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ORIGINAL PAPER

Retinal vessel diameters: Can they predict future risk of infertility in patients with varicocele?

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Summary ***Objective: The objective of this study was to assess the relationship between retinal vessel diameters, such as retinal arteriolar diameter, retinal venular diameter, and arteriolar/venular ratio (AVR), as clinical parameters of fertility in varicocele patients.***
Materials and methods: Sixty-eight (68) infertile varicocele men with abnormal semen parameters and sixty-one (61) varicocele normozoospermic men were included in the study. Moreover, fifty-eight (58) healthy normozoospermic men without varicocele were enrolled as a control group. For each participant, retinal vascular diameters were measured from the digital retinal photographs as a central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), and AVR. In addition, hormones (total testosterone and FSH), and semen parameters were assessed and correlated with retinal vessel diameters.
Results: The mean CRAE, CRVE, and AVR values were 147.8 ± 15.8 μm, 198.3 ± 39.3 μm, and 0.61 ± 0.01 in infertile varicocele patients, respectively. Significant difference of CRAE, CRVE, and AVR were found when comparing infertile varicocele patients with both varicocele and control normozoospermic male groups ($p = 0.01$, $p = 0.006$, and $p = 0.007$; respectively). Larger retinal venular caliber and smaller AVR ratio showed a significant inverse correlation with both sperm parameters and hormones (total testosterone and FSH) ($p < 0.05$). No significant correlations were found between CRAE with both sperm parameters and hormonal values (total testosterone and FSH) ($p > 0.05$).
Conclusions: Infertile patients with varicocele showed a significant relationship with the retinal vascular diameter (CRVE and AVR ratio). This finding supports recommendation for regular eye examinations in the varicocele population.

KEY WORDS: *Infertility; Reproductive hormone; Retinal vessels; Semen parameters; Varicocele.*

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INTRODUCTION

Varicocele is a vascular disease affecting approximately 25% of infertile men (1). Varicocele is thought to play a crucial role in the pathophysiology of infertility (2). Assessment of varicocele pathophysiology effects on fertility requires many non-invasive and efficient procedures.
The retinal microcirculation has allowed the physician to visualize the medical vascular diseases noninvasively in humans and examine their association with such vascular pathologies (3, 4). The role of retinal microvascular diameters has been investigated in many medical vascular diseases, such as cardiovascular, hypertension, diabetes, and kidney diseases (4-7). Previous published reports confirmed the strong association of the retinal vasculature changes with predication of cardiovascular risks and nephropathy (6, 7). Based on the association of varicocele with medical vascular diseases and its vasculature progressive nature, alterations in retinal vasculature will be assumed to occur in men with varicocele (8, 9). In a previous report, retinal vasculature parameters such as retinal microvascular diameters *central retinal venular equivalent* (CRVE), *central retinal arteriolar equivalent* (CRAE), and the *arteriolar-to-venular ratio* (AVR) have been emerged to have a clinical correlation with varicocele patients (10).
However, the possible relationship between retinal vasculature diameters and fertility was not investigated. In this study, we aimed to detect and evaluate the relationship of retinal vascular calibers with fertility-related parameters, including semen values, and reproductive hormone (serum total testosterone and FSH) in infertile patients with varicocele.

PATIENTS AND METHODS

Study population

The data of 413 infertile patients who were referred to our Andrology department were retrospectively analyzed. From these infertile men, only subjects with varicocele were selected and divided into two groups. Group 1 consisted of sixty-eight (68) infertile varicocele patients with *abnormal semen parameters* (ASP). Group 2 consisted of sixty-one (61) varicocele normozoospermic subjects. Fifty-eight (58) healthy normozoospermic men were enrolled in the study as a control group. None of the subjects in the control group had varicocele or scrotal symptoms.
Age was similar between the three groups.
The study was conducted according to the ethical guidelines of the Declaration of Helsinki for medical research and all procedures were approved by the *Institutional Review Board of the Faculty of Medicine at Jazan University, Saudi Arabia*. All participants gave a signed consent at enrollment.

No conflict of interest declared.

Selection criteria

Patients with varicocele and infertility with abnormal semen parameters were assessed according to WHO guidelines (2010) (11). Patients with other urogenital diseases, erectile dysfunction medications, sperm antibodies, and systemic vascular problems affecting the retina (e.g., cardiovascular disease, atherosclerosis, hypertension, and diabetes) were excluded. Patients with refractive error within ± 6.0 diopters equivalent sphere and within ± 3.0 diopters astigmatism were excluded. Patients with a history of glaucoma, trauma, or previous ocular surgery, and corneal opacity were also excluded. The female partners were also investigated for exclusion of the others causes of infertility, such as ovulatory problems, or tubal obstruction.

