European Review for Medical and Pharmacological Sciences 2023; 27: 9978-9986

Evaluation of Transforming Growth Factor Beta-1 in the vein wall of males with varicocele

M.Z. KESKIN[1], S. EKMEKCI[2], A.Y. ENGIN[3], E. ATES[4], M. UCAR[5], E. SIYEZ[6], E. KISA[1], C. YÜCEL[1], C. KÖSE[7], M.A. ERDOGAN[8], Y.Ö. İLBEY[9]

[1]Urology Clinic, Tepecik Training and Research Hospital, Izmir, Turkey
[2]Pathology Clinic, Tepecik Training and Research Hospital, Izmir, Turkey
[3]Cardiovascular Surgery Clinic, Tepecik Training and Research Hospital, Izmir, Turkey
[4]Urology Clinic, Adnan Menderes University Hospital, Aydın, Turkey
[5]Urology Clinic, Akdeniz University Hospital, Antalya, Turkey
[6]Urology Clinic, Buca Seyfi Demirsoy State Hospital, Izmir, Turkey
[7]Embrylogy Clinic, Tepecik Training and Research Hospital, Izmir, Turkey
[8]Department of Physiology, Faculty of Medicine, Izmir Katip Celebi University, Izmir, Turkey
[9]Urology Clinic, Bezmi Alem Vakıf University Hospital, Istanbul, Turkey

Abstract. – OBJECTIVE: This study aims to investigate potential differences in the presence of Transforming Growth Factor-Beta 1 (TGF-β1) between the vein walls of patients with varicocele and those of healthy individuals.

PATIENTS AND METHODS: The study comprised a total of 40 participants, divided into two groups. The control group (Group 1) consisted of 20 patients who underwent coronary bypass surgery, while the varicocele group (Group 2) included 20 patients scheduled for varicocelectomy. The cytoplasmic and nuclear staining patterns of TGF-β1 immunohistochemistry were assessed in tissue samples under light microscopy, identifying any differences in TGF-β1 presence between varicocele patient vein walls and normal (saphenous) veins.

RESULTS: The varicocele group demonstrated lower nuclear and cytoplasmic TGF-β1 staining rates compared to the control group. After controlling for the independent factor of age, significantly lower nuclear and cytoplasmic staining was still observed in the varicocele group.

CONCLUSIONS: This study is the first of its kind to compare TGF-β1 staining in the vein walls of varicocele patients and healthy individuals. Previous studies focusing on varicose veins reported elevated TGF-β1 expression. Contrarily, our study observed lower TGF-β1 expression in varicocele patient veins, marking a unique contribution to the field.

Key Words:
TGF-β1, Varicocele, Normal vein walls, Varicose vein wall.

Introduction

Varicocele is the most commonly diagnosed treatable condition related to male infertility. It holds a prevalence rate of 15% among the general population and ranges between 19-41% in the infertile population. Moreover, varicocele prevalence reaches 35% in the primary infertile population and escalates to 80% in the secondary infertile population[1]. Varicocelectomy is the most frequently employed treatment method for this condition[2]. While varicocelectomy has been proven to enhance semen parameters, its effect on pregnancy and paternity rates remains a contentious issue[3]. The primary factors contributing to the disruption of spermatogenesis include malfunctioning spermatic vein valves and an increase in scrotal temperature due to venous reflux[4].

Several factors are implicated in the etiology of varicocele. Recent reports[5,6] suggest that certain cytokines, growth factors [like Transforming Growth Factor Beta-1, (TGF-β1)] and extracellular matrix metalloproteinases-2 and -9 (MMP-2 and MMP-9) may trigger the onset of varicocele by promoting fibrosis in the vascular wall. Additionally, neurotransmitters such as nitric oxide have been found in high concentrations in the spermatic vein walls of varicocele patients[7]. The TGF-β family consists of three polypeptide members (TGF-β1, TGF-β2, TGF-β3)[8]. TGF-β1, in particular, has a profound impact on the cardiovascular system, especially affecting endothelial cells

Corresponding Author: Mehmet Zeynel Keskin, MD; e-mail: zeynel_akd@hotmail.com

(EC), vascular smooth muscle cells (VSMC), myofibroblasts, macrophages, and other cells of the hematopoietic system[9]. TGF-β1 not only facilitates the interaction between EC and VSMC, but also participates in the remodeling and synthesis of the extracellular matrix (ECM) in the vein wall. This eventually disrupts the collagen/elastin balance in the vein wall, reducing elasticity, promoting fibrosis, and leading to venous failure that results in varicocele[10].

