine body mass indexes in the health surveillance of workers [8]. Since serum creatinine levels may be associated with an increased risk of diabetes, it is recommended that blood creatinine levels should also be examined in screenings to identify those at high risk of diabetes [9]. A positive correlation was between creatinine level and working time, aspartate/alanine aminotransferase levels, and age. It is also necessary to check liver enzyme levels and evaluate renal function during periodic control examinations of workers exposed to metal fumes [10]. Hepatocytes are damaged in people occupationally exposed to heavy metal fumes; therefore, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase levels are increased in the bloodstream. It is also thought that liver functions deteriorate, and non-alcoholic fatty liver disease develops in these individuals due to gene interactions [11].

Considering all these, it is understood that the welding profession involves significant risks regarding employee health. Health surveillance of welders is critical to reveal these risks in all aspects and take appropriate measures regarding worker health. Welders are a suitable group for biological monitoring to measure occupational exposure tendency and to examine dose-response relationships. In this study, we aimed to determine the effects of metal fumes on various hematological parameters in welders and to reveal the potential health effects of occupational exposure.

MATERIALS and METHODS

The study was cross-sectional. It was conducted in Ankara Occupational and Environmental Diseases Hospital, a reference hospital for occupational diseases. The study population included male welding workers between the ages of 21 and 50 who applied to the hospital for periodic health examinations during 2021, had been working for at least one year, spent at least eight hours a day in welding work, and were between the ages of 21 and 50. There were no female welding workers among the applicants. As it affects the elimination of heavy metals; those who used any medication or herbal supplements such as vitamins, N-acetylcysteine, and lipoic acid, those with a history of alcohol consumption, those with chronic diseases, and those who had recently undergone surgery were excluded. Sample selection was not made, and all workers who met the inclusion criteria were in the study, which was conducted with 254 people.

Among the parameters examined in the study, data on age, employment duration, and smoking habits were obtained from the periodic examination forms. Height and body weight were measured during the examination, and body mass index was calculated. The results of routine complete blood count and serum biochemistry tests were evaluated. Blood parameters examined included hemoglobin levels, erythrocyte, leukocyte, and platelet counts, aspartate aminotransferase (AST), alanine aminotransferase (ALT), free prostate-specific antigen (PSA), creatinine and manganese levels, and erythrocyte sedimentation rates. Blood samples were collected in purple-capped EDTA tubes, and the tube was gently inverted and mixed 5-6 times as soon as the blood was collected to

avoid clot formation. Serum samples were collected in 10 ml red-capped 16x100 mm tubes without gel, centrifuged at 1500 x g for 10 min, and separated and stored until the analysis time. Inductively coupled plasma-mass spectrometry method was used to analyze the manganese samples. The Ethics Committee of Gazi University ethically approved the study, and informed consent was obtained from all participants.

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) for Windows 25.0. In the descriptive statistics section, categorical variables were presented as numbers and percentages, and continuous variables as mean ± standard deviation and median (minimum-maximum value). The data obtained were analyzed for normal distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data conforming to normal distribution were evaluated with the Student's t-test. In the case of non-normally distributed data, the Mann-Whitney U test was applied to determine the difference between the groups. In addition, Spearman and Pearson correlation tests were used to investigate the relationship between blood parameters and study duration. $p<0.05$ was considered statistically significant.

RESULTS

In our study, 40.6% of the welders were between 21 and 30, 28.7% were between 31 and 40, and 30.7% were between 41 and 50. 34.3% of the participants have been welding for less than five years, 29.8% for 5-9 years, 15.0% for 10-19 years, and 20.9% for 20 years or more. According to body-mass index values, 1.6% of the participants were underweight, 37.4% were average weight, 44.1% were pre-obese, and 16.9% were obese. 63.8% of the participants were smokers (Table 1). Hyperglycemia was present in 35.8%, polycythemia in 27.2%, thrombocytosis in 12.6%, elevated sedimentation in 11.4%, elevated alanine aminotransferase in 10.6%, leukocytosis in 7.1%, elevated aspartate aminotransferase in 5.9% and elevated free prostate-specific antigen in 5.9% (Table 2). When the blood parameters of the participants were analyzed, fasting blood glucose was 95.7±11.4, aspartate transaminase 19.6±6.6, alanine transaminase 27.2±16.1 and sedimentation rate 7.5±5.5 in those who had worked less than five years. In those who worked five years or more, these values were 100.4±25.5, 23.0±8.6, 31.1±19.2 and 9.1±5.4, respectively. The difference was statistically significant ($p<0.05$). No significant correlation existed between hemoglobin, platelet, manganese, and creatinine values in blood tests and working for five years or more (Table 3). In the blood test results, leukocyte, glucose, aspartate transaminase, alanine transaminase, and sedimentation rate values were correlated with total working time (Table 4).