Clinical examination

The varicocele diagnosis was confirmed by using 1993 WHO guidelines (12). Subclinical varicocele cases were excluded. Subjects of the control group underwent the same evaluation (13). The total testicular volume of all the participants was measured by Prader's orchidometer. All the subjects included in the study underwent detailed ocular examination including slit-lamp anterior segment evaluation (*Haag-Streit, Germany*), fundus examination with Volk 90 diopter, and measurement of intraocular pressure using the Goldmann applanation tonometer AT 900. The serum concentration of total testosterone, FSH, and glucose and semen parameters were determined by using standardized protocols (14, 15).

Retinal vessel measurements

All the participants had simultaneous stereoscopic color transparency centered on the optic disc (45° fields) with pharmacological mydriasis in the ophthalmic department. Most photography sessions coincided with the annual visits. The retinal arterioles and venules caliber were measured by an automatic computed system. By using *Knudtson et al.* formula, the retinal vessel diameters were calculated as *central retinal arteriolar equivalent* (CRAE) and *central venular equivalent* (CRVE) (16).
Arteriolar-venular-ratio (AVR) was calculated by using both the CRAE and CRVE, taking the mean of the results of right and left eye measurements. Assessment of the retinal vessels and other retinal diseases was performed by a single trained and certified examiner masked for participant characteristics.

Data analysis and statistics

Sample size calculation was based on the outcome of CRVE as the primary variable of interest. For this calculation, 9 infertile patients with varicocele, 9 normozoospermic patients with varicocele, and 9 healthy normozoospermic subjects without varicocele were enrolled. Mean CRVE values were 197 ± 29.4 µm in the infertile varicocele group, 173 ± 25.6 µm in the varicocele normozoospermic group, and 171 ± 19.2 µm in the control group. Based on these numbers and with an alpha = 0.05, it was estimated that 23 subjects per group were necessary to achieve 95% statistical power of the study. Furthermore, AVR was found 0.62 ± 0.02 in the infertile varicocele group, 0.78 ± 0.01 in the normozoospermic group with varicocele, and 0.79 ± 0.01 in the control group. Based on these data and with an alpha = 0.05, 52 subjects per group were necessary to achieve 95% statistical power of the study. Therefore, 68 patients with infertile varicocele group, 61 normozoospermic men with varicocele, and 58 healthy normozoospermic subjects (control group) were considered sufficient for the study.
The statistical evaluations were performed using IBM SPSS version 24.0 (*Armonk, NY*). Continuous variables were tested for the normality of distribution with the Kolmogorov-Smirnov test. Results were expressed as means ± *standard deviation* (SD). Differences in the means were compared using the Student's t-test and the Mann-Whitney U-test, when the data were normally and abnormally distributed, respectively. We performed the Fisher's exact test, and Chi-square test to determine any statistical difference between the two groups. The correlations between retinal vessel data, seminal parameters, hormonal values, and testicular volume were investigated using the Spearman rank correlation. The level of the p-value < 0.05 was considered statistically significant.

Results

Descriptive findings

The demographic data of the studied groups are shown in Table 1. The statistical analysis of the differences of demographic and clinical characteristics among the studied groups demonstrated no significant difference.

Table 1.
Demographic data and clinical characteristics of the studied groups.

Parameters	Varicocele with ASP *	Varicocele with normozoospermia	Control
Number of patients	68	61	58
Mean age (years)	28.7 ± 2.4	27.9 ± 1.6	29.1 ± 2.1
Varicocele grade			0
Grade 1	15 (22%)	16 (26%)	
Grade II	51 (75%)	44 (72%)	
Grade III	2 (3%)	1 (2%)	
Varicocele laterality			0
Left	56 (82%)	52 (87%)	
Right	12 (18%)	8 (13%)	

ASP: Abnormal semen parameters.

Study of retinal vessel diameters

The association between retinal vessel diameters with both seminal parameters and hormonal values are shown in Table 2.
The mean CRAE, CRVE, and AVR values were statistically significant different in infertile varicocele patients with abnormal semen values compared to normozoospermic patients with varicocele and control patients ($p = 0.01$, $p = 0.006$, and $p = 0.007$; respectively).
In varicocele infertile patients, CRVE showed a significant inverse correlation with sperm concentration, progressive motility and normal sperm morphology ($r = -0.337$, $p < 0.005$; $r = -0.289$, $p < 0.017$; $r = -0.239$, $p < 0.049$, respectively). In addition, CRVE had a significant inverse correlation with hormonal values ($r = -0.442$, $p < 0.000$

Table 2.
Changes in retinal vessels diameters, semen parameters, hormonal levels, and testicular volume of the studied groups.

