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Evaluate the Correlation between the Expression of Androgen Receptor Gene and level of Some Interluken in Semen Sample with Varicocele

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Abstract: A varicocele is an anomalous enlargement and twisting of the veins in the spermatic cord. Varicoceles are a prevalent condition in the general population and are often discovered during normal medical examinations. However, they are the most frequent correctable cause of male factor infertility. The aim of the current study was design to investigate the immunological parameters, gene expression profiles, and their correlations in varicocele and non-varicocele oligospermic patients compared to healthy controls, providing insights into the molecular mechanisms underlying male infertility. A total of 120 human male personals were involved in this study over the period from October 2023 to January 2024. Seminal fluid samples were collected from patients who were diagnosed with varicocele confirmed oligospermia (40 samples) and non-varicocele oligospermia (40 samples), in addition to 40 healthy males as control. Demographically, the distribution of the study groups according to age (20-35 and 36-50) among different groups showed no significant variation. The mean age was 34.97±6.5, 31.23±6.7 and 32.0±7.5 for control, varicocele, and non-varicocele oligospermia patients, respectively. According to body mass index, no significant differences in mean of BMI among groups was observed. With respect to alcohol consumption, all participants were non-alcohol consumer, while the distribution of smoking habit among groups revealed a significant difference for varicocele smoking patients (73%) and non-varicocele oligospermic smokers (77%) compared with the proportion of smoking control (22%). The level of IL-18 and Il-37 was estimated in the seminal plasma of tested groups using Enzyme Linked Immunosorbant Assay (ELISA) technique. The seminal plasma levels of the pro-inflammatory cytokine IL-18 were significantly elevated in both varicocele and non-varicocele oligospermic patients compared with control, suggesting its potential role in the pathogenesis of these conditions. Interestingly, the anti-inflammatory cytokine IL-37 was also significantly increased, possibly as a compensatory mechanism to counterbalance the pro-inflammatory state. Notably, the level of seminal anti-sperm antibodies (ASAbs) was significantly higher in varicocele patients compared to the other groups, indicating an autoimmune component in varicocele-related male infertility. The role of androgen receptor *(AR) gene* expression in varicocele disease was detected using reverse transcriptase polymerase chain reaction (RT-PCR). The fold of gene expression results by RT-PCR technique revealed a significant downregulation of (*AR*) gene expression in varicocele patients compared with control. In conclusion, no significant correlation was found between the expression of *AR* and the levels of IL-18, IL-37, or ASAbs, indicating that the alterations in these parameters may involve distinct molecular pathway.

Keywords: Varicocele, Androgen Receptor, Gene Expression , Interleukins 18 , Interleukins 37 , ASAb

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Introduction

The correlation between the expression of the androgen receptor gene and the levels of certain interleukins in semen samples of men with varicocele is a topic of significant interest in the field of male infertility research. Varicocele, a condition characterized by the abnormal enlargement and twisting of veins in the scrotum, has been associated with alterations in gene expression and inflammatory responses that can impact male fertility. The androgen receptor gene plays a crucial role in modulating cytokine levels, and its expression may be affected by factors such as hypoxia and oxidative stress associated with varicocele (1). Changes in *AR* gene expression can influence the activity of NF-κB, a key regulator of cytokine secretion, leading to increased inflammation in varicocele patients. Interleukins, such as IL-18 and IL-37, are important inflammatory markers that can be dysregulated in varicocele cases. Elevated levels of pro-inflammatory cytokines, as seen in varicocele-induced inflammation, can have detrimental effects on sperm function and spermatogenesis (2). Therefore, evaluating the correlation between *AR* gene expression and interleukin levels in semen samples from men with varicocele can provide valuable insights into the molecular mechanisms underlying infertility in these individuals. There are many studies about the androgen receptor and it association with infertility (3,4,5,6,7). This study aims to shed light on the interplay between the role of androgen receptor expression, cytokine regulation, and sperm health in the context of varicocele-related male infertility (8).

Materials and methods

Subject and sample collection:

Current study conducted on 120 male subjects over the period from October 2023 to January 2024. semen samples were collected from patients who were diagnosed with varicocele confirmed oligospermia (40 samples). The patients were diagnosed by hospital physicians using Doppler ultrasonography, patient aged 20-50 (The median age was 30) and non-varicocele oligospermia (40 samples) aged matched with varicocele patients. They were diagnosed for oligospermia using seminal fluid analysis.In addition Forty apparently healthy individuals as control with no history of infertility or oligospermia and without any abnormal sperms (confirmed by seminal analysis) with median age of 34 and age range between 20-50 years old (whose have at least one child). Specimens were obtained from patients attending the Higher Institute for the Diagnosis of Infertility and Assisted Reproduction Techniques – Al-Nahrain University / Baghdad, Al-Kadhimiya Teaching Hospital/Baghdad and Kamal Al-Samarrai Hospital/Baghdad. All information of demographic parameters, including age, body-mass index (BMI), alcohol consumption, smoking, family history and semen parameters, were collected with the medical reports of the patients. Participants enrolled in this study were informed about the purpose of the study and consent letters were received. The ethical permission to conduct the research was obtained from these hospitals and from the Ethical Committee of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies – Baghdad University.

Inclusion Criteria includes Subjects confirmed varicocele and sperm

concentration less than 15 million/mL while Subjects with reproductive organ surgery, diabetes and other acute diseases such as orchitis or ejaculatory duct obstruction were excluded.