DISCUSSION

In our cross-sectional study of welders admitted to a hospital where many occupational diseases in Turkey are detected, the participants' health examination results were evaluated, and the

Table 1. Sociodemographic characteristics of participants, Türkiye, 2022

	(n)	(%)
Age (n=254)		
21-30 years old	103	40.6
31-40 years old	73	28.7
41-50 years old	78	30.7
Working time (n=254)		
Less than 5 years	87	34.3
5-9 years	76	29.8
10-19 years	38	15.0
More than 20 years	53	20.9
Body-mass index (n=254)		
<18,5 kg/m2	4	1.6
18,5-24,9 kg/m2	95	37.4
25,0-29,9 kg/m2	112	44.1
>30,0 kg/m2	43	16.9
Smoking (n=254)		
Yes	162	63.8
No	92	36.2

Table 2. Laboratory characteristics of participants, Türkiye, 2022

	(n)	(%)
Hyperglycemia	91	35.8
Polycythemia	69	27.2
Thrombocytosis	32	12.6
Elevated ESR	29	11.4
Elevated ALT	27	10.6
Leukocytosis	18	7.1
Elevated AST	15	5.9
Elevated fPSA	15	5.9

Table 3. Blood test results according to participants' working time, Türkiye, 2022

	< 5 years (n=87)	≥5 years (n=167)
Hemoglobin (n=254) **		
Mean±SD	16.3±1.0	16.2±0.9
Median	16.3	16.3
Range (min-max)	13.6-18.5	12.0-18.8
p=0.451		
Leukocytes (n=254) *		
Mean±SD	7.4±1.6	8.0±2.2
Median	7.3	7.6
Range (min-max)	4.6-11.5	3.7-18.1
p=0.091		
Platelets (n=254) **		
Mean±SD	244.7±51.5	244.9±58.0
Median	247	236
Range (min-max)	145-400	145-400
p=0.987		
Glucose (n=254) *		
Mean±SD	95.7±11.4	100.4±25.5
Median	95	97
Range (min-max)	69-161	56-341
p=0.042		
Aspartate transaminase (n=254) *		
Mean±SD	19.6±6.6	23.0±8.6
Median	18	21
Range (min-max)	10-43	12-78
p=0.015		
Alanine transaminase (n=254) *		
Mean±SD	27.2±16.1	31.1±19.2
Median	22	26
Range (min-max)	9-89	9-155
p<0.001		
Sedimentation rate (n=254) *		
Mean±SD	7.5±5.5	9.1±5.4
Median	6	8
Range (min-max)	1-25	3-36
p=0.001		
Manganese (n=254) *		
Mean±SD	11.9±4.2	11.3±3.5
Median	11.6	11,1
Range (min-max)	4.8-23.0	4.7-24.0
p=0.353		
Creatinine (n=254) **		
Mean±SD	0.84±0.10	0.84±0.09
Median	0.84	0.83
Range (min-max)	0.6-1.1	0.6-1.1
p=0.798		

asdf * Independent samples t-test, **Mann-Whtitney U test

Table 4. Correlation of blood parameters with working time

	r	p
Hemoglobin	-0.032	0.614
Leukocytes	0.127	**0.043**
Platelets	-0.002	0.969
Glucose	0.202	**0.001**
Aspartate transaminase	0.176	**0.005**
Alanine transaminase	0.147	**0.019**
Sedimentation rate	0.205	**0.001**
Manganese	-0.077	0.219
Creatinine	-0.047	0.459

relationship between occupational exposure and blood parameters was examined. In addition, some chronic disease risk factors that may negatively affect the general health status of workers were also discussed. Our findings will be helpful in terms of revealing the effects of welding fumes on workers' health.

Approximately three-fifths of the welders in our study were smokers. 22.3% of the world population (36.7% of men) use tobacco products, and approximately 80% of the 1.3 billion users are in low- and middle-income countries [12]. In Turkey, the prevalence of smoking in men aged 15 years and older is 41.3% [13]. In studies conducted in Iran and Taiwan, the prevalence of smoking among welders is approximately one-third. Simultaneous cigarette and welding fumes exposure was associated with decreased pulmonary function [14,15]. In another study conducted in Turkey, welding increased the risk of chronic bronchitis by 2.8 times and smoking by 3.2 times [16]. The prevalence of smoking among welders in our study was higher than the population average. Smoking habits of certain occupational groups may be more common. Social interaction among welders or workplace culture may encourage smoking. Welders who are under stress due to their jobs may adopt smoking as a relaxation method.

In light of our findings, 44.1% of the participants were pre-obese, and 16.9% were obese according to their body mass index. The mean body mass index of the participants was 26.4. In European Union countries, the prevalence of pre-obesity in men was 43.9%, and the prevalence of obesity was 16.3% [17]. In Turkey, 39.7% of men aged 15 years and older are pre-obese, and 17.3% are obese [18]. In a study conducted in China to investigate the health

status of welders, 52.6% of welders were pre-obese or obese [19]. In a study conducted on welders in Canada, the prevalence of pre-obesity and obesity was 61% [20]. In Taiwan, welders' average body mass index was 25.2 [21]. In the United States, pre-obesity prevalence among workers was 36.1%, obesity was 22.0%, and the mean body mass index was 26.2. In advanced clerical and service sector workers, these prevalences were 50.4% and 27.3%, respectively, with an average BMI of 28.3 [22]. According to the average body mass index in the Netherlands, those working in the transportation sector are in the first place, with 25.2. Those working in the metal industry have an average body mass index of 24.7. The prevalence of overweight and obesity in this sector is 36.1% and 6.0%, respectively [23]. The prevalence of pre-obesity and obesity in welders in our study is similar to the population average and other studies conducted in the same occupational group. Working in a physically demanding job, with irregular or long working hours, may increase the tendency to gain weight. Lack of physical activity opportunities in some occupations may lead to obesity. Limited healthy eating options and irregular eating habits at work are also risky. We think that the prevalence of pre-obesity and obesity in welders is alarming in terms of worker health.