This study aims to determine whether a difference exists in the expression of TGF-β1 between the vein walls of varicocele patients and those of healthy individuals (saphenous vein).

Patients and Methods

Ethical Considerations

Our research was designed and conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Prior to initiating any research activity, we obtained approval from the Local Ethical Committee of Tepecik Training and Research Hospital (Approval No. 201858).

The process of enlisting participants was entirely voluntary. Detailed information about the nature of the study, its objectives, and the potential risks associated were provided to all potential participants. Only individuals who voluntarily decided to participate and provided written informed consent were included in the study.

The collection of tissue samples was carried out during routine varicocelectomy procedures. Specifically, we used the residual portions of the veins that remained following ligation. These portions would otherwise have been discarded. No additional surgical steps or interventions were performed solely for the purpose of our study. All participants were thoroughly briefed about this process and given explicit permission for their discarded tissues to be used for research. It was emphasized to the participants that this procedure would not impact their surgical outcome or overall health.

A unique identifier was assigned to each participant to ensure privacy and confidentiality. Any data that were collected were subsequently de-identified and securely stored. Throughout the study, we prioritized the welfare of our participants. All questions or concerns raised by participants were promptly and comprehensively addressed.

Collection of Tissue Samples and the Study Groups

This study involved a total of 40 cases, divided into two groups: Group 1, the control group, consisted of 20 patients who underwent coronary bypass surgery; and Group 2, the varicocele group, comprised 20 patients scheduled for varicocelectomy for left varicocele.

For Group 1, 1-cm samples of the normal vein wall were extracted from the saphenous vein, which was exposed during coronary bypass surgery. This served as the control tissue.

For Group 2, the standard varicocelectomy procedure was followed, where the varicose veins were ligated. The sections of varicose veins between the ligated ends, typically considered waste tissue and usually discarded, were utilized in our study. From these "waste" segments, we procured 1-cm samples for further analysis.

These samples were subsequently transferred to the pathology laboratory and preserved in 10% formaldehyde. The ethical review board and the patients were fully informed about the use of these "waste" vein tissues for research. All procedures were in accordance with ethical standards, as they did not involve any extra surgical steps or additional risks to patients. Our study methodology entailed additional analysis of leftover vein segments post-ligation, without any deviation from standard treatment protocols.

This procedure allowed us to investigate the levels of TGF-Beta in the wall of varicose veins in varicocele patients, contributing to a deeper understanding of the pathophysiology of varicoceles in male infertility.

Preparation of Tissues for Histopathological Evaluation

The transferred tissues were fixed in 10% formaldehyde for a period of twenty-four hours, after which they underwent standard paraffin embedding procedures and block preparation. From these blocks, 3-µm sections were extracted and mounted onto polylysine slides for subsequent immunohistochemical evaluation. These sections were then deparaffinized and treated with the TGF-β1 antibody (GeneTex, Irvine, CA, USA) using a universal kit, in conjunction with an automated staining system (Leica Bond-Max, Leica Biosystems, Nussloch, Germany). The light microscopic evaluation focused on assessing the cytoplasmic and nuclear staining patterns of the TGF-β1 immunohistochemistry in the tissues.

Histopathological Evaluation

The evaluation of immunohistochemical staining was conducted by a pathologist using a light microscope, comparing levels of cytoplasmic and nuclear staining between the control and varicocele groups. The rate of cytoplasmic staining was defined as the ratio of cells demonstrating cytoplasmic staining to the total number of cells within the section. Staining intensity was scored on a scale of 0 to 3, where 0 equated to no staining, 1 to weak staining, 2 to moderate staining, and 3 to strong staining[11]. Similarly, the rate of nuclear staining was calculated as the ratio of nuclei that displayed staining to the total number of nuclei within the section.

Statistical Analysis

All statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). The normality of the groups was assessed using the Shapiro-Wilk test. Statistical relationships between the groups were analyzed *via* the Student's *t*-test and Mann-Whitney U test. An Analysis of Covariance (ANCOVA) was employed to control for age as a confounding factor. A *p*-value lower than 0.05 was considered statistically significant.