Seminal ejaculates were collected into sterile cup following voluntary self-masturbation and processed with 2 hours after collection. The semen sample was split into two parts. The first part was centrifuged at 3000 rpm for 10 min and the pellet was suspended in ready to use phosphate buffer saline, then aliquot of 250 µL of the cell suspension (5 x 10^6 cells) was mixed with 750 µL of Trizol reagent in an Eppendorf tube for the detection *AR* expression level in sperms. The second part of the seminal fluid was centrifuged for 10 min at 3000 rpm to separate the seminal plasma, they were divided into aliquots and kept at -20°C until use for investigating the level of IL-18, IL-37 and anti-sperm antibody and level of antisperm antibody (ASAb).

Seminal fluid analysis

The analysis of semen samples was conducted according to Semen Analysis Criteria of WHO (2010) using automated semen analyzer. Parameters including sample volume, sperm concentration, total sperm count, progressive and non-progressive motile and immotile sperms were detected.

Quantitative real time PCR- for detection of *androgen receptor gene* expression

a. RNA extraction

The extraction of RNA from semen samples was achieved using TRIzol™ Plus RNA Purification Kit (ThermoFisher, USA). Then, RNA purity and concentration were determined by NanoDrop. The concentration of total RNA was determined by measuring the absorbencies of RNA at 260/280 nm using Nano drop spectrophotometer. RNA concentration was calculated according to the following formula(9):

RNA Concentration (µg/mL) = 40 x Dilution Factor x OD260 nm.

In order to generate first strand cDNA, 0.5 µg – 5 µg of total RNA was used in RT-PCR protocol. The accepted RNA purity ratio (OD260/OD280 nm) is commonly ranged 1.8 to 2.0 (9) .

b. **cDNA Synthesis from mRNA**

Reverse transcription of total RNA extracted from sperm cells to cDNA was achieved using EntiLink™1st Strand cDNA Synthesis Super Mix kit (ELK Biotechnology, USA). The procedure was carried out according to the manufacturer's instruction

c. Gene Expression procedure

Perform reverse transcription to convert RNA into cDNA using the EntiLink™ 1st Strand cDNA Synthesis Super Mix 4.

Design and use primers for the androgen receptor gene (AR) for PCR amplification 4 as shown in Table (1).

The kit components were mixed and subjected to brief centrifugation. All components were placed in an ice bath.

cDNA synthesis was carried out in a total volume of 30 µL containing the ingredients which were added in the order indicated in (Table 2).

Table (1): Designed primers for androgen receptor gene expression.

Primers	Sequence (5'-3')	Annealing Temp. (°C)	Product Size (bp)	Primers were designed in this study
AR (F)	CTGGATGAGGAACAGCAACC	59	152	
AR (R)	GCAGCTGAGTCATCCTCGTC			
B2M (F)	TGGGTTTCATCCATCCGACA	59	160	
B2M (R)	ACGGCAGGCATACTCATCTT			

Table (2): The ingredients mixed for cDNA synthesis.

Components	Reaction Volume
Total RNA (1 µg) in nuclease-free water	10 µL
2xEntiLink™ Synthesis Super Mix	10 µL
ddH2O	20 L

Quantitative real time polymerase chain reaction qRT-PCR protocol

The gene expression for d *AR* in sperm cells of varicocele and oligospermia patients was determined by qRT-PCR using HOT FIREPol® EvaGreen® qPCR Mix Plus assay. Beta -2- Microglobulin *(B2M)* expression was used as housekeeping gene (control primer). All samples were run in triplicates. The qRT-PCR reagents in (Table 3) were added to prepare the reaction mixture.

The ΔCt value was calculated for each sample:

ΔCt = Ct (target gene) - Ct (reference gene)ΔΔCt = ΔCt (experimental sample) – ΔCt (control sample)

Fold change = $2^{-\Delta\Delta Ct}$

Table (3): qRT-PCR reagents required to prepare the reaction mixture.

Component	20 µl Rxn
HOT FIREPol® EvaGreen® qPCR Mix Plus	4
Primer Forward (10 pmol μL^{-1})	1
Primer Reverse (10 pmol μL^{-1})	1
cDNA	5
Ultrapure-distilled water	13

qRT-PCR was carried out according to the amplification program in (Table 4).

Table (4): Amplification program of qRT-PCR for target genes and housekeeping gene.

Step	Temp.	Time	Circle
Initial denaturation	95°C	12 min.	1
Denaturation	95°C	15 sec.	40 cycles
Annealing	62°C	20 sec.	
Elongation (extension)	72°C	20 sec.	

Estimation level interleukins and ASA in seminal fluids

Measure the levels of interleukins IL-18, IL-37 as well as antisperm antibody in the semen samples using ELISA kits 7. Follow the ELISA kit protocols for interleukin quantification in the samples (10).

Statistical Analysis

The statistical analysis was performed using GraphPad Prism version 9.2 (GraphPad Software Inc., LaJolla, CA). One Way (Tukey's Test) were used to determine whether group variance was significant or not. Pearson coefficient r-value was employed to assess correlation. Chi-square was employed to test count variances. Receiver operating characteristic (ROC) curve analysis was performed to determine area under curve (AUC) and the optimum cut-off value of serum markers best prediction. Quantitative parametric data were subjected to

Shapiro-Wilk test to confirm the normal distribution and were expressed as mean ± SD and statistical differences were defined as * $p < 0.05$ and ** $p < 0.01$ (11).

