



RESEARCH ARTICLE

Evaluation of Seminal Plasma Chitotriosidase-1 Levels in A Samples of Iraqi Oligoasthenoteratozoospermic Infertile Men with & Without Varicocele

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ABSTRACT

Fertility problems nowadays was experienced in about 15% of couples of child-bearing age. In almost 50% of cases the identified cause is a male factor, as demonstrated by abnormal values of semen parameters according to WHO guidance, the main tool for the evaluation of male factor infertility is standard semen analysis. This study aimed to assess seminal plasma levels of Chitotriosidase-1 in infertile men mainly oligoasthenoteratozoospermic (OAT) patients with and without varicocele Patients. cross-sectional study was enrolled from July 2023 to June 2024 at Male Infertility Clinic of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University. Serum. Patients were divided into 2 groups, 40 with varicocele (unilateral or bilateral), 40 without a history of varicocele, and 20 fertile men as a control group who recently father for children. FSH, LH, Prolactin, and Testosterone, with history of primary or secondary infertility were measured and seminal plasma collected samples for Chitotriosidase-1. Our data revealed that there were no significant differences in serum gonadotropic hormone levels between the groups. However, infertile individuals with varicocele had lower sperm concentration, progressively motile sperms, and normal morphology sperms, as well as a higher percentage of immotile sperms compared to those without varicocele. Additionally, seminal plasma chitotriosidase-1 levels were significantly higher in patients with varicocele compared to those without, with the lowest levels found in the control subjects. Chitotriosidase-1 levels were higher in infertile males with varicocele as compared with that without varicocele.

INTRODUCTION

Male fertility is evaluated by standard semen analysis, which describes the sperm count, motility, and morphology. However, the WHO guide does not provide a complete explanation of what can and influences male reproductive potential. Semen analysis could be a good beneficial measure in both clinical and research settings, for the evaluation of male fertility status as well as monitoring spermatogenesis during, and following male fertility regulations and other interventions (1)

Male infertility can be classified according source as Male infertility could be either Primary infertility or Secondary infertility another classification was according to the pathophysiological changes in the male genital tract and other organs influences its function (2). Varicocele has been considered as one of the most correctable causes of male infertility, and highly treatable disease observed in 30-40% of men (3). Varicocele can cause direct cell damage might affect germ cells via increase intratesticular temperature, hypoxia, and increase oxidative stress that lead to impaired testicular tissue function, therefore the surgical treatment, and / or antioxidants supplements are not completely effective to improve seminal fluid parameters (4,14)

Diagnoses of varicocele was depended on two factors, firstly History of infertility state such as how many years with, is it primary or secondary, age and fertility status of female partner, presentation only with infertility, presence of pain, swelling, or any genital tract abnormalities; secondly Physical examination to the infertile man, and evaluation of varicocele done clinically according to clinical varicocele grading (5). The clinical grading system for varicocele has been as follow: Grade1: Small, not visible, hard to detect without a medical professional. Grade2: Moderate, not visible, felt while standing. Grade3: Large, easily visible through the scrotum, easily felt while standing (6).

Medical treatment: recommended for low grade subclinical varicocele, and subnormal semen parameters, give Antioxidant and vitamins (7,15) while Surgical treatment: varicocelectomy is the gold standard treatment of varicocele (8). Chitotriosidase-1 is one of family members of chitinase enzymes, synthesized by the activated macrophages in response to inflammatory processes. There is few evidence showing that serum Chitotriosidase-1 (CHIT1) activity is increased in patients with newly diagnosed, untreated, and uncomplicated type 2 DM (T2DM) (9). This study aimed to assess seminal plasma levels of Chitotriosidase-1 in infertile men mainly oligoasthenoteratozoospermic (OAT) patients with and without varicocele.

MATERIALS AND METHODS

Patients: 100 male partners attended the out-patient consultation clinic of High Institute of Diagnosis and Assisted Reproductive Technique, of age group between (20-63) years old presented with history of male infertility and subnormal seminal fluid analysis parameters. Inclusion criteria: all infertile men with and without varicocele, with subnormal seminal fluid parameters. Exclusion criteria: Azoospermic males with obstructive Azoospermia, infertility associated hypogonadotropic hypogonadism, and any other male genital tract abnormality causing infertility.

Patient groups: Eighty male of the visitors to the male infertility consultation clinic of High Institute of Diagnosis of infertility and Assisted Technologies were enrolled in this study, 1st group, 40 infertile males with history of primary or secondary male infertility with presence of varicocele unilateral and bilateral, the 2nd group, other 40 infertile males with multiple degrees of oligoasthenoteratozoospermia OAT according to the WHO guidance of SFA seminal fluid analysis parameters, and the 3rd group, 20 visitors who had normal SFA parameters and father-children before as a control.