Parameters	Varicocele with ASP	Varicocele with normozoospermia	Control	P value *
CRAE (µm)	147.8 ± 15.8	138.7 ± 9.3	136.5 ± 7.5	0.01
CRVE (µm)	198.3 ± 39.3	171.4 ± 28.4	169.8 ± 24.2	0.006
AVR	0.61 ± 0.01	0.77 ± 0.02	0.79 ± 0.02	0.007
Semen concentration (million/mL)	12.9 ± 5.7	24.8 ± 13.2	27.5 ± 11.5	0.004
Progressive motility (%)	21.7 ± 8.2	36.9 ± 11.3	38.4 ± 13.7	0.005
Morphology (% of normal)	14.7 ± 4.9	24.8 ± 13.9	26.6 ± 12.3	0.01
Total serum testosterone (ng/mL)	6.3 ± 1.6	13.7 ± 5.7	12.1 ± 8.3	0.04
FSH (mIU/mL)	15.1 ± 6.7	5.7 ± 2.6	6.1 ± 2.1	0.001
Total testicular volume (mL)	16.2 ± 4.8	28.7 ± 15.4	29.9 ± 18.2	0.002

** P-value (Comparison between varicocele group with ASP and both varicocele and control normozoospermic groups)*
Values are presented as mean ± SD.
ASP: Abnormal semen parameters; AVR: Arteriolar-to-venular ratio; CRAE: Central retinal arteriolar equivalent; CRVE: Central retinal venular equivalent.

Table 3.
Correlation of infertile varicocele patients' retinal vessels diameters with semen parameters, hormones levels, and testicular volume.

Parameters	CRAE (µm)		CRVE (µm)		AVR	
	R	P *	R	P *	R	P *
Sperm concentration (million/mL)	-0.221	0.071	-0.337	0.005	-0.360	0.003
Progressive motility	- 0.191	0.119	-0.289	0.017	-0.323	0.007
Morphology (% of normal)	- 0.160	0.192	-0.239	0.049	-0.284	0.019
Total serum testosterone (ng/mL)	- 0.182	0.137	-0.442	0.000	-0.305	0.011
FSH (mIU/mL)	-0.229	0.060	-0.338	0.005	-0.367	0.002
Total testicular volume (mL)	-0.135	0.272	-0.145	0.237	-0.213	0.081

R = Correlation coefficient.
** P-value (by Spearman rank correlation).*
AVR: Arteriolar-to-venular ratio; CRAE: Central retinal arteriolar equivalent; CRVE: Central retinal venular equivalent.

for total testosterone, and r = -0.338, p < 0.005 for FSH). A significant inverse association was found between AVR and sperm and hormonal values (r = -0.360, p < 0.003 for sperm concentration; r = -0.323, p < 0.007 for progressive sperm motility; r = - 0.284, p < 0.019 for normal sperm morphology; r = -0.305, p < 0.011 for total testosterone; and r = -0.367, p < 0.002 for FSH). On the contrary, CRAE did not have any significant correlation with sperm and hormonal values (Spearman rank correlation coefficient, p = 0.071 for sperm concentration, p = 0.119 for progressive sperm motility, p = 0.192 for normal sperm morphology, p = 0.137 for total testosterone, and p = 0.060 for FSH) (Table 3).

Retinal vessel diameters (CRAE, CRVE, and AVR) had no significant correlation with total testicular volume (Spearman rank correlation coefficient p > 0.05) (Table 3). Furthermore, no significant correlation was found between the laterality and grade of varicocele with retinal vasculature diameter (p > 0.05) (data not shown). However, an increase in retinal venular calibration (CRVE) was prominent in the higher grade of varicocele, although it was not observed in the retinal arteriole. Statistically significant differences for seminal values (sperm concentration, progressive sperm motility, and normal sperm morphology), hormonal values (serum total testosterone and FSH), and total testicular volume were observed in both normozoospermic patients with varicocele and controls compared to the infertile patients with varicocele (p < 0.05) (Table 2).

Discussion

This study showed a significant association between CRVE diameter and sperm parameters in infertile varicocele patients. Based on this finding, we provide another evidence for the role of medical vascular diseases in the pathophysiology of both varicocele and retinopathy. This finding is consistent with the increased prevalence of peripheral varicose veins, and ectasic changes in the coronary arteries in varicocele patients (17, 1). Moreover, the changes of retinal vasculature caliber have been demonstrated and identified as markers in several vascular diseases (4-5, 18). The presence of larger retinal vascular caliber in patients with varicocele is initiated by the effect of hypoxia on retinal blood flow leading to retinal vasodilation. The dilatation of retinal micro-vasculature may also be affected by higher levels of inflammatory biomarkers and by local endothelial *nitric oxide* (NO) synthesis in vascular beds which is a potent vasodilator (19). These primary vascular pathogenetic processes are also involved in the pathogenesis of infertility (2, 9, 20, 21).