Results

The mean age±standard deviation (min-max) for all participants was 43.03±19.03 years (ranging from 19 to 79 years), 59.5±9.01 years for the control group (ranging from 48 to 79 years), and 25.59±7.43 years for the varicocele group (ranging from 19 to 46 years). In our study, the ages of the participants in the varicocele group were statistically lower ($p<0.001$) (Table I).

Differences in nuclear staining were evident across the groups. The intensities of nuclear and cytoplasmic staining within the same samples were comparable, yet the nuclear staining rate varied between cases. For the nuclear staining measure, the median value across all patients was 85 (ranging from 0-100). In the control group, the median was 100 (ranging from 100-100) as all patients in this group exhibited a nuclear staining level of 100 (as shown in Figure 1). For the varicocele group, the median was 10 (ranging from 0-70), as illustrated in Figures 2, 3, and 4. Contrary to the expectations drawn from the existing literature, our results demonstrated lower rates of nuclear staining with TGF-β1 in the varicocele group ($p<0.001$). Regarding the cytoplasmic staining measure, the median value was 2.5 (ranging from 1-3) for all patients, 3 (ranging from 2-3) in the control group (as shown in Figure 1), and 2 (ranging from 1-3) in the varicocele group (as shown in Figures 2, 3, and 4). Once again, our results contradicted the expectations from the literature, showing lower rates of cytoplasmic staining with TGF-β1 in the varicocele group ($p<0.001$).

Correlational analysis was employed to compare the age parameter with the nuclear and cytoplasmic staining rates. It was discovered that age had a positive correlation with both of these measures [$p<0.001$, Correlation Coefficient (CC) for nuclear staining: 0.832, $p<0.001$; CC for cytoplasmic staining: 0.707] as presented in Tables II and III.

Our control group (Group 1) consisted of older patients who had undergone bypass surgery for coronary artery disease, while the patient group (Group 2) was composed of patients scheduled for varicocelectomy due to infertility. This led us to question if the unexpected lower TGF-β1 levels in the varicocele group were linked to their lower average age. Consequently, we explored whether significant differences persisted between the study groups regarding nuclear and cytoplasmic staining with TGF-β1 when age was controlled as an independent factor. For this, we utilized an analysis of covariance (ANCOVA).

Table I. Age data of patients.

Age (Years)	Group I (Control) (n=20)	Group II (Varicocele) (n=20)	All Patients (n=40)
Mean	59.5	25.59	43.03
Std. Dev.	9.01	7.43	19.03
Minimum	48	19	19
Maximum	79	46	79

p-value<0.001 between group I and II.

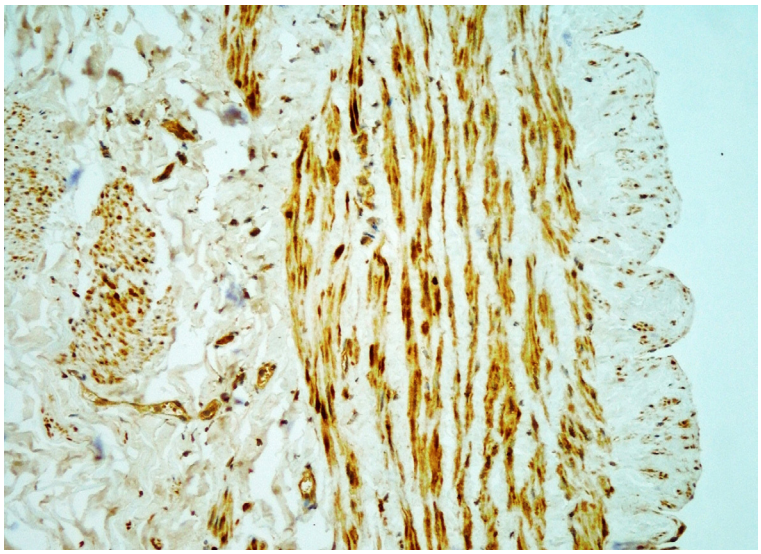

Figure 1. Saphenous vein (control group), 100% nuclear and 3+ cytoplasmic staining, TGF-β1 (DAB, x200).