According to the results of the complete blood count performed in our study, leukocytosis was in 7% of the participants, and a weak correlation was between the total working time of the participants and the leukocyte count. In a study conducted in the United States of America, a significant increase in total leukocyte count was found in non-smoking welders immediately after exposure to welding fumes. Acute systemic inflammatory reactions were observed hours after welding fume exposure [24]. In a case-control study conducted in Ghana, total leukocyte count did not show a significant difference between welders and non-welders [25]. In our study, high smoking frequency may lead to leukocytosis. Leukocytosis may be natural, but prolonged or persistent high white blood cell levels may indicate health problems. More comprehensive studies are needed to understand the effects of occupational exposure on leukocyte levels.

Our study found that liver enzyme levels were higher in welders who had worked for five years or more compared to those who had worked for less than five years, and these levels were correlated with total working time. In a similar study conducted in Turkey investigating the effect of manganese exposure on the erythropoietic system, AST and ALT levels were higher in welding workers than in-office workers [26]. In a study conducted on healthy workers in a ferromanganese refinery in China, AST and ALT levels were higher in welding and production workshop workers than in other worker groups, such as drivers and cooks [27]. A study conducted in Poland found that serum AST and ALT levels were significantly different in foundry workers compared with the control group [28]. In Iran, AST and ALT levels were significantly higher in miners than in the control group [29]. Increased concentrations of AST and ALT enzymes have been reported in mice exposed to welding fumes [30]. Although the type and solubility level of heavy metals to which welders are exposed vary depending on the type of welding process and welding electrode, an increase in AST and ALT levels may be observed in all workers exposed to multiple heavy metals due to the development of liver damage. It may not only be due to the welding work of the workers but may also indicate other health problems. Nevertheless, based on the findings in the literature, welders are a risky group in terms of liver health.

Our study found that fasting blood glucose levels were higher in welders who worked for five years or more than those who worked for less than five years, and these levels were correlated with total working time. A study conducted on shipyard workers in Taiwan found that heavy metal exposure disrupted glucose homeostasis, and welders had higher fasting blood glucose levels than administrative workers [31]. A study investigating the potential association of metal levels with diabetes risk in workers in complex coal furnaces in China showed that high levels of many metals measured in urine were associated with hyperglycemia and diabetes risk [32]. Similar results were also in another study evaluating toxic metals in biological samples of diabetic patients [33]. In a study investigating the relationship between metal exposure and cardiometabolic risk factors in young men of African descent living in different countries, it was reported that insulin resistance and oxidative stress may be induced by metal exposure in breathing air and may lead to abnormal glucose metabolism

and increase the risk of developing diabetes [34]. As seen, researchs shows that metal exposure can trigger diabetes risk factors and effect glucose homeostasis. Metals, which cumulatively accumulate more in the body as the duration of work increases, may increase inflammation at the cellular level due to their toxic effects and lead to hyperglycemia over time.

CONCLUSION

The majority of the welders in our study were found to be smokers. The prevalence of overweight and obesity among the participants was considerable. A correlation was found between working time liver enzyme levels and fasting blood glucose values. As in all occupational groups, health surveillance, workplace environment measurements, and organizational arrangements are essential for welders. Periodic health examinations are necessary to assess health problems accurately. These examinations can help diagnose respiratory problems caused by smoking and welding fumes. Weight monitoring can help identify employees at risk of obesity. Parameters such as liver enzyme levels and blood glucose should also be checked. Early detection can help end exposure and start treatment as soon as possible. Welders should be trained about the occupational health and safety risks they face. They should be made aware that they should not smoke. Workers should be given accurate nutrition information and encouraged to develop healthy behaviors. Support can be provided to employees through smoking cessation programs. Providing exercise in the workplace can help prevent obesity or promote weight loss. These activities can also have positive effects on metabolic health. Appropriate measures should be taken to make workplace environments healthier and safer. In this context, measures to reduce exposure should be prioritized. The effectiveness of ventilation systems is vital in determining the distribution and levels of harmful substances, such as welding fumes, in the work environment. It is essential to install better ventilation systems or use personal protective equipment. Regulating working hours and exposure time can help workers rest and reduce their exposure. All this will be possible if employers fulfill their responsibilities and obligations concerning occupational health and safety.

Author contribution

Study conception and design: VM, RA and PÇ; data collection: PÇ; analysis and interpretation of results: VM and FB; draft manuscript preparation: VM, RA and İM. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Gazi University Ethics Committee (Protocol no. 2023-1306).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Does proptosis effect the meibography in patients with thyroid eye disease?

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ABSTRACT

Objective: The study aimed to compare the meibographies of the eyes of patients with thyroid eye disease who have varying degrees of proptosis.

Materials and Methods: Charts of patients with thyroid eye disease between January 2019 to January 2022 were retrospectively reviewed. Patients with mild and inactive thyroid eye disease, and with 1 mm or more difference in measurements of Hertel exophthalmometer between the eyes were included in the study. The eye of each patient with higher proptosis was included in the study group while the other eye with lower proptosis was included in the control group. The area of meibomian gland loss was evaluated using meibography (Sirius; CSO, Florence, Italy).

Results: Total of 28 eyes of 14 patients were evaluated. Mean meibomian gland dropout area for the upper eyelid was 17.91± 15.37% in the study group and 14.43± 8.61% in the control group. Mean meibomian gland dropout area for the sum of upper and lower eyelid was 44.76± 23.16% in the study group and 43.03± 21.59% in the control group. Mean meibomian gland dropout area for the upper eyelid and also for the sum of upper and lower eyelid were higher in the study group than the control group; however, these results were not significant ($p=0.540$ and 0.865, respectively). On the other hand, the Pearson correlation test results suggested a significant correlation between the two groups; for the upper eyelid ($p<0.001$, $r=+0.670$) and also for the sum of upper and lower eyelids ($p<0.001$, $r=+0.768$).

