Evaluation the Effect of Interleukin-6 and Tumor Necrosis Factor in Semen Quality of Infertile Men with Varicocele.

تقييم تاثير انترلوكين 6وعامل نخر الورم في نوعية السائل المنوي لدى الرجال العقيمين والمصابين بالقيلة الدوالية الخصوية

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الخلاصة

الهدف: تقييم تأثير ارتفاع مستوى الانترلوكين interleukin-6 و عامل نخرالورم Tumor necrosis factor في بلازما المني على نوعية السائل المنوي لدى الرجال العقمين المصابين بالقيلة الدوالية الخصوية.

المنهجية : دراسة وصفية أجريت في مركز الخصوبة والعقم التخصصي في مدينة الصدر الطبية في مدينة النجف الاشرف خلال الفترة الاول من نيسان لغاية الاول من حزيران 2014 تم اختيار عينة غرضية (غير احتمالية) مكونة من (75) رجل يعاني من العقم المصابين بالقيلة الدوالية الخصوية و(25) أصحاء كعامل سيطرة ومقارنة تراوحت اعمارهم (20-40) سنة تم استعمال استبانة تتضمن فترة الزواج وسلامة العينة من الامراض المزمنة مثل مرض السكري و الاورام السرطانية وكذلك الامراض الجنسية الانتقالية , تم جمع المعلومات من خلال المقابلة الشخصية للمرضى و تم تحليل النتائج باستخدام T –test وZ- test.

النتائج: أظهرت نتائج الدراسة ان 42% من الرجال المصابين بالقيلة الدوالية الخصوية يعانون من ارتفاع مستوى بروتينات التحركة 6 في بلازما المني وكذلك أظهرت هذه الدراسة ان هناك علاقة عكسية R= -0.58 بين إرتفاع مستوى بروتينات التحركة 6 في بلازما المني مع انخفاض حركة النطف التقدمية و كذلك وجود علاقة عكسية R= -0.30 بين ارتفاع مستوى بروتينات التحركة 6- في بلازما المني مع نقصان تركيز النطف(مليون/ مل). وكذلك أظهرت نتائج الدراسة ان 41% من الرجال المصابين بالقيلة الدوالية الخصوية يعانون من ارتفاع مستوى عامل نخرالورم في بلازما المني وكذلك أظهرت هذة الدراسة ان هناك علاقة عكسية R= -0.17 بين أرتفاع مستوى عامل نخرالورم في بلازما المني مع انخفاض حركة الحيوانات المنوية و كذلك وجود علاقة عكسية R= -0.25 بين ارتفاع مستوى عامل نخرالورم في بلازما المني مع نقصان في تركيز النطف.

ألاستنتاج: أستنتجت الدراسة ان هنالك علاقة بين الرجال العقيمين والمصابين بالقيلة الدوالية الخصوية و ارتفاع تركيز انترلوكين6 وعامل النخر الورم في بلازما المني , وكذلك استنتجت هذة الدراسة ان زيادة في تركيز انترلوكين6 وعامل نخر الورم يؤثر سلبيا علي نوعية السائل المنوي والمتضمنة تركيز النطف و حركة النطف التقدمية .

التوصيات: اوصت الدراسة بأن جميع الذكور الذين يعانون من العقم يجب ان يتاكدوا من وجود الدوالي من خلال الفحص السريري.واوصت الدراسة على ان جميع المصابين بالدوالي ويعانون من العقم يجب قياس مستوى IL-6و كذلك TNFفي السائل المنوي.

Abstract:

Objective: The study was designed to evaluation of effect Interleukin-6 and Tumor necrosis factor in seminal plasma to semen quality in infertile men with varicocele.