Results and discussion

Demographic distribution of the study groups

Distribution according to age

The distribution of the study groups according to age was shown in (Table 5). No significant differences were observed based on age distribution between patient groups (Varicocele and non-varicocele oligospermia) and apparently healthy subjects. The total number of varicocele and non-varicocele oligospermia patients within age range of 20-35 years was 25(63%) and 29 (73%), respectively. Whereas the lowest number of patients was noted within the age range of 36-50 years, 15 (37%) for varicocele patients and 11 (27%) for non-varicocele oligospermia

Table (5): Distribution of sample study according to age in control and patients' groups.

Age Dist.	No. (%)			Chi Square	Sig.	*p* Value
	Varicocele	Oligospermia	Control			
20 – 35	25 (63%)	29 (73%)	21 (53%)	3.4	NS	0.1815
36 – 50	15 (37%)	11 (27%)	19 (47%)			
Total	40 (100%)	40 (100%)	40 (100%)			

NS: Non-Significant.

The mean age of different groups showed no significant differences. The mean age was 34.97±6.5, 31.23±6.7 and 32.0±7.5 for control, varicocele, and non-varicocele oligospermia patients, respectively. Recent studies have shown an increasing prevalence of varicocele and oligospermia in younger male patients. Varicocele is the abnormal enlargement of the veins in the scrotum and is one of the leading causes of male infertility. Oligospermia refers to low sperm count which can also lead to infertility(12, 33, 34).

Traditionally, varicocele was thought to be a condition affecting older males. However, newer research indicates that varicocele is now being detected more frequently in adolescents and men in their 20s and 30s. One study found that over 15% of boys aged 10-19 had varicocele detected on physical exam. The causes for the rising prevalence in younger males are not entirely clear but factors like increased screening, obesity, environmental toxins and hormones have been proposed (13). Similarly, oligospermia or low sperm count was conventionally believed to be a problem for older males. But recent sperm analysis data showed that sperm counts have been declining in younger men worldwide. A number of theories have been put forth - including increased exposure to environmental pollutants and endocrine disrupting chemicals during critical developmental windows. Lifestyle factors like stress, smoking and alcohol use also likely contribute to poorer sperm parameters in young men. Many reports involving parametric studies on patients with non-varicocele oligospermia should that age distribution ranged from 35 to 45 years(14).

Distribution according to body-mass index (BMI)

The BMI was calculated among different groups. Results in (Table 6)

clearly indicated that no significant differences in mean BMI among control, varicocele, and non-varicocele oligospermia patients. All groups were within the overweight category.

Table (6): Mean ± SD BMI in control and patients' groups.

Mean ± SD BMI in kg/m^2			Sig.	*p* Value
Varicocele	Oligospermia	Control		
29.6 ± 5.0	28.8 ± 4.8	28.3 ± 3.34	NS	0.5237

NS: Non-Significant.

While several studies have found an association between BMI and varicocele/oligospermia (17), some research suggested there may be no significant BMI differences between these patients and normal fertile controls (35),(36). A study examined BMI over 2106 men with male infertility including varicocele/ oligospermic patients and 200 age-matched fertile controls showed that the mean BMI 26.4 kg/m^2 (15). The average BMIs in this study was within the overweight range which was in concurrence with the results obtained. In contrast, a meta-analysis involved 11 studies showed that obese males have a low risk of varicocele, while overweight males have a protective impact against varicocele (16).

Distribution according smoking

Smoking is one of the most causes of long-term negative effects on human health. The distribution of smoking habit among studied groups revealed a significant ($p < 0.0001$) differences for varicocele smoking patients (73%) and non- varicocele oligospermic smokers (77%) compared with the proportion of smoking control (22%) (Table 7). The prevalence of smoking patients varicocele and non- varicocele oligospermia was significantly higher than non-smoking patients, indicating that smoking is a risk factor for the progression of varicocele and non-varicocele oligospermia.

Table (7): Distribution of varicocele, non-varicocele oligospermia patient and control according to smoking.

Smoking	No. (%)			Chi Square	Sig.	*p* Value
	Varicocele	Oligospermia	Control			
Yes	22 (73%)	23 (77%)	9 (22%)	26.7	**	<0.0001
No	8 (27%)	7 (23%)	31 (78%)			
Total	40 (100%)	40 (100%)	40 (100%)			

**** $p < 0.01$.**

Distribution according to family history and other diseases

The distribution of patients according to family history of the disease showed that all patients in different groups have no family history of varicocele or non-varicocele oligospermia diseases. In addition, no varicocele or non-varicocele oligospermic patients were not experienced any other disease not male infertility related diseases. Research by Al-Kandari *et al*., (18) revealed that 13.5% of male infertility cases including varicocele and oligospermia showed positive family history, indicating the influence of genetic factors on male infertility.

Detection of seminal plasma level of IL-18 and IL-37

The seminal plasma level of IL-18 and IL-37 among varicocele and non-varicocele oligospermic patients were detected in comparison with control group using ELISA technique. According to (Table 8). The level of IL-18 in varicocele and non-varicocele oligospermic patients was significantly increased compared with control with 1.2 (p = 0.0008) and 1.3 (p < 0.0001) fold increase, respectively. No significant variation in IL-18 level was recorded between varicocele and non-varicocele oligospermic patients.

Table (8): Mean ± SD seminal plasma IL-18 level in control and patients' groups.

