

Assessment of Serum Levels of YKL40 in Men with Varicocele

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Abstract

When the pampiniform and internal spermatic veins dilate, it is known as varicocele. Among sub fertile males, varicocele is the most frequent abnormality to be discovered. A 40 kDa secreted mammalian glycoprotein known as Chitinase-3-Like-1 (CHI3L1) binds chitin but lacks the enzyme capability to catalyse chitin's degradation. YKL40 is a neutrophil-produced cytokine that has been linked to a variety of inflammatory disorders. It also serves as a biomarker for endothelium disease. This study's goal is to find out whether endothelial dysfunction has a role in the development of varicocele by measuring blood levels of YKL40 in patients with varicocele. Thirty healthy men with no clinically evident varicocele were matched for age and verified by scrotal Doppler Ultrasonography with enzyme-linked immunosorbent assay to have high serum YKL40 values (ELISA). A patient's and a control's age ranged from 25 to 50. Varicocele patients had greater serum YKL40 levels than healthy controls. Endothelial dysfunction has a role in varicocele development, as shown by this finding.

Key words: Varicocele, YKL40, ELISA.

1. Introduction

When the pampiniform and internal spermatic veins dilate, it is known as varicocele. One of the most frequent abnormalities among sub fertile males is varicocele [15].

Among the many mediators released by the endothelium are endothelin-1, endothelial nitric oxide synthase (Enos), and prostaglandin. The endothelium is a thin innermost layer of flat cells. It used to be assumed that it was a mechanical obstruction. They discovered that this tissue regulates blood vessel tone, cell proliferation, and leukocyte-thrombocyte interactions with the vessel wall [2].

A 40 kDa secreted mammalian glycoprotein known as Chitinase-3-Like-1 (CHI3L1) binds chitin but lacks the enzyme capability to catalyse chitin's degradation. Neutrophils are capable of producing the inflammatory cytokine YKL40, which has been linked to many illnesses [11].

Endothelial dysfunction is a medical term that refers to things like an imbalance in the levels of various endothelium-derived relaxing and constricting substances or a change in the metabolism of readily accessible nitric oxide (NO). It acts as both a mechanical and biological barrier between the blood and the vascular wall, which is made up of endothelium. Secretion of chemicals that either relax or constrict ECs [12].

Because nitric oxide and endothelin-1 are essential for controlling vascular tone, an imbalance between EDRF and EDCF in varicocele is a key factor in varicocele pathophysiology. Nitric oxide is an EDRF [12].

Because of venous stasis and endothelial dysfunction, the endothelium of the varicose spermatic vein may be exposed to hypoxic conditions [5].

This study's goal is to find out whether endothelial dysfunction plays a role in the development of varicocele by measuring blood levels of YKL40 in individuals with the condition.

2. Patients and Methods

The current study is a case-control study conducted at Dermatology & Andrology and Clinical & chemical Pathology departments in Benha University. It included fifty varicocele patients and age-matched thirty healthy males with no detectable varicocele clinically and this was confirmed by Scrotal Doppler Ultrasonography.

Participants gave their informed written consent before enrollment and the study was approved by the Research Ethics Committee, Faculty of Medicine, Benha University. Patients included in the study were between the ages of 25 and 50 years. Diabetics, smokers, hypertensives and obese patients were excluded.

All participants were subjected to

1. Full history taking and clinical examination

History taking and clinical examination were done to exclude other possible causes of endothelial dysfunction such as diabetes hypertension and smoking and age more than 50 years.

2. Semen collection

- Semen samples were collected via masturbation following a minimum of 3 days and maximum of 5 days abstinence from sexual activity.
- The specimen was collected in sterile, wide-mouthed containers to minimize collection error.
- Specimens were collected at laboratory at room temperature.

3. Semen analysis

- Semen analysis was performed according to the WHO manual in a standardized way.
- Semen samples were analyzed within 1 hour of collection.
- Sperm parameters were considered normal when sperm concentration was >15 million/ml of semen, motility was >32%, and normal sperm forms were >4% by WHO (2010) criteria.