Conclusion: This study showed differences regarding meibographic changes between control and study group. Further studies with larger series are needed to confirm these results.

Keywords: Meibography, meibomian gland morphology, thyroid eye disease

INTRODUCTION

Thyroid eye disease (TED) is one of the most common orbital inflammatory disease, moreover it is the most common cause of unilateral and bilateral proptosis seen in adults [1]. The clinical presentation of TED includes proptosis, lid retraction, lid lag, lagophthalmus, restrictive extraocular myopathy, optic neuropathy, and inflammatory changes of the ocular surface [2]. Dry eye is often the primary culprit behind ocular surface discomfort in these individuals [3]. Prior research has suggested that, alongside inflammation, factors such as tear film evaporation and osmolarity due to proptosis and widened lid fissures could contribute to dry eye symptoms in these patients [4]. However, the precise mechanism detailing the connection between TED and dry eye remains incompletely understood.

The study was designed to compare the meibographies of the eyes of patients with TED who have varying degrees of proptosis in between the eyes. Thus, it was aimed to investigate one of the factors contributing to the pathophysiology of dry eye in TED patients.

MATERIALS and METHODS

Charts of patients with TED who were admitted to our hospital between January 2019-January 2022 were retrospectively reviewed. Patients with mild and inactive TED, and with 1 mm or more difference in measurements of Hertel exophthalmometer (Handaya, Tokyo, Japan) between the eyes were included in the study. The eye of each patient with higher proptosis was included in the study group while the other eye with lower proptosis was included in the control group. The area of meibomian gland loss was evaluated using meibography (Sirius; CSO, Florence, Italy). The upper and lower eyelids' tarsal conjunctival surfaces were inverted, and a minimum of eight images of meibomian glands were captured to choose the most distinct image. All the measurements were done by the same physician. The study was approved by the Ankara Bilkent City Hospital Ethics Committee (E1-23-4564) and conducted in accordance with the principles of the Helsinki Declaration.

The distribution of the data was evaluated with the Kolmogorov–Smirnov test. Student's T-test and Mann-Whitney U test were used for differences between the groups. Pearson correlation coefficients were used to study the association between study and control groups. A value of $P \leq 0.05$ was considered significant. Statistics were made using SPSS 25.0 program.

RESULTS

A total of 28 eyes from 14 patients were assessed, comprising 9 (64.3%) women and 5 (35.7%) men. The mean age of the patients was 49.30±11.25 years. The mean Hertel exophthalmometry value was 19.36 ±4.22mm for the study group and 17.79 ±3.87mm for the control group.

The mean meibomian gland dropout area for the upper eyelid was 17.91± 15.37% in the study group and 14.43± 8.61% in the control group, while for the sum of upper and lower eyelids, it was 44.76± 23.16% in the study group and 43.03± 21.59% in the control group. Although the mean meibomian gland dropout area for both the upper eyelid and the sum of upper and lower eyelids was higher in the study group compared to the control group, these differences did not reach statistical significance (p=0.540 and p=0.865, respectively) (Table 1).

On the other hand, the results of the Pearson test indicated a significant correlation between the two groups: for the upper eyelid ($p<0.001$, $r=+0.670$) and also for the sum of upper and lower eyelids ($p<0.001$, $r=+0.768$).

Table 1. Meibomian gland dropout area in eyes with greater proptosis (study group) and lesser proptosis (control group)

	Study Group	Control Group	P
Meibomian gland loss area (upper eyelid) (%)	17.91±15.37	14.43±8.61	0.540
Meibomian gland loss area (lower eyelid) (%)	26.85±12.75	28.60±15.80	0.788
Meibomian gland loss area (sum of lower and upper eyelid) (%)	44.76±23.16	43.03±21.59	0.865

DISCUSSION

TED is one of the most common orbital inflammatory disease, moreover it is the most common cause of unilateral and bilateral proptosis seen in adults [1]. The clinical presentation of TED includes proptosis, lid retraction, lid lag, lagophthalmus, restrictive extraocular myopathy, optic neuropathy, and inflammatory changes of the ocular surface [2]. Dry eye is often the primary cause of ocular surface discomfort in individuals with TED [3]. Prior studies suggest that, apart from inflammation, tear film evaporation and osmolarity due to proptosis and widened lid fissures may contribute to dry eye symptoms [4]. However, the exact mechanism elucidating the link between TED and dry eye remains incompletely understood.

Earlier investigations examining the correlation between dry eye and TED found notable distinctions in meibography scores between individuals with TED and healthy individuals [5-7]. Moreover, the meibography scores of TED patients had a positive association with exophthalmos and palpebral fissure height in some studies [7, 8].

Recent studies have identified a link between meibomian dysfunction caused by systemic and ophthalmologic conditions and inflammation. TED is recognized as an inflammatory disorder, with previous research indicating inflammation in both the ocular surface and eyelids [9]. Is the pathogenesis of the dry eye seen in TED patients due to inflammation or does the degree of proptosis also contribute to the pathophysiology?

The investigation of mechanical effects revealed that blinking exerts shearing forces, reducing tear viscosity and facilitating the ejection of lipid from the meibomian orifices [10]. Consequently, incomplete blinking due to proptosis and eyelid retraction in TED patients may lead to obstructive meibomian gland disease. This obstruction could be one among multiple factors contributing to dry eye in individuals with TED.