Interleukins	Mean ± SD IL Level in pg/Ml			Sig.	*p* Value
	Varicocele	Oligospermia	Control		
IL-18	5708 ± 834[a]	6175 ± 831[a]	4867 ± 919[b]	**	<0.0001
IL-37	23979 ± 4968[a]	21289 ± 7854[a]	11846 ± 3544[b]	**	<0.0001

****: p < 0.01, SD: Standard Deviation. Different letters (a, b) are significantly different in row at p < 0.05.**

The seminal level of Il-37 was significantly (p < 0.0001) elevated in varicocele and non-varicocele oligospermic patients with 2.02 and 1.8 times increase compared with control group, respectively. No significant differences in IL-37 level was detected between varicocele and non- varicocele oligospermic patients. The study examines the levels of IL-37 and IL-18 in the semen of infertile males with varicocele and oligospermia. In this case-control study, increased levels of seminal IL-37, also known as IL-1F7, which is a type of anti-inflammatory cytokine produced by macrophages. Furthermore, a substantial rise in the semen pattern of IL-18 in varicocele and non-varicocele oligospermia. The present study agreed with Zeinali *et al*., (19) who reported an increased level of both IL-18 and IL-37 in infertile men with varicocele. Similar results were reported by Camargo *et al*., (20) which the level of IL-18 was dramatically decreased in in male post-varicocelectomy patients, indicating that varicocelectomy enhanced sperm morphology and reduced inflammatory activity in seminal plasma within six months following the surgery.

In varicocele patients, the increased seminal IL-18 levels are thought to be associated with the inflammatory process and oxidative stress induced by venous stasis and hypoxia within the testes. The dilated veins in varicocele can lead to reflux of metabolites and toxins, triggering an inflammatory response and the production of IL-18 (21).

Similarly, non-varicocele oligospermic patients also exhibit higher seminal IL-18 levels compared to control. This result was in agreement with pervious Iraqi study which indicated the high expression of IL-18 in seminal fluid of infertile patients with oligospermia compared with control(22). The underlying mechanisms are not fully understood but may involve inflammatory processes, oxidative stress, and impaired spermatogenesis contributing to the dysregulation of IL-18 production.

In varicocele patients, the elevated seminal IL-37 levels are thought to be a compensatory mechanism in response to

the pro-inflammatory state induced by venous stasis, oxidative stress, and the production of inflammatory mediators like IL-18. It was suggested that IL-37 is released by macrophages in semen fluid and has the ability to inhibit excessive inflammatory reactions, specifically the pro-inflammatory effects of IL-18. Consequently, the primary role of IL-37 is to facilitate a negative feedback process that enhances sperm motility.

Similarly, non-varicocele oligospermic patients also exhibit higher levels of seminal IL-37, suggesting a potential role of inflammation and the body's attempt to regulate the inflammatory response in the pathogenesis of oligospermia (23).

Detection of seminal plasma level of anti-sperm antibodies (ASAbs)

The level of ASAbs was detected in all tested groups. Based on statistical analysis, the level of ASAbs in varicocele patients (29120 ± 21540 pg/mL) was significantly increased ($p < 0.0001$) compared with control (6034 ± 4651 pg/mL) and non-varicocele oligospermic patients (9847 ± 8284 pg/mL). More interestingly, no significant differences were recorded between control and oligospermia groups with respect to ASAbs level (Table 9).

Table (9): Mean ± SD seminal plasma ASAbs level in control and patients' groups.

ASAbs	Mean ± SD IL Level in pg/mL			Sig.	*p* Value
	Varicocele	Oligospermia	Control		
	29120 ± 21540[a]	9847 ± 8284[b]	6034 ± 4651[b]	**	<0.0001

****: $p < 0.01$, SD: Standard Deviation. Different letters (a, b) are significantly different in row at $p < 0.05$.**

These results came in agreement with Bozhedomov *et al*. (24) which were indicated that the higher level of ASAbs in varicocele patients demonstrated a decrease anti-sperm immune response 3 months after varicocelectomy operation. In varicocele patients, the increased levels of seminal ASAbs are believed to be a consequence of the disruption of the blood-testis barrier (BTB) and the exposure of sperm antigens to the immune system. The BTB is a physiological barrier that separates the seminiferous tubules from the systemic circulation, creating an immune-privileged environment for spermatogenesis. In varicocele, the venous stasis and increased hydrostatic pressure can compromise the integrity of the BTB, allowing sperm antigens to leak into the bloodstream and trigger an autoimmune response.

Receiver operating characteristic (ROC) analysis of IL-18, IL-37 and ASAbs

To assess the diagnostic significance of IL-18, IL-37 and ASAbs, ROC analysis was performed. The elevated level of IL-18 in varicocele patient groups occupied a significant area under curve (AUC), which was 0.7628 ($p = 0.0005$). At a cut-off value of 5154 pg/mL, the sensitivity and specificity of IL-18 were 80 and 63%, respectively (Figure 1).