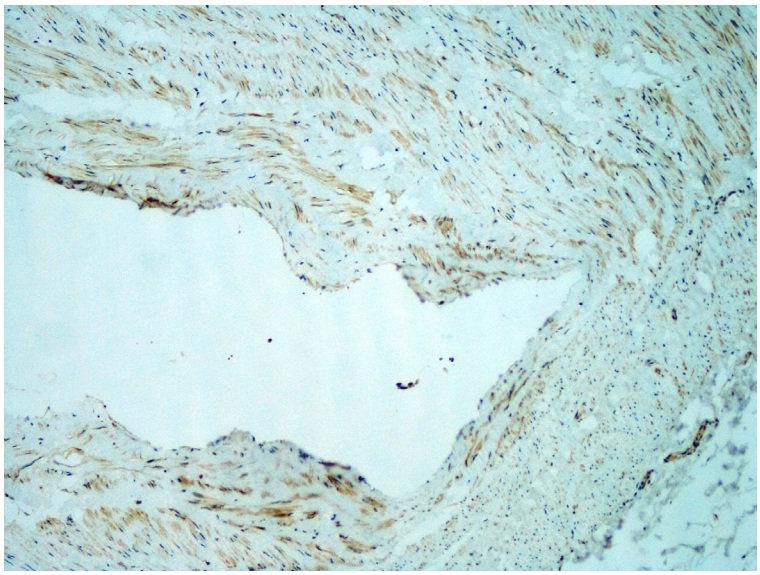

Figure 2. Varicocele vein, no nuclear staining and 1+ cytoplasmic staining, TGF-β1 (DAB, x100).

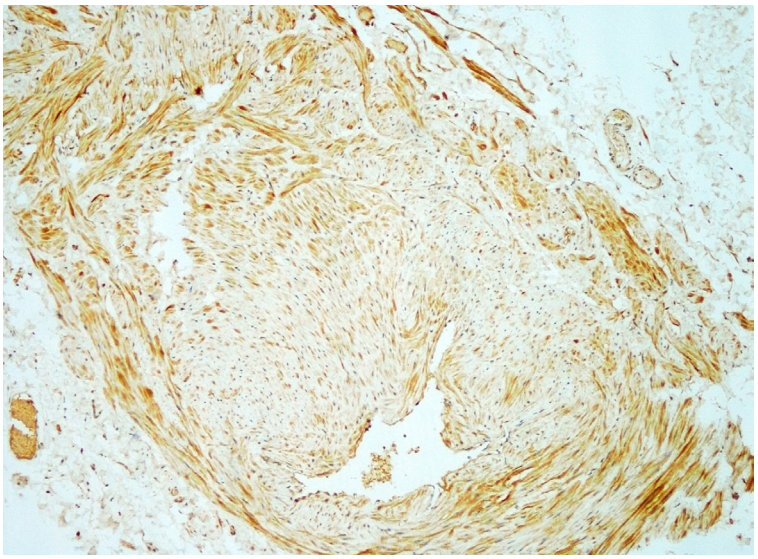

Figure 3. Varicocele vein, 10% nuclear staining and 2+ cytoplasmic staining, TGF-β1 (DAB, x100).