Methods: A descriptive correlation study was conducted in specialized infertility center / teaching AL-Sadder medical city in Najaf city from first of April 2014 to the first of June 2014.Sample was selected that consisted of (75) infertile men with varicocele, and (25) healthy subject as control, their age between (20-40) years old. A questionnaire format and observational checklist were used which including marriage period, excluding from chronic disease like diabetic, cancer, sexual transmitted disease. The data were collected through the utilization of questionnaire and the structure interview technique with varicocele patients. Data was analyzed by using T-test and Z-test

Results: The result of the study sample have revealed that 42% from all subjects has elevated Interleukin-6 concentration in seminal plasma ,and also the elevation Interleukin-6 level in seminal plasma has inversely correlation R = -0.58 with decreased in sperm motility ,also has inversely correlation R = -0.30 with low sperm count. From other hand 41% from all subjects has elevated Tumor necrosis factor levels in seminal plasma ,and also the elevation Tumor necrosis factor concentration in seminal plasma has inversely correlation R = -0.17 with decreased in sperm motility ,also has inversely correlation R = -0.25 with low sperm count.

Conclusions: They study concluded there was a significant difference between elevation of Interleukin-6 and Tumor necrosis factor levels in seminal plasma in infertile men with varicocele, and they study concluded there was a significant correlation between increased levels of Interleukin-6 and Tumor necrosis factor in seminal plasma with reduce sperm count and motility .

Recommendations: the study recommended that: Varicocele examination and diagnosis should be done to all infertile male.Varicocele patients with abnormal seminal fluid examination should be estimated concentration of Interluekin-6 and Tumor necrosis factor in seminal plasma

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INTRODUCTION

Infertility is defined as inability of couples to achieve pregnancy following one year of unprotected intercourse .By this criterion, infertility affects 13% - 18% of couples and male factors account for up to half of all cases[(1)].

One of male infertility cause is varicocele which is present in 2% - 22% of the adult male population. In men with abnormal semen analysis , the prevalence of varicocele reach 25 % ,of cases ,varicocele have been linked to serious of events such as : biochemical changes in the epididymal fluid , a stasis of internal spermatic vein , elevated scrotal temperature , testicular hypoxia , and retrograde blood flow of renal and adrenal [(2)].

Varicocele , which is the distension of the pampiniform venous plexus , usually on the left side , result from inversion of blood flow in the internal spermatic vein . It is the most common cause of male infertility[(3)].

IL6 –like factor has been shown to be produced by Sertoli cells is responsive to interleukin 1 alpha (IL1α), Lipopolysaccharide LPS and latex beads but also to FSH and testosterone .The action of IL6 in testis is reduces transferrin secretion in Sertoli cells .Moreover, IL6 inhibits spermatogenesis and reduces sperm motility. Increase levels of IL6 in seminal plasma have been reported to be associated with infertility [(4)].

TNFα has been reported to be secreted by germs cells. By using a bioassay, northern blot analysis and in situ hybridization.[(5)] Have shown that spermatids (round) and pachytene spermatocytes produce TNFα. Somatic cells do not appear to produce TNFα, but further studies are required to confirm this observation. Human seminal plasma contains both TNFα and soluble TNFα receptors [(6)].

The presence of TNFα receptors has been demonstrated in Leydig, and Sertoli cells TNFα receptors in the testis are probably under the control of growth factors and hormones. TNFα receptors are enhanced by FSH, EGF and bFGF [(7)].

Aims of study

This study was conducted to evaluate the relationship of varicocele with other potential immunological and hormones parameters in male infertility by using the fallowing objectives:

1. Estimation of Interleukin-6 (IL-6), and Tumor necrosis factor (TNF) in seminal plasma.
2. Study of correlation between concentration of IL-6 and TNF , in seminal plasma with seminal fluid parameter include sperm count , sperm motility and semen viscosity .

METHODOLOGY:

This study was performed during the period from First of April 2014 to First of June 2014.All planned investigation was carried out on 75 infertile subjects with varicocele. These subjects were selected from people attended the specialized center for infertility in Al-sadder medical city in Najaf and satisfied the full requirements of the study these compared with 25 healthy men as control group subjects. Careful history was obtained from patients including age, duration of married, varicocele .

The selections of subjects for this study depended on much exclusion include.

- Patient with cancer, Hypogonadism , Testis Injured and Patient and that previously infected with gonorrhea.