4. Scrotal Doppler ultrasound

- Scrotal Doppler US was performed with the patient in the supine position. The scrotum was supported with a rolled towel and warmed ultrasound gel was placed on the scrotum.

5. Assessment of Serum levels of YKL-40

5 ml of venous blood sample was taken after overnight fasting by clean venipuncture using disposable plastic syringe and put in plain tube (without anticoagulant) then left at room temperature for 30 minutes until clotting. The tube was centrifuged (at 1500 rpm for 15 minutes). The separating serum was aliquoted and stored at -20°C until subsequent assay. A double-antibody sandwich ELISA (Enzyme Linked Immune Sorbent Assay) was used to detect serum level of YKL-40 using a commercial Human YKL-40 ELISA Kit for research use only (Cat #: 201-12-2064, SunRedBio, China).

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 23. Categorical data were

presented as number (percentage) and chi-square test was used to analyze them, while quantitative data were expressed as mean \pm standard deviation (SD); Student's "t" test, Chi-Square test and Mann-Whitney U test were used to analyze them respectively. Correlation was assessed by Spearman's correlation coefficient (ρ). Receiver operating characteristic (ROC) curve was used to determine the cutoff values of serum YKL-40 level with optimum sensitivity and specificity in early prediction of AV. P-value $\leq .05$ was considered the accepted level of significance in this work.

3. Results

The present study was conducted on 50 males suffering from varicocele (group I) and 30 age-matched healthy males as a control group (group II) and the collected data were analyzed using suitable statistical methods.

Laboratory investigations:

Serum level of YKL-40 was significantly higher among patients with varicocele when compared to controls ($p < 0.05$) as shown in Table (1) Fig.(1).

Table (1) Comparison between the two studied groups according to serum level of YKL40 .

YKL40 LEVEL ng/ml	GROUP I (N = 50)	GROUP II (N = 30)	U	P
Min. – Max.	12.71 – 98.37	15.29 – 88.81		
Mean \pm SD.	43.33 \pm 25.0	26.92 \pm 17.63	442.0*	0.002*
Median (IQR)	37.14(20.32–63.03)	20.29(17.21–24.26)		

U: Mann Whitney test .

p: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

Group I: Patients with Varicocele .

Group II: Control .

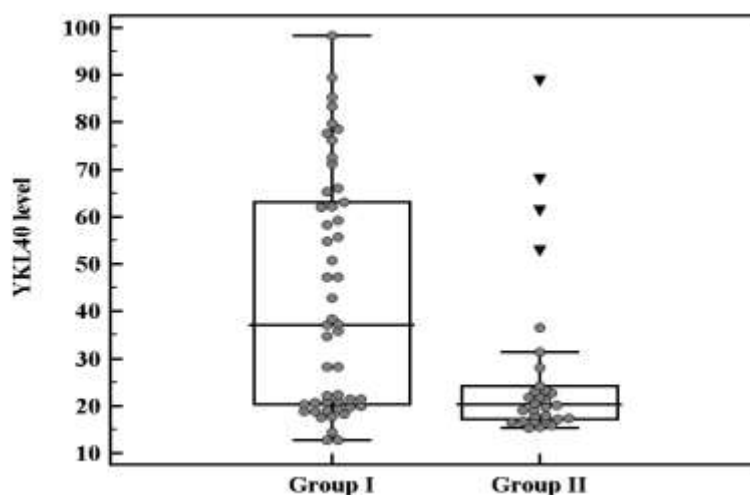


Fig. (1) Comparison between the two studied groups according to YKL40 level .

There were statistically significant decrease in motility and sperm count while there was significant increase in abnormal forms in patients than controls ($p < 0.001$) Table (2) Fig.(2) .

Table (2) Comparison between the two studied groups according to semen analysis .