In the lights of aforementioned; this study examined the attributes of the meibomian glands in mild-inactive TED patients who had different levels of proptosis in between the eyes. Thus, it was aimed to investigate one of the factors contributing to the pathophysiology of dry eye in TED patients. In the study, it is found that the mean meibomian gland dropout area for both the upper eyelid and the sum of upper and lower eyelids was higher in the more proptotic eyes of the patients with TED compared to the less proptotic eyes; however, these differences did not reach statistical significance. On the other hand, the results of the Pearson test revealed a significant correlation between the two groups: for the upper eyelid ($p<0.001$, $r=+0.670$) and also for the sum of upper and lower eyelids ($p<0.001$, $r=+0.768$). Consequently, the importance of these findings deserves to be confirmed in larger-scale studies.

The study has several limitations. To start, the study followed a retrospective design and had a limited sample size. Another limitation is the lack of data on diagnostic tests for dry eye and blinking mechanism. However this study was designed considering that there may be meibographic differences between the eyes of TED patients who have different amounts of proptosis in between the eyes that are exposed to different biomechanical conditions with each blinking movement. The study revealed meibography changes through a comparison among more proptotic eyes and less proptotic controls. It is believed that this finding warrants further investigation.

CONCLUSION

This study identified differences in meibographic changes between the eyes of TED patients with varying degrees of proptosis between both eyes. Further studies with larger series are necessary to validate these findings.

Author contribution

Study conception and design: SBE; data collection: SBE and DS; analysis and interpretation of results: SBE and DS; draft manuscript preparation: SBE. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ankara Bilkent City Hospital Ethics Committee (E1-23-4564/27.12.2023).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

The biochemical effect correlated with pulmonary dysfunction and complications in obese patients

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ABSTRACT

Objective: Obesity, an epidemic metabolic disorder, is associated with various biochemical, inflammatory, oxidative and immunological pathways. We aimed to investigate the biochemical effect correlated with pulmonary dysfunction and complications in overweight and obese patients.

Material and Methods: We aimed to evaluate retrospectively the effect of biochemical parameters on pulmonary dysfunction and complications in 79 overweight and obese patients.

The correlative effect of biochemical values, including CRP, and spirometric measurements, such as forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1), on pulmonary dysfunction and complications in 79 overweight and obese patients seen in the outpatient clinic were evaluated. Body mass index (BMI), FEV1 and FVC, and total biochemistry values, including creatinine, AST, ALT and CRP values were correlated among each other.

Results: Low FVC levels, leukocytosis, high AST and ALT levels, and comorbidities of obesity are significantly associated with high BMI values by univariate analysis in these patients. Higher AST levels are significantly correlated with higher leucocyte counts, and both AST and ALT levels are significantly correlated with platelet counts.

Conclusion: We investigated the effect of biochemical parameters on pulmonary dysfunction and complications in obese patients. Obesity can be helpful to categorize high-risk patients with low FVC levels in the context of respiratory diseases and high AST and ALT levels for other comorbidities as steatohepatitis, diabetes mellitus and coronary artery disease. This study sheds light on future research on obese patients for prognosis of these diseases, because of their biochemical profile correlation with pulmonary dysfunction and complications.

Keywords: Biochemical parameters, pulmonary function, complications, obesity

INTRODUCTION

Obesity is an epidemic metabolic problem and defined as a body mass index (BMI) greater than 30 kg/m^2. The worldwide prevalence of obesity has increased gradually in the last years, with the major health and economic burdens, because of its comorbidities, such as malignancies, metabolic syndrome, diabetes mellitus (DM), steatohepatitis (MESH), hypertension (HT) and cardiovascular diseases (CAD) [1-3]. These complications are caused by mechanical changes and impairment of anti-oxidant mechanisms via adipocytes accumulation, especially in the chest and abdominal regions, and as well as adipocytes-derived pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α), tumor growth factor (TGFβ), interleukin-1-β (IL-1β), and interleukin-6 (IL-6) [4,5].

Obesity, a preventable and treatable metabolic condition, is associated with chronic inflammation through the cascade of adipocytes-derived pro-inflammatory adipokines and adipokine-derived reactive oxygen species. Because of its complex pathophysiology, this condition has a high mortality and morbidity rate. In this metabolic process, there are both immune (immune cells) and non-immune (adipocytes) inflammatory changes via oxidative stress imbalance, which is triggered by genetic, epigenetic and environmental disturbances. Chronic inflammation is caused by activation of the innate immune system, which promotes a pro-inflammatory state and adipokine-derived oxidative stress, eventually systemic acute-phase response. Similar to other organs, chronic inflammation in the airways initiates the pathological process of respiratory diseases. This inflammatory process is ended up with an irreversible lipotoxic tissue damage, which induces chronic airway obstruction, bronchitis and systemic pulmonary dysfunction [6,7].

The mechanical effects of obesity on pulmonary physiology and the functional effect of adipose tissue as an endocrine organ producing chronic systemic inflammation via cytokines and effects on central respiratory control result in the comorbidity of obesity as pulmonary dysfunction. Obesity causes mechanical compression of the diaphragm, lungs, and chest cavity, which can lead to restrictive pulmonary damage. It also significantly interferes with respiratory function by decreasing lung volume, particularly the expiratory reserve volume and functional residual capacity. The ineffectiveness of the respiratory muscles reduces strength and increases pulmonary resistance. All these factors lead to inspiratory overload, which increases respiratory effort, oxygen consumption, and respiratory energy expenditure [8,9].

Besides chronic obstructive pulmonary disease (COPD), obesity plays a key role in the development of obstructive sleep apnea and obesity hypoventilation syndrome [10].