For each patient the following test were carried out

- Routine seminal fluid analysis (SFA) .by using machlar chamber for determent sperm concentration (sperm/ml) and percentage sperm progressive motile
- Estimated concentration (IL-6) and (TNF) in seminal plasma by special kits of – Enzyme –Linked – Immune- Sorbet - Assay ELISA

Seminal fluid sample

Semen sample collection and delivery.

The following instructions for sample collection and delivery were based on WHO .2010 recommendations

1- The sample was collected after a minimum of 48 hours and no longer than 7 days of sexual abstinence.

2- Two semen samples were collected for initial evaluation.

3- Ideally the sample was collected in the privacy of a room near the laboratory. If not, it should be delivered to the laboratory within 1h after collection.

4- The sample was obtained by masturbation and ejaculated into a clean, wide- mouthed glass or plastic container.

5-Ordinary condoms must not be used for semen collection because they may interfere with the viability of spermatozoa.

6- Incomplete samples were not analyzed, particularly if the first portion of the ejaculate is lost.

RESULTS:

Table (1) Percentage of elevation Interluekin-6 and Tumor necrosis factor in seminal plasma of infertile men with varicocele

Studies parameter	Percentage %	P Value
Interleukin-6	42%	Sign <0.05
Tumor necrosis factor	41%	Sign < 0.05

Table (1) Also shows the percentage of elevated concentration of IL-6 was (42%) in seminal plasma, Also table (1) referred the percentage of elevated concentration of TNF was (41%) in seminal plasma.

The IL-6and TNF levels in SP were measured by ELISA test. Based on information from kits. The normal value of IL- 6 and TNF concentration in SP. IL-6 was 7.8 – 10.0 pg/ml and TNF was 4.6 – 12.4 pg/ml.

Table (2) Correlation between increased concentrations of IL 6 in SP and semen parameters.

Parameter studies	M±SD	R	P value
IL 6	17.1±10.6		
Sperm motility	37.4±20.3	- 0.58	Sign <0.05
Sperm count	34.3±10.6	- 0.30	Sign <0.05

M = mean
SD= standard division
R = correlation coefficient

Table (2) shows the elevate concentration of IL-6 in SP has strong inversely correlation R = (- 0.58) with sperm motility, Also this table was shown the elevate concentration of IL 6 in SP has inversely correlation R = (- 0.30) with sperm count this correlation.

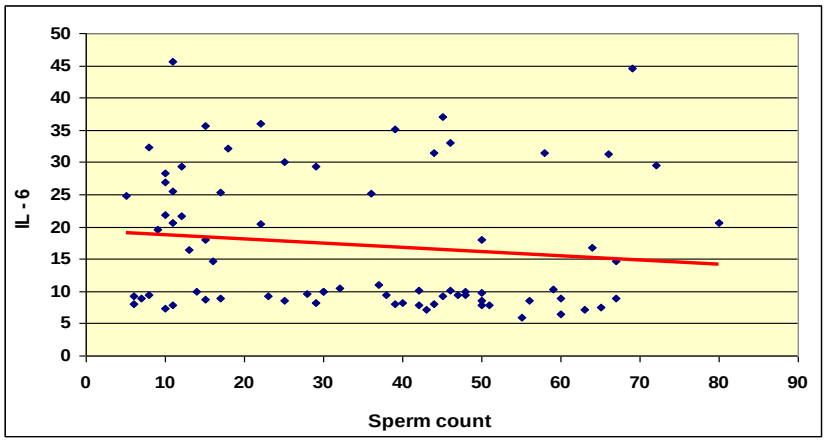

Figure (1) Correlation between the concentration of IL6 in SP with sperm concentration in infertile men with varicocele .