Semen analysis	Group I (n = 50)	Group II (n = 30)	U	p
Abnormal forms (%)				
Min. – Max.	6.0 – 30.0	1.0 – 5.0		
Mean ± SD.	17.92 ± 7.09	2.67 ± 1.21	0.0*	<0.001*
Median (IQR)	18.0(12.0 – 23.0)	3.0(2.0 – 4.0)		
Sperm count(×10×⁶)				
Min. – Max.	4.99 – 40.0	14.50 – 60.29		
Mean ± SD.	11.38 ± 5.24	30.78 ± 13.48	37.50*	<0.001*
Median (IQR)	10.88(8.30 – 13.46)	27.60(18.60 – 40.70)		
Motility				
Min. – Max.	9.0 – 50.0	20.0 – 66.0		
Mean ± SD.	25.19 ± 10.45	51.27 ± 10.58	72.50*	<0.001*
Median (IQR)	26.50(15.0 – 32.80)	53.50(44.0 – 59.0)		

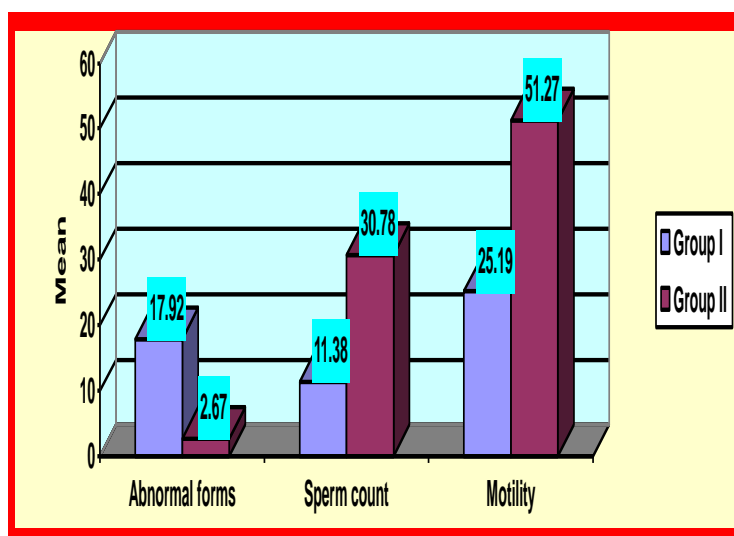
U: mann whitney test .

P: p value for comparing between the studied groups

*: statistically significant at $p \leq 0.05$

Group I: Varicocele patient .

Group II:Control .

**Fig. (2)** Comparison between the two studied groups according to semen analysis.

There was statistically significant positive correlation between serum level of YKL40 and age while there was no statistically significant correlation between serum level of YKL40 level and BMI in varicocele patients ($P > 0.05$ Table (3) Fig. (3) .

Table (3) correlation between serum level of YKL40 and both age and BMI in group 1 (n=50).

	YKL40 level	
	r_s	p
Age (years)	0.321	0.023*
BMI	0.155	0.283

R_s : Spearman coefficient

*: Statistically significant at $p \leq 0.05$

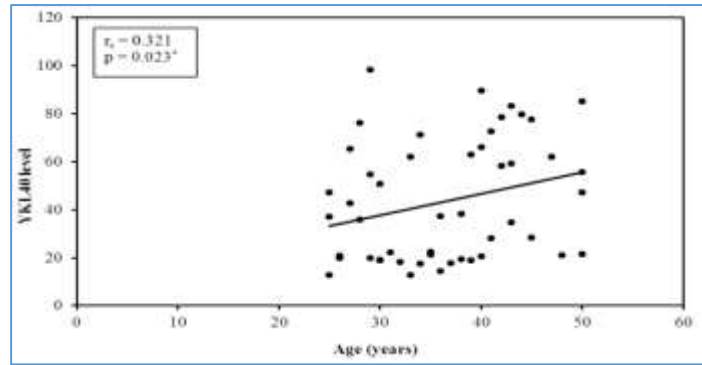


Fig. (3) Correlation between serum level of YKL40 level and age in group

There were statistically significant positive correlations between serum level of YKL40 and abnormal forms while there was significant negative correlation between serum level of YKL40 and motility in group I ($p < 0.05$) Table (4) Fig. (4 ,5).