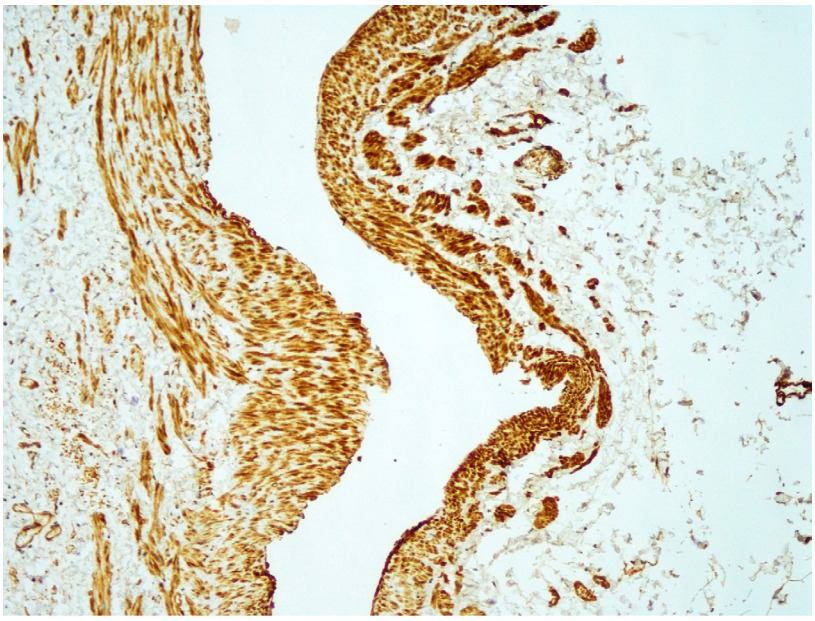

Figure 4. Varicocele vein, 90% nuclear staining and 3+ cytoplasmic staining, TGF-β1 (DAB, x100).

Table II. Bivariate-correlation analysis of age and nuclear staining.

		Age (year)	Nuclear staining (%)
Age (year)	Pearson's Correlation	1	.832**
	Sig. (2-tailed)		.000
	N	40	40
Nuclear staining (%)	Pearson's Correlation	.832**	1
	Sig. (2-tailed)	.000	
	N	40	40

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

Table III. Bivariate-correlation analysis of age and cytoplasmic staining.

			Age (year)	Cytoplasmic staining (degree)
Spearman's rho	age (year)	Correlation Coefficient	1.000	.707**
		Sig. (2-tailed)	.	.000
		N	40	40
	cytoplasmic staining (degree)	Correlation Coefficient	.707**	1.000
		Sig. (2-tailed)	.000	
		N	40	40

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

Upon analysis, we found that even after adjusting for age, nuclear and cytoplasmic staining were significantly lower in the varicocele group [$p<0.001$, partial eta-squared (ηp^2)=0.579 for nuclear staining; $p<0.001$, partial eta-squared (ηp^2)=0.338 for cytoplasmic staining] (Tables IV and V).

Discussion

Cytokines from the TGF-β family are implicated in numerous functions, including proliferation, differentiation, apoptosis, embryonic development, angiogenesis, wound healing, and many others[12]. The TGF-β family, encompassing

Table IV. Nuclear Staining when the age factor is under control.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	**53,932,574[a]**	**2**	**26,966.287**	**106,249**	**.000**	**.869**
Intercept	5,616,729	1	5,616.729	22,130	.000	.409
Age	16,523	1	16,523	.065	.800	.002
Group	**11,159,952**	**1**	**11,159.952**	**43,971**	**.000**	**.579**

[a]R Squared=.869

Table V. Cystoplasmic Staining when the age factor is under control.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	**14,755[a]**	**2**	**7,378**	**34,780**	**.000**	**.685**
Intercept	8,270	1	8,270	38,987	.000	.549
Age	.039	1	.039	.184	.671	.006
Group	3,468	1	3,468	16,350	.000	.338

[a]R Squared=.869

over 30 structurally similar polypeptide growth factors in mammals, is typically categorized into two groups. The first, referred to as the TGF-β-like group, comprises TGF-β1, TGF-β2, TGF-β3, activin, inhibin, and several growth differentiation factors (GDFs). The second group includes Bone Morphogenetic Proteins (BMPs), various GDF cytokines, and the Anti-Müllerian Hormone (AMH)[8].

TGF-β1, a member of the TGF-β family, exerts the most potent effect on the cardiovascular system. It is found in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), myofibroblasts, macrophages, and other cells of the hematopoietic system[9]. TGF-β1 plays a crucial role in vein growth and function[13]. It not only affects ECs and VSMCs but also orchestrates their interactions, demonstrating both angiogenesis-promoting and -inhibiting capabilities[14,15]. TGF-β1 is a pivotal regulator in the synthesis (for example, collagen, elastin) and remodeling of the extracellular matrix (ECM)[15-17]. This function of TGF-β1 in relation to the ECM has spurred discussions about its potential role in structural abnormalities that may occur in the vascular wall, such as varicose structures and fibrosis[10,15,18]. Some studies[19,20] have observed an increase in TGF-β1 expression in VSMCs and ECs in response to heightened stress in the vein wall due to diseases like hypertension (HT). These studies[19,20] concluded that TGF-B1 undertakes remodeling to mitigate this stress in the vein wall. As a result, increased levels of TGF-β1 expression in the vein wall (originating from ECs, VSMCs, myofibroblasts) were expected in structural pathologies such as chronic venous failure, varicose vein formation, and arterial plaque. However, this increased expression, while remodeling, also induces fibrosis[19-21].