The vasodilation of the retinal micro-vessels was observed before the onset of microvascular complications of chronic kidney diseases, such as nephropathy (22). Similarly, larger venular caliber was related to dyslipidemia, which may reflect a proinflammatory state associated with obesity (23). Noteworthy, generalized retinal arteriolar narrowing is shown to be a reflection for future onset of systemic hypertension (18). These previous clinical studies provide evidence that retinal vasculature changes may precede the development of clinical varicocele.

Consequently, the CRVE can play a role as an early prediction marker for the severity of varicocele. Changes of AVR are more predictive than either arteriolar or venular diameter alone. In this study a smaller AVR was significantly correlated to sperm parameters. Smaller AVR values are related mainly to venular dilatation than to arteriolar dilatation. Therefore, prominent dilatation was recognized in retinal veins than in retinal artery in varicocele patients (10).

However, arteriolar diameters are usually measured against venular ones during ophthalmoscopy clinical examination. This procedure may underestimate the arteriolar caliber and consequently the severity and complications of varicocele. Also, smaller AVR was associated and affected by the inflammatory mediators (5). Despite these previous studies, AVR values are more sensitive than CRVE alone to determine the risk of varicocele (10).

In this study, we found a significant association of the caliber of retinal vessels with both total serum testosterone and FSH hormones. The potential role of the hormonal imbalance, induced by varicocele, on the retinal microvascular dilatation was supported in our series (24) showing a negative correlation between retinal vasculature parameters (CRVE and AVR) and the reproductive hormones in infer-

tile varicocele patients. These retinal vasculature changes may be attributed to the testosterone imbalance associated with testicular insufficiency in men with varicocele. In fact, the severity of these hormonal changes, in this study, may be related to the greater loss of testicular volume in varicocele patients as compared to the normozoospermic healthy subjects. The diminished testicular function is associated with a substantial increase in the FSH and decrease of testosterone levels, resulting in an inverse ratio between serum testosterone and FSH levels. On the other hand, testosterone has a negative direct effect on the vessel vasculature through regulation of the vascular tone and regulation of apoptosis in vascular endothelial cells. On the contrary, it was reported that testosterone did not have any influence on micro-circulation and even that its inhibition improves microvascular dilatation (25). Since the vascularity of the testis plays a crucial role in normal testicular function (17), the retinal vessel parameters assessed in this study can be used as a prognostic clinical marker for the gonadal function in varicocele men.

Our results demonstrated that most varicocele patients with abnormal semen values had higher CRVE and smaller AVR. Therefore, high CRVE and smaller AVR could be used as a negative prognostic factor for spermatogenesis quality in varicocele patients and young normal adults. The hemodynamic retinal arteriolar and venular caliber can be determined noninvasively and measured quantitatively, which may allow monitoring of clinical outcomes of varicocele.

However, there are some limitations of this study.

The number of participants might have been larger. Also, this study did not have data about intraocular pressure which may influence retinal vascular caliber measurements. Furthermore, photographs were not synchronized with both the fertility and *Color-Doppler ultrasonography* (CDUS) evaluation because vessel diameter may change because of systemic blood flow changes. Our study did not assess the relationship between diameters of the spermatic vessel and the retinal vessels because of the high risk of false-negative and false-positive diagnoses related to CDUS performed by different operators. These misinterpretations are related mainly to the mobility of the spermatic cord vessels and to the patient position during measurements (standing or lying). In addition, quantitative data of the scrotal veins (maximum diameter and the presence, velocity, and duration of reflux) were lacking in the reports of sonographic examinations of all participants with clinically diagnosed varicocele (13). Moreover, different cutoff considered and controversies about the diagnostic criteria for varicocele in Doppler procedures cause difficulty in the evaluation of the results. In consideration of all the previous described influential factors, *Ediz et al.* found that the maximum spermatic vein diameters measured during the Valsalva maneuver by CDUS were not significantly correlated with any of the sperm parameters (26) although *Mahdavi et al.* reported that sperm parameters correlated with CDUS findings in patients with varicocele (27).

Finally, our results may not be applicable in patients with subclinical varicocele.

The strengths of this study include high magnification of digital fundus images with correction for refractive errors and the use of the automatic computed system for quantitative measurement of the retinal vascular parameters.

Conclusions

Our results revealed that changes in retinal vascular parameters have a relationship to both seminal parameters and hormonal values. The clinical implication of our results in varicocele patients is that the assessment of retinal arterioles and venules may be a possible prognostic marker for varicocele outcome. However, there is a great need for further experimental investigations with a larger number of patients.

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