Table (4) Correlation between serum level of YKL40 and semen analysis in group I (n= 50).

	YKL40 level	
	r_s	p
Abnormal forms (%)	0.965	<0.001*
Sperm count($\times 10^3$)	-0.204	0.156
Motility	-0.281	0.048*

R_s : Spearman coefficient

*: Statistically significant at $p \leq 0.051$

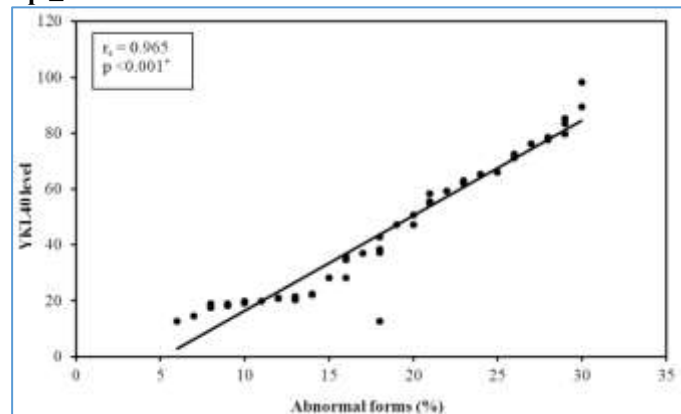


Fig. (4) Correlation between serum level of YKL40 level and abnormal forms (%) in group 1

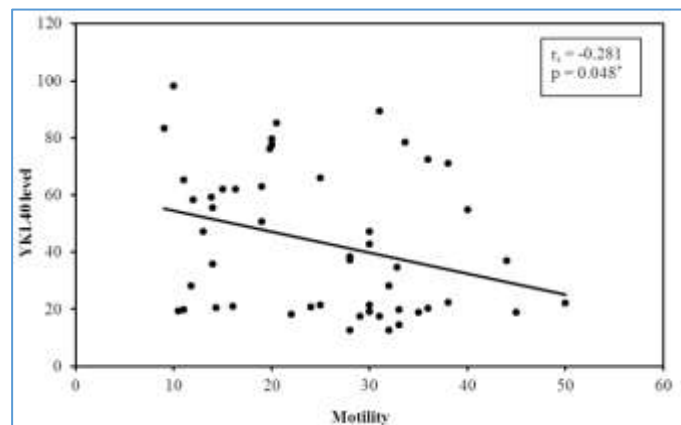


Fig. (5) Correlation between serum level of YKL40 and motility in group I .

4. Discussion

Male infertility is often caused by varicocele, a benign growth on the testicles. Unilateral left varicocele occurs in 15% of the general population and in 41% of infertile women. It is a common condition. A link has been shown between varicocele and infertility, and women who undergo varicocelectomy procedures have reported improved semen quality [9].

In addition to macrophages and chondrocytes, YKL-40 is a glycoprotein produced by other cell types as well as certain kinds of cancer cells. Cells with high cellular activity express more YKL-40 in healthy adult human tissue [14].

Inflammation and endothelial dysfunction are both marked by the protein YKL-40, which is thought to be involved in tissue remodelling during inflammation and in angiogenic processes that mediate the infiltration, differentiation, and maturation of macrophages [5].

elevated YKL-40 has been found in a wide range of illnesses and conditions such as diabetes mellitus and cancer, as well as in cardiovascular and immune disorders and inflammatory diseases of various etiologies such as osteoarthritis, hepatitis, ulcerative colitis, and Crohn's disease [18].

The research included 50 men with varicocele and 30 otherwise healthy men of similar age and sex as a control group. All of the patients were referred by the Benha University Hospitals' Dermatology Department's outpatient clinic.