Given that varicocele pathology is a disorder related to the vein wall, an increase in TGF-β1 is anticipated. Yet, no studies in the existing literature have directly investigated this subject. Kisa et al[22] explored the relationship between TGF-β and Epidermal Growth Factor (EGF) levels in varicocele and seminal plasma. In this study, patients were divided into three groups. The first group consisted of patients with varicocele exhibiting oligoasthenoteratozoospermia, the second group included patients with varicocele, and the third group served as the control comprising subjects without varicocele. This study discovered significantly higher TGF-β levels in the seminal plasma of groups 1 and 2 compared to the control group. Although higher EGF levels were detected in the two varicocele groups compared to the control group, the difference was not statistically significant. Interestingly, this study found no correlation between age and levels of TGF-β and EGF in seminal plasma. Furthermore, semen parameters were observed to have a negative correlation, specifically with TGF-β levels[22]. In another study, Cheng et al[2] induced experimental varicocele in

rats by ligating the left renal vein and found that subcutaneous EGF treatment resulted in a more substantial improvement in semen parameters compared to varicocelectomy alone. The most significant improvement was observed in the group that received both varicocelectomy and EGF treatment[2]. Kowalewski et al[23] also reported higher mRNA levels of TGF-β RII in the varicose group compared to the control group, as expected.

In our study, we conducted an immunohistochemical evaluation of vein tissues collected from both the varicocele and control groups. Contrary to expectations drawn from existing literature, we observed that the vein walls of patients with varicocele exhibited less cytoplasmic and nuclear staining compared to those of normal vein walls.

In a study conducted by Pascual et al[16], saphenous vein tissues obtained during coronary bypass procedures composed the control group, while varicose vein tissues from patients undergoing varicose vein surgery formed the study group. The authors assessed both latent and mature TGF-β1 levels independently. The study found reduced levels of latent TGF-β1 in the varicose vein wall compared to the healthy vein wall when age was not factored in. Furthermore, when age was taken into consideration, the control group aged over 50 exhibited higher latent TGF-β1 levels than the under-50 control group. However, strikingly lower latent TGF-β1 levels were found in the over-50 varicocele group compared to their under-50 counterparts. In contrast, mature TGF-β1 displayed an inverse trend, with higher expression identified in the varicose group when age was not factored in. Additionally, both the control and varicose groups showed an increase in mature TGF-β1 expression correlating with age[16].

This aforementioned study aligns closest with our own in terms of materials and methods. However, it diverges as its study group consisted of vascular walls inclusive of varicose veins, while our study group comprised infertility patients undergoing varicocele surgery. Despite the rich literature investigating TGF-β1 levels, our literature review found no studies comparing TGF-β1 levels in vein walls of varicocele patients and healthy vein walls, making our study the first to undertake this approach.

In the study by Pascual et al[16], the mean ages for the control group aged 50 years and below, the control group aged above 50 years, the varicose group aged 50 years and below, and the varicose group aged above 50 years were found to be 38.4±5.8, 71.2±10.6, 39.4±7.8 and 60.7±9.4 years, respectively. In our study, due to the inclusion of infertility patients with varicocele, the patients were significantly younger. The median (min-max) age values for the control and study groups were determined as 59 (48-79) and 23 (11-46) years respectively.

Although there are studies[22] suggesting that aging does not affect TGF-β1 expression, they are outnumbered by publications indicating an increase with age[16,23]. Notably, in our study, even after controlling for age, the varicocele group demonstrated significantly lower nuclear and cytoplasmic staining.

Cytokines in the TGF-β family are involved in various functions such as proliferation, differentiation, apoptosis, embryonic development, angiogenesis, wound healing, and many others[12]. The TGF-β family in mammals comprises more than 30 polypeptide growth factors with similar structures, subdivided into two major groups[8]. Among these, TGF-β1 has a potent effect on the cardiovascular system, playing key roles in vein growth and function, as well as extracellular matrix (ECM) synthesis and remodeling[9,13,15-17].