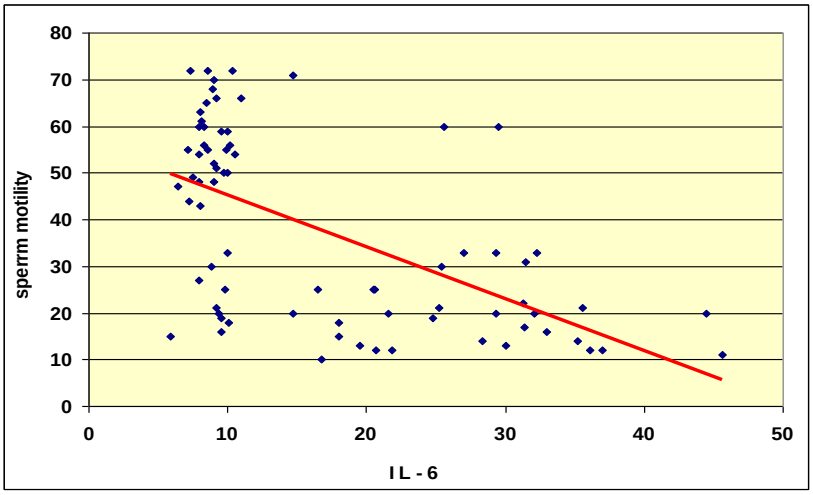

Figure (2) Correlation between the concentration of IL6 in SP with sperm motility in infertile men with varicocele .

Table (3) Correlation between increased concentrations of TNF in SP with seminal fluid parameter.

Studies parameter	M±SD	R	Tc	Tt	P value
TNF	18.8±10.2				
Sperm motility	37.4±20.3	- 0.17	1.47	1.96	No sign
Sperm count	34.3 ± 20.6	- 0.25	2.2	1.96	sign

M = mean
SD= standard division **Tt = t-table**
R = correlation coefficient **Tc= t-calculate**

Table (3) was reflected the correlation between increased levels of TNF in SP with seminal fluid parameter.

From table (3) No significant difference and correlation R= (– 0.17) between elevated TNF levels in SP with reduce sperm motility. Also table (5) reflected significant difference and inversely correlation R= (- 0.25) and between elevated TNF levels in SP and low sperm count.

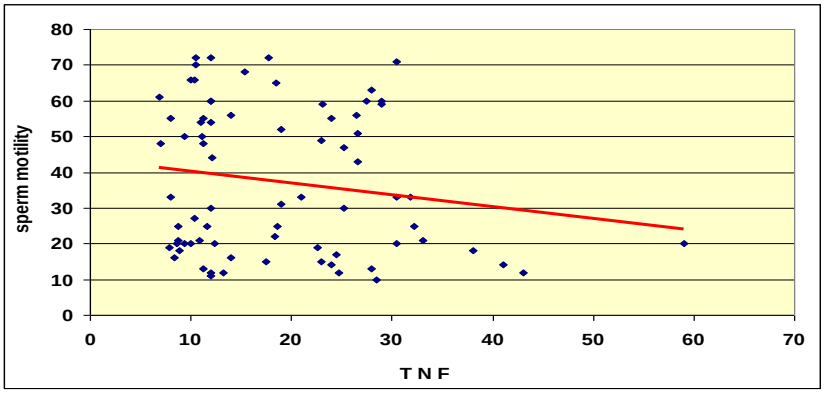

Figure (3) Correlation between the concentrations of TNF in SP with sperm motility

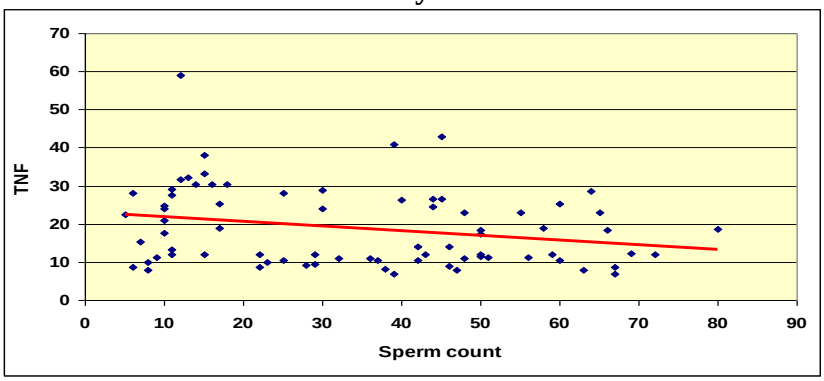

Figure (4) .Correlation between the concentrations of TNF in SP with sperm count.