The research revealed for the first time a link between the endothelial dysfunction marker YKL40 and varicocele.

The Clinical and Chemical Pathology Department of Benha University Hospitals collected and examined samples for the examination of semen and the serum level of YKL-40.

Varicocele patients had significantly greater YKL-40 levels than healthy controls, according to the findings of this research.

That's because endothelial dysfunction contributes to varicocele development.

This discovery that the endothelium is more than just a physical barrier between blood and the tissue underneath has been made apparent since 1980, when it was discovered that the endothelium is also an endocrine organ that can manufacture and release several metabolically active chemicals. It is crucial for the endothelium to emit relaxing and contracting elements to keep the circulatory system in balance [16].

In endothelial dysfunction, there is an imbalance between the relaxing and contracting factors derived from the endothelium (EDRFs) (EDCF). Varicocele is one of several vascular diseases that may be caused by an imbalance in the body's vascular tone [4].

When it comes to varicocele, there are increased levels of relaxing endothelial factors like Nitric Oxide (NO), prostacyclin (PG), and hyperpolarizing endothelial factors like angiotensin-11 and endothelin-1, as well as prostaglandins like thromboxaneA2 (TXA2),

prostaglandin H2 (PGH2), and prostaglandin F2a (PGF2), as well as reactive oxygen [1].

It's also possible that the endothelium in varicose spermatic veins is under hypoxic circumstances because of the vein's tendency to stay closed off, which causes higher venous pressure. Endothelial damage occurs as a result of the low oxygen levels, which sets off a chain reaction of inflammatory processes. [5].

Innate and adaptive immune responses include endothelial cells (ECs), which produce cytokines and chemokines that attract phagocytes to the infection site [8].

Endocrine disruptors such as endothelial cells play a critical part in the onset of inflammation and continue in their influence over it. The phenotypic of ECs becomes activated during an inflammatory event. In general, there are two kinds of EC activation. It's important to remember that type I activation is a brief but intense reaction. It sets off the endothelial-leukocyte-platelet interaction. There are two types of activation: type I and type II. Type I activation is characterised by a rapid reaction, whereas type II activation is more persistent. Tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1), and interleukin-6 (IL-6) produced mostly from activated leukocytes, as well as chemokines such monocyte chemoattractant protein 1 and IL-8, are the main mediators of type II activation [19].

YKL-40, an inflammatory biomarker, is produced by vascular endothelial cells in response to the cytokines generated by these cells. Macrophages, neutrophils, chondrocytes, vascular smooth muscle cells, and hepatic stellate cells all express and release YKL-40. YKL-40 may have a role in inflammation, both acute and chronic, as well as in a wide range of clinical diseases that result in tissue fibrosis [6].

Researchers found a statistically significant difference in aberrant forms, sperm count, and motility between varicocele patients and controls in this research. In comparison to controls, patients had lower sperm counts and motility and higher levels of aberrant sperm. These findings matched those of [3], who showed how varicocele impairs the quality of sperm.

Scrotal heat, hypoxia, renal and adrenal metabolite reflux, hormonal abnormalities, and the development of anti-sperm antibodies are all potential pathophysiological processes implicated in varicocele-induced male infertility [9].

Serum YKL-40 levels had a strong positive connection with aberrant forms of the sperm and a substantial negative correlation with motility in varicocele patients, according to the results of the present research. These findings were in line with those of [7], who proposed that YKL-40 was a multifunctional pro-inflammatory protein with significant roles in immunity and inflammation and the control of cell proliferation, differentiation and apoptosis.

In addition, we found a strong connection between YKL40 serum levels and age in our research. This was in line with the findings of [10] who found that the inflammatory cytokine YKL-40 was present in

atherosclerotic lesions, a condition associated with ageing.

Endothelial dysfunction in varicocele may be detected by looking for the protein YKL-40, which was found in this research.

This suggests that endothelial dysfunction may play a role in varicocele by increasing the serum level of YKL40.

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