Method: The seminal fluid samples were taken in the lab. Of the Institute after abstinence period between (3-7) days for three groups. The seminal plasma was prepared after doing direct SFA to the semen and centrifuged in 3000 gr for 8 minutes, the supernatant aspirated by pipette to the Eppendorf tube and the samples stored frozen under -20 C° to be examined later on after completing all samples collection.

The sample once taken should be stored in temperature between 27-37 degree centigrade, and standard direct semen tests, macroscopic appearance (appearance, semen volume, PH, and, liquefaction time), left for 30-60 minutes for complete liquefaction. After complete liquefaction of semen, prepare wormed slide and by a micropipette take the semen and drops of semen put on the slide and covered with pre-wormed cover slip (22x22) under light microscope for each sample.

Microscopic testing done for sperm concentration, sperm motility, sperm vitality; if motility is low, sperm numbers, sperm morphology, agglutination, non-sperm cells; leukocytes, round and germ cells.

Seminal fluid analysis done directly on semen sample collected by the patient himself after giving him instruction of how to collect whole sample of ejaculate. examine sperm abnormalities, normal SFA should be within these parameters: Volume, Total sperm count, Motility, and Morphology.

ELISA testing by using specific kits for the biomarkers we are searching for and we want to evaluate Chit-1, FSH (follicular stimulating hormone), S. LH (luteinizing hormone), S. Testosterone, S. Prolactin, and for some patients send for serum. TSH (thyroid stimulating hormone) if there was suggestive history.

RESULTS

Comparison of seminal fluids parameters between the studied groups

Our data as see in figure -1 showed that the control subjects showed significantly higher ($p < 0.001$) as compared with infertility male with or without varicocele; sperms concentration (27.05 ± 2.21 vs. 8.71 ± 0.83 vs. 8.79 ± 0.80), progressively motile sperms percent (47.05 ± 2.95 vs. 18.37 ± 2.03 vs. 18.94 ± 1.49), total PMS per ejaculate (27.20 ± 2.98 vs. 7.28 ± 1.26 vs. 7.43 ± 0.52), and morphologically normal sperms percent (26.50 ± 1.12 vs. 8.03 ± 0.80 vs. 9.09 ± 1.43).

On another hand, immotile sperms percent was significantly lower ($p < 0.001$) among control subjects (34.00 ± 2.37 vs. 59.74 ± 3.76 vs. 59.63 ± 2.69). However, there were no significant differences in non-progressively motile sperms between the 3 studied groups (21.89 ± 1.88 vs. 21.43 ± 1.62 vs. 18.95 ± 1.86 ; $p = 0.555$).

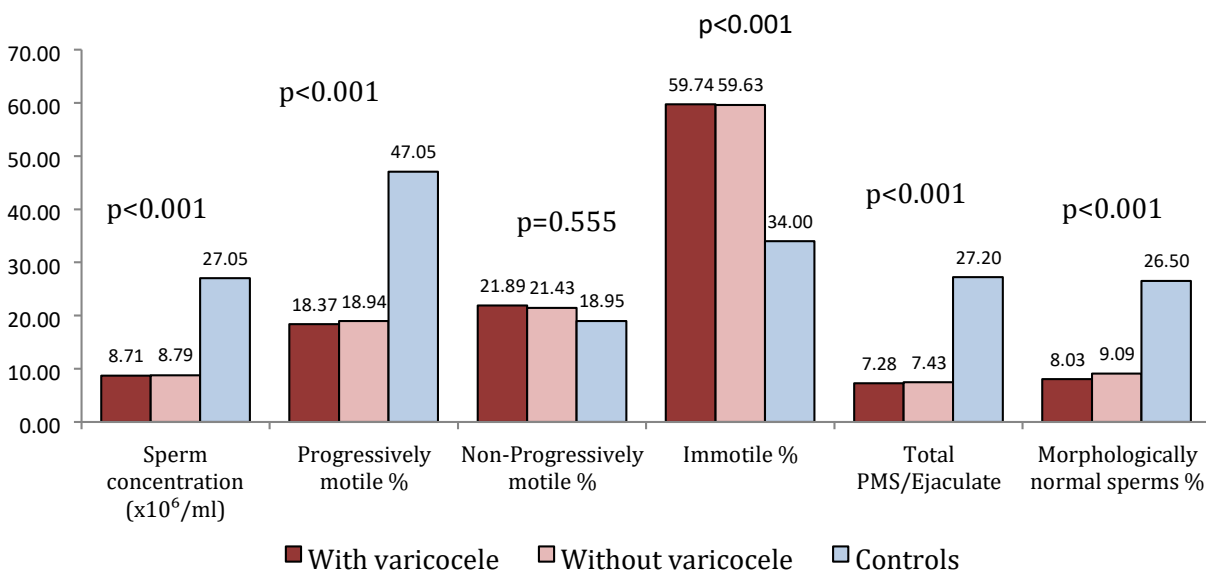


Figure 1: Comparison of seminal fluids parameters between the studied groups

Comparison of hormonal levels between the studied groups

There were no significant differences ($p > 0.001$) when Comparison of hormonal levels, FSH level (7.86 ± 1.79 vs. 8.03 ± 2.82 vs. 6.82 ± 0.60), LH (3.91 ± 0.40 vs. 3.36 ± 0.44 vs. 3.16 ± 0.29), testosterone (5.71 ± 0.46 vs. 5.92 ± 0.52 vs. 5.64 ± 0.58) and prolactin levels (16.58 ± 1.65 vs. 19.51 ± 3.85 vs. 14.58 ± 1.97) between the three studied groups as illustrated in figure 2.