Previous studies[19,20] have noted that stress-induced pathologies, such as hypertension (HT), increase TGF-β1 expression in the vein wall. Interestingly, varicocele pathology, a disorder concerning the vein wall, is yet to be comprehensively studied for TGF-β1 expression, despite the prevalent understanding that it should increase in such pathological states. Notably, our study stands as a novel contribution to this area of research, as it evaluates TGF-β1 expression directly in the vein walls of varicocele patients for the first time.

Our findings, however, contradict the expected increase in TGF-β1 expression. Immunohistochemical evaluation of vein tissues from our study and control groups showed less TGF-β1 cytoplasmic and nuclear staining in the varicocele patients' vein walls than in healthy vein walls. Although these results diverge from previous findings in the related vein wall pathologies[19,20,22], they add a fresh dimension to the understanding of TGF-β1's role in varicocele, sparking critical discussions about its complex and context-specific functions.

Limitations

Our study's novelty comes with limitations; the evaluation of TGF-β1 expression was solely based on immunohistochemistry, leaving out the quantitative assessment that a western-blot approach could have provided. The lack of distinction be-

tween latent and mature TGF-β1 forms and the absence of investigation into other members of the TGF-β family also narrow the scope of our findings. However, these limitations pave the way for future investigations, which we believe will benefit from integrating immunohistochemistry with western blotting and other comprehensive methodologies for a more thorough evaluation of TGF-β1's role in varicocele and other venous disorders.

Conclusions

Our study provides a novel perspective to the existing literature by comparing TGF-β1 staining between the vein walls of varicocele patients and healthy vein walls. In contrast to the literature that reported an increased TGF-β1 expression in varicose vein walls, our results revealed a reduction in TGF-β1 staining in the vein walls of varicocele patients. This observation suggests a potentially different role for TGF-β1 in the pathophysiology of varicoceles than what is currently understood. Thus, our results suggest that further research is required to better understand the precise role of this complex cytokine in the context of varicocele. Such investigations could potentially enhance our understanding of the pathophysiology of varicoceles.

Informed Consent
In compliance with ethical standards, informed consent was obtained from all individual participants included in the study. All participants were adequately informed about the nature, benefits, and risks of the study, and written consent was provided. This procedure was carried out prior to the commencement of any research activities.

Ethics Approval
All procedures were carried out following the receipt of approval (201858) from the Local Ethics Committee at Tepecik Training and Research Hospital.

ORCID ID
Mehmet Zeynel Keskin: 0000-0002-9206-5586
Sumeyye Ekmekci: 0000-0003-1607-500X
Ayşen Yaprak Engin: 0000-0002-0763-2719
Erhan Ates: 0000-0002-9677-5673
Murat Ucar: 0000-0001-6977-7430
Ender Siyez: 0000-0002-4448-0515
Erdem Kısa: 0000-0002-4728-3808
Cem Yücel: 0000-0003-0838-9199
Can Köse: 0000-0001-7351-7981
Mumin Alper Erdogan: 0000-0003-0048-444X
Yusuf Özlem İlbey: 0000-0002-1483-9160

Acknowledgements
We would like to express our gratitude to all the participants of this study. We would also like to thank our colleagues and the staff in our departments for their invaluable support throughout the process of this research. Although they may not meet the authorship criteria, their contributions to this work are greatly appreciated.

Conflict of Interest
There is no conflict of interest. All authors declare no competing financial disclosure.

Funding
None.

Authors' Contributions
M.Z.K conceived of the presented idea, investigated the study, and supervised the manuscript. S.E and C.K performed the analyses. M.Z.K and A.Y.E and E.A. conceived and planned the experiments. M.U. and E.S processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. MAE, edited the text and manuscript, E.K, C.Y and Y.Ö.İ discussed the results and commented on the manuscript. All authors discussed the results and contributed to the final manuscript.