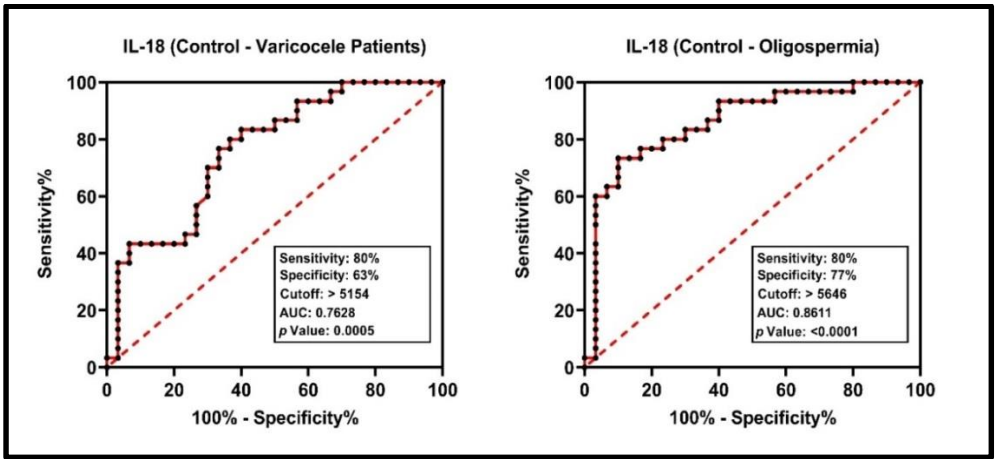

Figure (1): Receiver operating characteristic (ROC) analysis of IL-18 level among varicocele and non-varicocele oligospermic patients showing area under curve (AUC), *p* value, sensitivity, specificity, and cut-off value.

The AUC of IL-37 was also calculated between control and varicocele patient group. The elevation of IL-37 in varicocele patients showed a strong significant prognostic power with AUC of 0.9822 ($p < 0.0001$) and the best sensitivity of 100% and specificity of 93% were achieved at cut-off value of 17134 pg/mL. In non-varicocele-oligospermic patients, the AUC was 0.93 indicate a significant a strong prognostic prediction ($p < 0.0001$) and the best sensitivity of 90% and specificity of 73% were achieved at cut-off value of 13997 pg/mL (Figure 2).

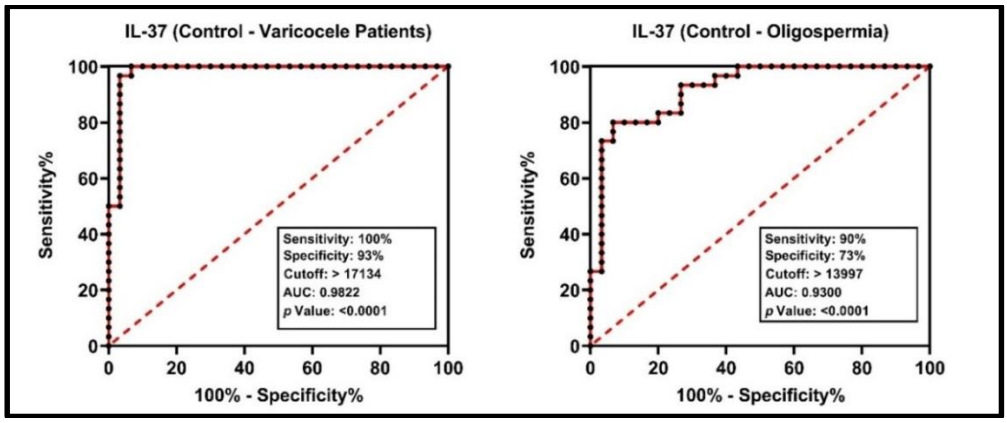

Figure (2): Receiver operating characteristic (ROC) analysis of IL-37 level among varicocele and non-varicocele oligospermic patients showing area under curve (AUC), *p* value, sensitivity, specificity, and cut-off value.

ROC analysis of ASAbs was detected between control and varicocele patient group. A significant ($p < 0.0001$) fair predictive AUC of 0.8322 and the calculated cut-off point of 9236 pg/mL gave the best sensitivity of 77% and specificity of 93%. In oligospermic patients, AUC was 0.6367 indicated a very poor insignificant diagnostic power with best sensitivity of 60% and specificity of 43% that achieved 4651 pg/mL (Figure 3).

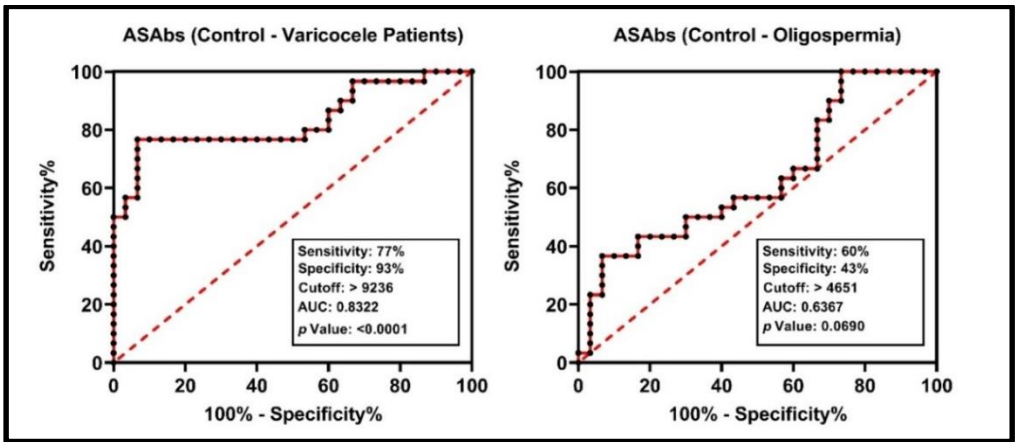

Figure (3): Receiver operating characteristic (ROC) analysis of ASAbs level among varicocele and non-varicocele oligospermic patients showing area under curve (AUC), *p* value, sensitivity, specificity, and cut-off value.