DISCUSSION

Correlation between elevated concentration of IL-6 and seminal fluid parameter.

The data obtained from table (1) shown the percentage of increased concentration. Of IL 6 in SP was (42%) of infertile men with varicocele and that has significant difference (P value < 0.05) when compared with control subjects. This results were in agreement with observation of [8] the seminal fluid of subfertile men with varicocele were shown to contain increased concentration of IL 6 and decreased concentration of transferrin .

Table (2) reflected the effect of increased levels of IL 6 in SP on the sperm motility and sperm count . The IL6 secreted from sertoli cells of testis [9] Table (2) Shown that there was an inversely correlation between high level of IL-6 in SP and reduced sperm count. Increased IL- 6 amount cause inhibition of the production of sertoli cells transferrin. Transferrin is responsible for transportation of Iron through the blood- testis barrier. Iron one of main element that stimulated dividing spermatocyte and spermatide **[10].**

The present study was in agreement with that found by **[11].** Decreased Iron supply as consequence of transferrin deficiency , result in decreased cell division and caused reduce in sperm count or oligozoospermia .

Also table (2) explained the correlation between high levels of IL6 in SP with reduce sperm motility (R = - 0.58) in strong inversely correlation , this correlation has significant difference (P value <0.05) when Compared Tc with Tt . IL-6 inhibits spermatogenesis and reduces sperm motility **[12].**

Our study data was in agreement with **[13].** they observed that the elevate concentration of interleukin -1 , interleukin -6 and soluble IL-6 receptors in seminal plasma was associated to(Asthinozoospermia).

The tumor necrosis factor(TNF) family includes two structurally and functionally-related protein , TNFα / cahectin and TNFβ/ lymphotoxin TNFα is synthesized as a preprotein . TNFα has been reported to be secreted by germs cells including spermatid and spermatocyte, human SP contains both TNFα and solubleTNFα receptors **[13].**

TNFα receptors has been demonstrated in Leydig and Sertoli cells, TNFα receptors are enhanced by FSH and growth factor EGF and bFGF [14] .

Depended on the obtained results from table (1) .The (41%) percentage of elevate TNF levels in SP of infertile men with varicocele and this Percentage has significant difference (P value < 0.05) when compared with control subjects . But the data obtained from table (3) was explained that there was no significant difference (P value > 0.05) correlation R (– 0.17) Between elevated levels of TNF in SP with sperm motility .

The motility of sperm means the movement or swimming of sperm in semen this process controlled by many enzymatic system persist mostly in accessory sex gland. The present study was in agreement with finding of [15] in which the presence TNF and IL2 in seminal plasma were not correlated with sperm motility .Whereas [16] .found that the ability of TNFα to cause a reduction in sperm motility is controversial . The Eiserman believed was incompatible with result of present study.

Also table (3) shown the significant difference (P < 0.05) and inversely correlation R (-0.25) between elevated TNF levels in SP with low sperm count . TNFα receptors has been demonstrated in Leydig and Sertoli cells. [17]From other hand the spermatogenesis initial in seminiferous tubules under action of Leydig and Sertoli cells therefore , can be shown a negatively correlation between TNFα and sperm count .

The result from present study was in agreement with finding by [18] the found the administration of TNFα to male induce a dramatic decline in testosterone levels and provokes sever seminiferous epithelium damage .

Also the present study was agreed with [19] data in which , the sertoli cells Insulin – Link growth factors(IGF-I) a stimulating factor for both steroidogenesis and germ cell development , the action of TNFα in testis which result to reduces FSH action on Aromatase activity and inhibin in sertoli cells , decreased in testosterone levels and reduces(IGF-I) bioavailability , Finally the TNFα has inhibitory effect on testicular function [20].

CONCLUSION

There was a significant correlation between increased levels of Interleukin-6 and Tumor necrosis factor in seminal plasma with reduce sperm concentration and motility .

RECOMMENDATIONS:

The study recommended that:

1. Varicocele examination and diagnosis should be done to all infertile male.
2. Varicocele patients with abnormal seminal fluid examination should be estimated concentration of Interluekin-6 and Tumor necrosis factor in seminal plasma.

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