Recent literature has shown that obesity is associated with impaired pulmonary function and increased risk of respiratory diseases [6,10-21]. Bantula et al. and Zammit et al. indicated that asthmatic patients with obesity have severe disease, need intense treatment, harder to treat and have frequent acute attacks [5,6].

BMI and waist-to-hip ratio (WHR) are both inversely associated with lung function, as assessed by forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) [14]. By using inverse-variance weighting to estimate the causal association of BMI and BMI-adjusted WHR with FVC, FEV1, FEV1/FVC, and asthma, Liu et al. found that increased BMI is causally related to decreased FVC and FEV1, and increased BMI-adjusted WHR could lead to lower FVC value and higher risk of asthma. Higher BMI and BMI-adjusted WHR were suggested to be causally associated with higher FEV1/FVC [14].

Therefore, we aimed to pursue a better understanding of the causal relationship among obesity, pulmonary dysfunction and diseases, and investigate the biochemical effect correlated with pulmonary dysfunction and complications in obese patients.

MATERIAL and METHODS

This study is a retrospective study. Patients over the age of 18 who applied to Lokman Hekim Chest Diseases outpatient clinic between January 2018 and February 2024 and having BMI values of 20-24.99, 25-34.99 and higher than 30, which is equivalent to normal, overweight and obesity (respectively) were included in the study with an

ethical approval of Lokman Hekim University Ethical Committee Approval No: 2024/74. Patients under the age of 18 and pregnant, who have a history of malignancy, active systemic disease, collagen tissue disease, interstitial lung disease, COPD, obstructive sleep apnea, obesity hypoventilation syndrome and those who use drugs that could affect complete blood count and total biochemistry parameters were excluded from the study.

Obese patients over 18 years old and retrospective analysis of the files of patients admitted to the outpatient clinic. The files and résumés of these patients were examined. Pulmonary function tests were recorded from their files. The patients' admission complete blood counts and total biochemistry parameters, including creatinine, aspartate transaminase (AST) and alanine transaminase (ALT), and acute phase reactant as C-reactive protein (CRP) results were examined from their files.

We evaluated retrospectively the effect of biochemical parameters on pulmonary dysfunction and complications in 79 overweight and obese patients, compared with healthy weight people.

The correlative effect of biochemical values, CRP and spirometric measurements, such as FEV1, FVC and FEV1/FVC on pulmonary dysfunction and complications in obese patients seen in the outpatient clinic were evaluated. BMI, FEV1, FVC and CRP values and total biochemistry values were correlated among each other.

FEV1 (lt), FVC (lt) and FEV1/FVC levels were measured by spirometry.

In their initial blood tests, which were obtained at admission, following parameters were studied: White Blood Cells (WBC) numeric value (normal range 4.6-10.2 x10^3/μL), neutrophil numeric value (normal range 1800-7700/μL), lymphocyte numeric value (normal range 1500-4000/μL), platelet numeric value (normal range 142-450x10^3/μL), creatinine value (normal range 0.5-1.2 mg/dL), CRP value (normal range 0-0.5 mg/dL), ALT value (normal range 10-40 IU/L) and AST value (normal range 15-50 IU/L) [22].

WBC, neutrophil and lymphocyte counts were measured by fluorescent flow scatter [22]. Platelet counts were measured by electric impedance [22].

Serum creatinine levels were measured by alkaline picrate based assay (Beckman) [22].

Serum CRP levels were measured by nephelometric/ turbidometric method (Beckman) [22].

Serum ALT levels were measured by IFCC method-LDH without pyridoxal 5 phosphate (P5P) (UV without P5P) (Beckman) [22].

Serum AST levels were measured by IFCC method-MDH without P5P (UV without P5P) (Beckman) [22].

In order to determine the number of samples, a power analysis (α =0.05, β =0.80) was performed by taking a study with similar methodology and a sample size of n=75 was obtained [7], a total sample of 79 people was obtained at the end of the study.

Analysis was performed with the SPSS v.25 software. Significance was set at $p < 0.05$ for all comparisons/analyses. Continuous data were summarized with mean ± standard deviation values, categorical data were summarized with frequency (n) and relative frequency (%). Univariate comparisons for continuous data were performed with the Mann-Whitney U test due to the fact that parametric assumptions were not met for any of the comparisons or variable sets. Categorical data distributions were compared with the Pearson Chi-square or the Fisher's exact test depending on assumptions.

RESULTS

11 female (5 (BMI 20-24.99), 4 (BMI 25-34.99) and 2 (BMI higher than 35)) and 68 male (36 (BMI 20-24.99), 28 (BMI 25-34.99) and 4 (BMI higher than 35)) healthy weight, overweight and obese patients were included in our study (Table 1).

Median age were 59.12 (BMI 20-24.99), 56.87 (BMI 25-34.99) and 59.5 (BMI higher than 35) years old (Table 1).

Low FVC levels, high WBC counts (leukocytosis), high AST and ALT levels are significantly associated with high BMI values by univariate analysis in these patients (Table 1).

Comorbidities of obesity including hypertension (HT), diabetes mellitus (DM), Coronary arterial disease (CAD) are significantly associated with high BMI values, by univariate analysis (Table 1).