Relative expression of *AR gene*

The concentration of RNA isolated from seminal fluid of varicocele, non-varicocele oligospermic patient and seemingly healthy individual was around 18.53 ng/µL. The process involves converting total RNA into complementary DNA (cDNA) in order to measure it using quantitative real-time PCR. This measurement is used to calculate the relative gene expression, which depends on the relationship between the Ct (Cycle threshold) value of the target gene in patients and the control group, as well as the internal control gene, also known as the (*B2M*) housekeeping gene (25). The result of *AR* gene expression showed a significant (p <0.0001) downregulation of *AR* expression in both varicocele and non-varicocele oligospermic patients compared with control. The fold of *AR* gene expression in patients was 0.22±0.21, 0.29±0.2 and 1.00 ±0.00[a] respectively (Figure 4).

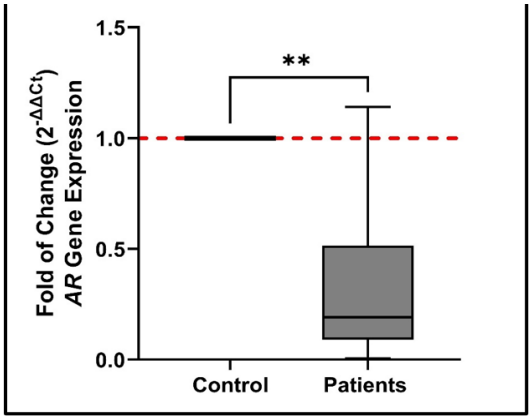

Figure (4): Mean ± SD fold of change of *AR* gene expression in varicocele patients compared with healthy control.
**** (P<0.01)**

The (AR) is a nuclear receptor that plays a crucial role in male reproductive function and fertility. It is expressed in various cell types within the testis, including Sertoli cells, Leydig cells, and spermatogenic cells. The AR mediates

the actions of androgens, particularly testosterone, which are essential for spermatogenesis, sperm maturation, and the development and maintenance of male reproductive organs(26).

Several studies have reported downregulation or decreased expression of the *AR* gene in testicular tissues of varicocele patients compared to fertile controls(37),(38). The downregulation of *AR* gene expression in varicocele patients is believed to contribute to the impairment of spermatogenesis and the development of oligospermia (low sperm count) and asthenospermia (reduced sperm motility). The decreased *AR* expression in testicular cells can lead to reduced androgen signaling, which is essential for various stages of sperm production and maturation (27).

The underlying mechanisms that contribute to the downregulation of *AR* gene expression in varicocele patients are not fully understood, but several factors have been proposed:

Oxidative stress: Varicocele is associated with increased oxidative stress within the testis, which can lead to DNA damage and alterations in gene expression, including the *AR* gene (28). Hypoxia: The venous stasis and impaired blood flow in varicocele can result in hypoxic conditions within the testis, potentially affecting the expression of various genes, including the *AR*(29). Inflammation: Varicocele is often accompanied by an inflammatory response, and pro-inflammatory cytokines have been shown to downregulate *AR* expression in certain cell types (22). Hormonal imbalances: Varicocele can disrupt the normal hormonal balance, including testosterone levels, which may impact *AR* expression and signaling (30).

The downregulation of *AR* gene expression in varicocele patients has important clinical implications. Reduced androgen signaling can contribute to impaired spermatogenesis, leading to subfertility or infertility. Additionally, the assessment of *AR* expression levels in testicular tissues may serve as a potential biomarker for evaluating the severity of varicocele and its impact on male fertility (31), as shown in (Figure 5).

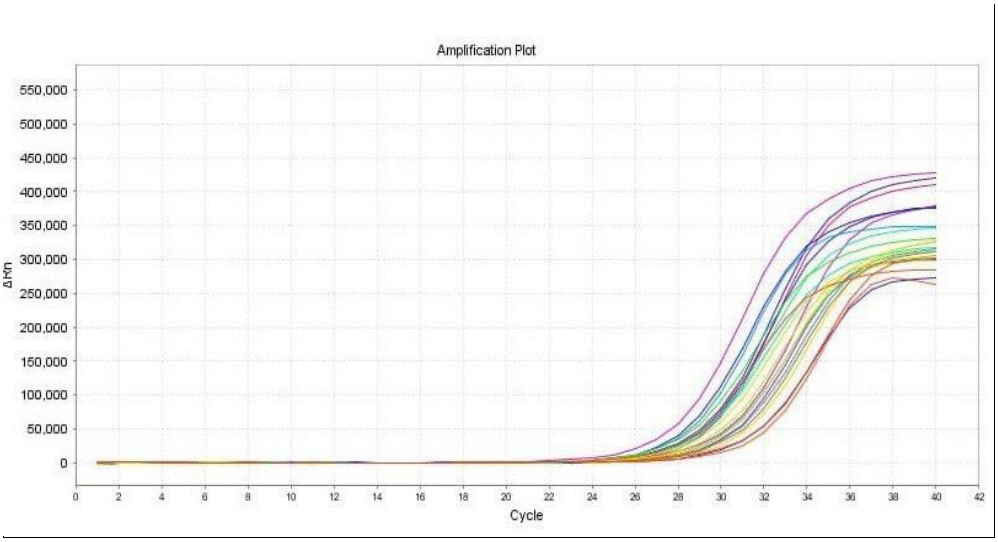

Figure (5): The amplification plot of *AR* gene.