References

1) Naughton CK, Nangia AK, Agarwal A. Pathophysiology of varicoceles in male infertility. Hum Reprod Update 2001; 7: 473-481.
2) Cheng D, Zheng XM, Li SW, Yang ZW, Hu LQ. Effects of epidermal growth factor on sperm content and motility of rats with surgically induced varicoceles. Asian J Androl 2006; 8: 713-717.
3) Evers JLH, Collins JA. Assessment of efficacy of varicocele repair for male subfertility: a systematic review. Lancet 2003; 361: 1849-1852.
4) Bae K, Shin HS, Jung HJ, Kang SH, Jin BS, Park JS. Adolescent Varicocele: Are Somatometric Parameters a Cause? Korean J Urol 2014; 55: 533.
5) Dogan F, Armagan A, Oksay T, Akman T, Aylak F, Bas E, Dursun M, Altug U, Bilgi C. Impact of micronised purified flavonoid fraction on increased malondialdehyde and decreased metalloproteinase-2 and metalloproteinase-9 levels in varicocele: outcome of an experimentally induced varicocele. Andrologia 2014; 46: 380-385.
6) Serra R, Buffone G, Costanzo G, Montemurro R, Scarcello E, Stillitano DM, De Franciscis S, Colacurci N. Altered metalloproteinase-9 expression as least common denominator between varicocele, inguinal hernia, and chronic venous disorders. Ann Vasc Surg 2014; 28: 705-709.
7) Ozbek E, Turkoz Y, Gokdeniz R, Davarci M, Ozugurlu F. Increased nitric oxide production in the

spermatic vein of patients with varicocele. Eur Urol 2000; 37: 172-175.

8) Weiss A, Attisano L. The TGFbeta superfamily signaling pathway. Wiley Interdiscip Rev Dev Biol 2013; 2: 47-63.

9) Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. J Cell Sci 2003; 116: 217-224.

10) Ruiz-Ortega M, Rodríguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. Cardiovasc Res 2007; 74: 196-206.

11) Li H, He G, Yao H, Song L, Zeng L, Peng X, Ou C, Sun R, Li Q. TGF-β Induces Degradation of PTHrP Through Ubiquitin-Proteasome System in Hepatocellular Carcinoma. J Cancer 2015; 6: 511-518.

12) Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003; 425: 577-584.

13) Goumans MJ, Ten Dijke P. TGF-β Signaling in Control of Cardiovascular Function. Cold Spring Harb Perspect Biol 2018; 10: a022210.

14) Goumans MJ, Liu Z, Ten Dijke P. TGF-beta signaling in vascular biology and dysfunction. Cell Res 2009; 19: 116-127.

15) Serralheiro P, Soares A, Almeida CMC, Verde I. TGF-β1 in Vascular Wall Pathology: Unraveling Chronic Venous Insufficiency Pathophysiology. Int J Mol Sci 2017; 18: 2534.

16) Pascual G, Mendieta C, García-Honduvilla N, Corrales C, Bellón JM, Buján J. TGF-beta1 upregulation in the aging varicose vein. J Vasc Res 2007; 44: 192-201.

17) Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human Mast Cell Chymase and Leukocyte Elastase Release Latent Transforming Growth Factor-β1 from the Extracellular Matrix of Cultured Human Epithelial and Endothelial Cells. J Biol Chem 1995; 270: 4689-4696.

18) Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med 2008; 233: 109-122.

19) O'Callaghan CJ, Williams B. Mechanical strain-induced extracellular matrix production by human vascular smooth muscle cells: role of TGF-beta(1). Hypertens 2000; 36: 319-324.

20) Qi YX, Jiang J, Jiang XH, Wang XD, Ji SY, Han Y, Long DK, Shen BR, Yan ZQ, Chien S, Jiang ZL. PDGF-BB and TGF-β1 on cross-talk between endothelial and smooth muscle cells in vascular remodeling induced by low shear stress. Proc Natl Acad Sci U S A 2011; 108: 1908-1913.

21) Willems M, Olsen C, Caljon B, Vloeberghs V, De Schepper J, Tournaye H, Van Saen D, Goossens E. Transcriptomic differences between fibrotic and non-fibrotic testicular tissue reveal possible key players in Klinefelter syndrome-related testicular fibrosis. Sci Rep 2022; 12: 21518.

22) Kısa Ü, Başar MM, Ferhat M, Çağlayan O. Seminal plasma transforming growth factor- B (TGF-B) and epidermal growth factor (EGF) levels in patients with varicocele. Turkish J Med Sci 2008; 38: 105-110.

23) Kowalewski R, Malkowski A, Sobolewski K, Gacko M. Evaluation of transforming growth factor-beta signaling pathway in the wall of normal and varicose veins. Pathobiology 2010; 77: 1-6.