Table 1. Summary and comparison of patient characteristics with respect to Body mass index (BMI) groups by using univariate analysis of possible risk factors in 79 patients

		Body mass index			P value
		25-29.9	30-34.9	>35	
Sex	Female	5 (12.2%)	4 (12.5%)	2 (33.3%)	0.360
	Male	36 (87.8%)	28 (87.5%)	4 (66.7%)	
Age (years)		59.12 ± 7.28	56.87 ± 9.03	59.5 ± 13.13	0.514
FEV1 (%)		48.85 ± 14.38	51.75 ± 13.19	50.33 ± 10.33	0.577
FEV1 (L)		1.2 ± 0.43	1.27 ± 0.36	1.22 ± 0.35	0.583
FVC (%)		57.34 ± 6.86	59.59 ± 7.5	59.33 ± 9.31	0.278
FVC (L)		2.04 ± 0.65	2.17 ± 0.62	1.55 ± 0.57	0.050
FEV1/FVC		84.98 ± 8.6	84.13 ± 7.27	78.83 ± 5.56	0.108
WBC (mm^3)		9.29 ± 2.28	9.48 ± 2.35	12.19 ± 2.47	0.050
Platelet count (mm^3)		238.37 ± 106.48	228.66 ± 127.46	233 ± 46.59	0.700
Neutrophil count (mm^3)		6.16 ± 1.87	6.23 ± 1.98	7.33 ± 1.92	0.411
Lymphocyte count (mm^3)		1.39 ± 0.6	1.43 ± 0.57	1.39 ± 0.46	0.875
CRP (mg/L)		10 ± 13.16	8.26 ± 7.66	12.39 ± 10.06	0.433
Creatinine (mg/dL)		1.05 ± 0.52	1.16 ± 0.65	0.91 ± 0.27	0.734
CAD	No	36 (87.8%)	28 (87.5%)	1 (16.7%)	**<0.001**
	Yes	5 (12.2%)	4 (12.5%)	5 (83.3%)	
HT	No	30 (73.2%)	23 (71.9%)	1 (16.7%)	**0.018**
	Yes	11 (26.8%)	9 (28.1%)	5 (83.3%)	
DM	No	32 (78%)	27 (84.4%)	2 (33.3%)	**0.023**
	Yes	9 (22%)	5 (15.6%)	4 (66.7%)	
AST (IU/L)		22.8 ± 10.89*	33.08 ± 14.88	42.37 ± 7.48	**<0.001**
ALT (IU/L)		24.29 ± 11.12*	33.72 ± 15.1	49.33 ± 14.73	**0.001**

Continuous data reported as mean ± standard deviation, categorical data reported as n (%).

Continuous data comparisons performed with the Kruskal-Wallis test, categorical data compared with Pearson chi square test.

*Significantly lower compared to both other groups (Bonferroni correction).

FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, WBC: white blood cell count, CRP: c-reactive protein, CAD: coronary artery disease, HT: hypertension, DM: diabetes mellitus, AST: aspartate transaminase, ALT: alanine transaminase.

FEV1 levels are significantly correlated with older age, and FVC levels are significantly correlated with FEV1 levels (Table 2).

Lympocytes counts are significantly correlated with older age (Table 2).

Liver function parameters, such as AST and ALT levels, are significantly correlated with each other. AST levels are significantly correlated with WBC counts, and both AST and ALT levels are significantly correlated with platelet counts (Table 2).

DISCUSSION

Obesity is a metabolic disorder with high morbidity and mortality. This disorder is caused by the consequence of an excessive adipose tissue accumulation. M1 macrophages' infiltration in adipose tissue of obese patients as well as overexpression of inflammatory adipokines are the major causes of obesity-related chronic inflammation "metainflammation". The inflammatory process is triggered by adipose tissue-derived adipokines, such as increased pro-inflammatory leptin and decreased anti-inflammatory adiponectin levels, and increased levels of pro-inflammatory cytokines, such as IL-1β, IL-6, TNFα, and TGFβ. For instance, in asthma, pro-inflammatory cytokines, such as IL-4, IL-5, IL-13, and IL-33, maintain the lean state. Obesity increases asthma risk and severity. Macrophage activation, age and sex affects immunometabolism in obese asthma patients [23-25]. Weight loss reduces inflammation, so improves asthma prognosis and lung function [5,26].

Table 2a. Correlations among continuous variables (age, FEV1, FVC, FEV1/FVC and WBC) examined by using correlation analysis in 79 patients

		Age	FEV1	FVC	FEV1/FVC	WBC
Age (years)	r	1				
	p	.				
FEV1 (L)	r	-.253*	1.000			
	p	**.025**	.			
FVC (L)	r	-.204	.357**	1.000		
	p	.072	**.001**	.		
FEV1/FVC	r	-.136	.053	.076	1.000	
	p	.233	.642	.508	.	
WBC (mm^3)	r	-.099	.135	.164	.141	1.000
	p	.384	.236	.149	.215	.
Platelet count (mm^3)	r	-.071	-.018	.079	-.020	.118
	p	.531	.877	.488	.863	.300
Neutrophil count (mm^3)	r	.052	.151	.132	.080	.723**
	p	.652	.185	.246	.485	**.000**
Lymphocyte count (mm^3)	r	-.356**	.066	-.002	.058	.138
	p	**.001**	.562	.987	.609	.225
CRP (mg/L)	r	-.153	.053	-.043	-.037	.080
	p	.178	.642	.706	.748	.484
Creatinine (mg/dL)	r	-.198	.025	.026	.069	.029
	p	.081	.827	.823	.547	.803
AST (IU/L)	r	-.055	-.054	.012	-.107	.270*
	p	.628	.635	.918	.350	**.016**
ALT (IU/L)	r	-.101	-.002	-.041	-.096	.186
	p	.376	.988	.719	.401	.100

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Besides activation of pulmonary NLRP3 inflammasome causes increased infiltration and activation of neutrophils, resulting in NETosis, worsening pulmonary symptoms, decreased lung function, and increased steroid resistance in obese asthma patients [27].