Correlation between *AR* gene expression and immunological parameters

The relationship between *AR* gene expression and the seminal level of IL-18, IL-37 and ASAbs was detected. Results in (Table 10), showed that a non-significant positive correlation was observed between *AR* gene expression and IL-18 level (r = 0.1428, *p* = 0.5481). With respect to IL-37 level, no correlation with *AR* gene expression was detected (r = 0.0531, *p* = 0.8241). In addition, a non-significant positive relationship was noted between *AR* gene expression and ASAbs level (r = 0.1782, *p* = 0.4523).

Table (10): The value of the coefficient correlation r between *AR* gene expression and immunological parameters in varicocele patients.

Parameters	*AR* Gene Expression	
IL-18	r Value	0.1428
	p Value	0.5481 NS
IL-37	r Value	0.0531
	p Value	0.8241 NS
ASAbs	r Value	0.1782
	p Value	0.4523 NS

While varicocele is associated with decreased *AR* gene expression in testicular tissues and altered levels of IL-18, IL-37 and ASAbs, the mechanisms underlying these changes appear to be independent of each other. The downregulation of *AR* is thought to be influenced by factors such as oxidative stress, hypoxia, and hormonal imbalances, rather than directly related to the inflammatory cytokine levels. Similarly, the increased levels of IL-18 and IL-37 in varicocele patients are likely related to the inflammatory processes and the body's attempt to regulate the inflammatory response, respectively, but do not seem to directly impact or correlate with *AR* expression

References

1. Abd, Z. H.; Thamer, Q. A. R. and Altaha, M. A. (2016). Clinical patterns and major causes of male infertility in Fallujah city. Al-Anbar Medical Journal, 13(1): 96-106.
2. Adams, Y.; Afoko, A. A. and Amidu, N. (2022). Effect of varicocelectomy on semen parameters of men seeking infertility treatment in Tamale, Ghana. Open Journal of Urology, 12(1): 7-26.
3. Khadhim, M. F. and Abdul-Hassan, I. A. (2017). Association of Androgen Receptor Gene Polymorphisms at three SNPs and their Haplotypes with Severe Oligozoospermia Risk in Iraqi Patients. Iraqi journal of biotechnology, 16(1): 16- 28.
4. Khadhim, M. F. and Abdul-Hassan, I. A. (2017). Association of Androgen Receptor Gene Polymorphisms at three SNPs and their Haplotypes with Severe Oligozoospermia Risk in Iraqi Patients. Iraqi journal of biotechnology, 16(1).
5. Kadhim, M. H. K. S. J. (2022). Estimation of Estrogen Receptor Beta Gene Expression and Its Correlation with Some Hormones Parameters in Samples of Iraqi Infertile Men. Iraqi journal of biotechnology, 21(2): 207-214.
6. Namaa, D. S. (2020). Genetic Polymorphisms of PLCζ and PAWP Genes in Infertile Men that Undergo ICSI Protocol. Iraqi journal of biotechnology, 19(2).
7. Sadoon, A.; Kadhim, S. J. and Nadhim, A. (2023). Study the correlation between androgen receptor gene polymorphism (rs1337082) with male infertility in samples of Iraqi who abuse anabolic steroids.

8. Agarwal, A.; Cho, C. L.; Esteves, S. C. and Majzoub, A. (2017). Reactive oxygen species and sperm DNA fragmentation. Translational andrology and urology, 6(4) 695-696.
9. Okamoto, T. O. S. H. I. H. I. R. O. and Okabe, S. U. S. U. M. U. (2000). Ultraviolet absorbance at 260 and 280 nm in RNA measurement is dependent on measurement solution. International Journal of Molecular Medicine, 5(6): 657-666.
10. Al-Ani, S. M.; Al-Kasser, E. A. and Al-Aboosy, F. M. (2021). The Etiological Factors of Infertility among Couples Attending the Infertility Clinic of Baghdad Teaching Hospital during the Years 2013 and 2014. Iraqi Medical Journal, 67(1): 26-30.
11. Watson, J.; Fitzallen, N.; Fielding-Wells, J. and Madden, S. (2018). The practice of statistics. International handbook of research in statistics education, 105-137.
12. Kimura, M.; Nagao, K.; Tai, T.; Kobayashi, H. and Nakajima, K. (2017). Age is a significant predictor of early and late improvement in semen parameters after microsurgical varicocele repair. Andrologia, 49(3): e12620.
13. Chung, J. M. and Lee, S. D. (2018). Current issues in adolescent varicocele: pediatric urological perspectives. The World Journal of Men's Health, 36(2): 123-131.
14. Agarwal, A.; Srivastava, A.; Fathima, F. and Lodhi, B. (2023). Insight into epidemiology of male infertility in central India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 12, 215-220.5, 27-32.
15. Nielsen, M. E.; Zderic, S.; Freedland, S. J. and Jarow, J. P. (2006). Insight on pathogenesis of varicoceles: relationship of varicocele and body mass index. Urology, 68(2): 392-396.
16. Xiao-Bin, G.; Fang-Lei, W.; Hui, X.; Cheng, Y.; Zhi-Xuan, C.; Zhi-Peng, H., *et al*. (2020). The association between body mass index and varicocele: A meta-analysis. International braz j urol, 47(1), 8-19.
17. Handel, L. N.; Shetty, R. and Sigman, M. (2006). The relationship between varicoceles and obesity. The Journal of urology, 176(5), 2138-2140.
18. Al-Kandari, A. M.; Al-Enezi, A. N.; Ibrahim, H. and Alkandari, O. (2020). A population-based study of the epidemiology and the risk factors for male infertility in Kuwait. Urology Annals, 12(4): 319-323.
19. Zeinali, M.; Hadian Amree, A.; Khorramdelazad, H.; Karami, H. and Abedinzadeh, M. (2017). Inflammatory and anti-inflammatory cytokines in the seminal plasma of infertile men suffering from varicocele. Andrologia, 49(6): e12685.
20. Camargo, M.; Ibrahim, E.; Intasqui, P.; Belardin, L. B.; Antoniassi, M. P.; Lynne, C. M., *et al*. (2022). Seminal inflammasome activity in the adult varicocele. Human Fertility, 25(3): 548-556.
21. Ates, E.; Ucar, M.; Keskin, M. Z. and Gokce, A. (2019). Preoperative neutrophil-to-lymphocyte ratio as a new prognostic predictor after microsurgical subinguinal varicocelectomy. Andrologia, 51(2): e13188.
22. Al-Marzoqi, A. H.; Hussein, H. J.; Al-Janabi, H. S. O.; Ibraheem, H. K. and Al Taee, Z. M. (2013). Role of interleukin 1, IL-18 and tumor necrosis factor alpha levels in seminal plasma of infertile males in Hillah patients. J. Bio Agr Health, 3(3): 165-171.
23. Ojo, O. A.; Nwafor-Ezeh, P. I.; Rotimi, D. E.; Iyobhebhe, M.; Ogunlakin, A. D. and Ojo, A. B. (2023). Apoptosis, inflammation, and oxidative stress in infertility: A mini review. Toxicology Reports, 10: 448-462.
24. Yang, F.; Li, J.; Dong, L.; Tan, K.; Huang, X.; Zhang, P., *et al*. (2021). Review of vasectomy complications and safety concerns. The world journal of men's health, 39(3): 406-418.
25. Schmittgen, T. D. and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. Nature protocols, 3(6): 1101-1108.
26. Al-Ani, S. M.; Al-Kasser, E. A. and Al-Aboosy, F. M. (2021). The Etiological Factors of Infertility among Couples Attending the Infertility Clinic of Baghdad Teaching Hospital during the Years 2013 and 2014. Iraqi Medical Journal, 67(1): 26-30.
27. Alenzi, M. J. (2021). Synergetic effect of hookah smoking on varicocele-associated

male reproductive impairment in the Saudi community in Al Jouf region, Saudi Arabia. Urology annals, 13(3): 205-209.
28. Alkan, İ.; Yüksel, M.; Canat, H. L.; Atalay, H. A.; Can, O.; Özveri, H., e*t al*. (2018). Superoxide anion production by the spermatozoa of men with varicocele: relationship with Varicocele grade and semen parameters. The world journal of men's health, 36(3): 255-262.
29. Al-Kandari, A. M.; Al-Enezi, A. N.; Ibrahim, H. and Alkandari, O. (2020). A population-based study of the epidemiology and the risk factors for male infertility in Kuwait. Urology Annals, 12(4): 319-323.
30. Almayyahi, A. O.; Attar, F. S.; Altimari, A. Y. and Usama, A. (2020). The Effect of Varicocelectomy in Improving Premature Ejaculation in Patients with Both Premature Ejaculation and Varicocele. Basrah Journal of Surgery, 26(2): 32-37.
31. Alnajjar, A. F. (2022). Prevalence of Male Infertility in Kirkuk City, Iraq. Journal of Pharmaceutical Negative Results, 13(1): 89-94.
32. Schmittgen, T. D. and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc,* 3**,** 1101-8.
33. Hsiao, W.; Rosoff, J. S.; Pale, J. R.; Greenwood, E. A. and Goldstein, M. (2011). Older age is associated with similar improvements in semen parameters and testosterone after subinguinal microsurgical varicocelectomy. Journal of Urology, 185, 620–625.
34. Zini, A.; Azhar, R.; Baazeem, A. and Gabriel, M. S. (2011). Effect of microsurgical varicocelectomy on human sperm chromatin and DNA integrity: a prospective trial. International Journal of Andrology, 34, 14–19.
35. Nielsen, M.E.; Zderic, S.; Freedland, S.J. and Jarow, J.P. (2006). Insight on pathogenesis of varicoceles: relationship of varicocele and body mass index. Urology, 68:392-6.
36. Najari, B. B.; Katz, M. J.; Schulster, M. L.; Lee, D. J.; Li, P. S. and Goldstein, M. (2016). Increased body mass index in men with varicocele is associated with larger spermatic vein diameters when supine. Urology, 89, 40-44.
37. Sheehan, M. M.; Ramasamy, R. and Lamb, D. J. (2014). Molecular mechanisms involved in varicocele-associated infertility. Journal of Assisted Reproduction and Genetics, 31, 521-526.
38. Zalata, A. A.; Mokhtar, N.; Badawy, A. E.-N.; Othman, G.; Alghobary, M. and Mostafa, T. (2013). Androgen Receptor Expression Relationship with Semen Variables in Infertile Men with Varicocele. The Journal of Urology, 189, 2243-2247.