Asthma and obesity are two diseases bridged by inflammation. In obesity-related metainflammation, adipocytes and M1 macrophages produce inflammatory cytokines including IL-6, TNF-α, IL-1β, and monocyte chemoattractant protein (MCP-1). By the trigger of NLRP3 activation, M1 macrophages can secrete pro-inflammatory cytokines such as IL-1β, IL-18, MCP-1, TNF-α, and IL-6 into the circulation [5]. Obesity increases airway hyperresponsiveness via the TNF-alpha pathway and treating obesity induces recovery [28]. Obese adipose tissue modulates proinflammatory responses of airway epithelial cells via neutrophilia and monocytosis [29]. Adipose tissue macrophage populations and inflammation are associated with systemic inflammation-related complications such as pulmonary dysfunction [30] and insulin resistance in obesity [31].

In our study, the synergistic effect of low FVC levels and leukocytosis, especially neutrophilia and monocytosis, are significantly associated with high BMI values by univariate analysis in overweight and obese patients without any pulmonary diseases (Table 1).

Additionally, the synergistic effect of low FVC levels and high AST and ALT values are significantly associated with high BMI values (Table 1). AST levels are significantly correlated with WBC counts (Table 2). Comorbidities of obesity including HT, DM and CAD are significantly associated with high BMI values (Table 1). As a summary of our study, obesity alone is associated with pulmonary dysfunction, leukocyte (monocyte and neutrophil) infiltration (possibly metainflammation) and liver dysfunction.

Table 2b. Correlations among continuous variables (platelet, neutrophil and lymphocyte count, CRP, Creatinine, AST and ALT) examined by using correlation analysis in 79 patients

		Platelet count	Neutrophil count	Lymphocyte count	CRP	Creatinine	AST	ALT
Age (years)	r							
	p							
FEV1 (L)	r							
	p							
FVC (L)	r							
	p							
FEV1/FVC	r							
	p							
WBC (mm^3)	r							
	p							
Platelet count (mm^3)	r	1.000						
	p	.						
Neutrophil count (mm^3)	r	.054	1.000					
	p	.634	.					
Lymphocyte count (mm^3)	r	.132	.051	1.000				
	p	.247	.652	.				
CRP (mg/L)	r	-.176	.054	.092	1.000			
	p	.120	.633	.421	.			
Creatinine (mg/dL)	r	-.011	-.006	-.044	.004	1.000		
	p	.924	.955	.701	.969	.		
AST (IU/L)	r	-.257*	.183	.155	-.071	-.095	1.000	
	p	**.022**	.106	.173	.536	.407	.	
ALT (IU/L)	r	-.309**	.110	.088	-.054	-.106	.881**	1.000
	p	**.006**	.336	.443	.635	.354	**.000**	.

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Obese patients have increased asthma risk, and obese asthmatic patients have more symptoms, more frequent and severe exacerbations, reduced response to several asthma medications, and decreased quality of life [26,32,33]. On the other hand, obesity related central airway collapse can be independent of asthma phenotype [34]. Airway mechanics can be altered in both obesity and/or asthma [35]. BMI has significant effects on all of the lung volumes [36-39], and the greatest effects were on functional residual capacity and expiratory reserve volume [40]. In addition, obesity decreases total respiratory system compliance primarily because of decreased lung compliance, with only mild effects on chest wall compliance. Obesity is associated with impaired gas transfer with decreases in oxygenation and varied but usually mild effects on diffusing capacity for carbon monoxide, while the carbon monoxide transfer coefficient is often increased [41].

Metabolically healthy obesity had better lung function [38]. Metabolically healthy obesity was associated with attenuated FVC and FEV1 decline in middle-aged people [38]. This shows the necessity for the synergistic effect of abnormal biochemical metabolism and pulmonary dysfunction on obesity, similar to our study.

The most important advantages of our study are: (1) All BMI levels are included in the study. (2) None of the patients had malignancy, active systemic disease, collagen tissue disease, interstitial lung disease, COPD, obstructive sleep apnea, obesity hypoventilation syndrome. Therefore, the direct influence of obesity on biochemical values and pulmonary dysfunction could be evaluated. (3) Since the number of studies conducted in mid-aged (median age= 58.5) overweight and obese people without any pulmonary disease is less, we think that our findings can contribute to the demonstration

of the correlative effect of biochemical values and pulmonary dysfunction in Turkish overweight and obese patients. However, some potential limitations should be considered when interpreting the results. First, the results cannot be generalized to the whole population, as it is a single-center study. Secondly, the small number of participants prevented detailed comparison of the correlative effect of biochemical values and pulmonary dysfunction; hence, it may have unfavorably affected the statistical analyses.

Besides the correlation between body fat and BMI is not constant. Therefore, assessing body fat distribution with measurements, such as waist circumference or waist-to-hip ratio may improve evaluation and diagnosis of obesity [42].

In conclusion, the synergistic effect of low FVC, high AST and ALT levels and leukocytosis may help predict the complications (comorbidities), such as respiratory diseases, HT, DM, CAD and MESH, in overweight and obese patients. Further studies are necessary to assess whether the correlative effect of biochemical values and pulmonary dysfunction can help explain the pathophysiological mechanisms of obesity-related inflammatory complications.

Author contribution

Study conception and design: ESG and BC; data collection: ESG and BC; analysis and interpretation of results: ESG and BC; draft manuscript preparation: BC. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Lokman Hekim University Ethical Committee (Approval No: 2024/74/23.02.2024).

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Conflict of interest

The authors declare that there is no conflict of interest.

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