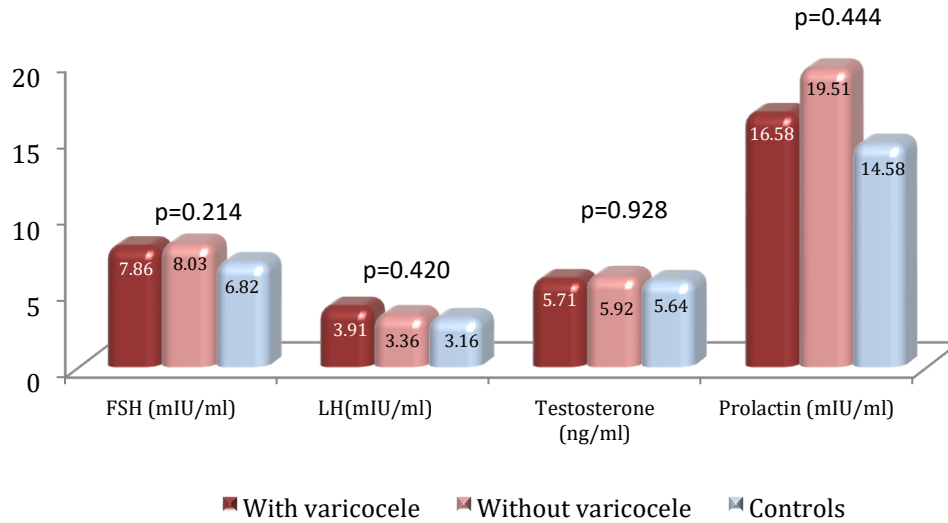


Figure 2: Comparison of hormonal levels between the studied groups

Comparison of seminal plasma chitotriosidase-1 between the studied groups

Our result showed that Seminal plasma chitotriosidase-1 level was significantly higher ($p < 0.001$) among patients with varicocele as compared that patient without and control subjects (1416.0 ± 43.3 vs. 635.7 ± 17.2 vs. 524.3 ± 35.2) and as presented in table 4-3, figure 4-3 and figure 4-3.

Table 1: Baseline seminal plasma chitotriosidase-1 levels

Chitotriosidase-1 (pg/ml)	Mean \pm SE	P value
Control	524.3 ± 35.2	< 0.001
Patients with varicocele	1416.0 ± 43.3	
Patients without varicocele	635.7 ± 17.2	

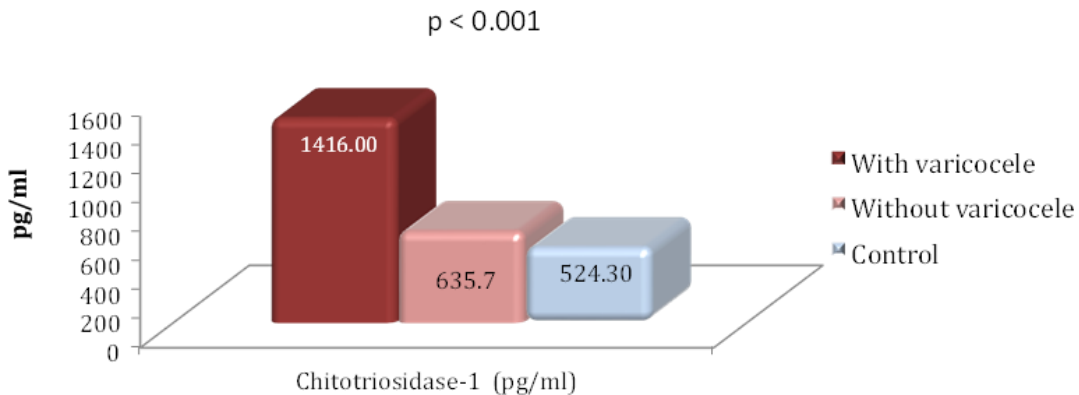


Figure 3: Comparison of seminal plasma chitotriosidase-1 between the studied groups

DISCUSSION

Male infertility has long been associated with varicoceles, even though it is still debated whether these conditions are related to sperm damage and infertility. Regarding well-known inflammatory indicators, including pro-inflammatory cytokines IL-1 and IL-6 as well as total antioxidative status

(TAS), which indicates the potential to preserve the oxidative-antioxidant balance in semen, non-standard inflammatory mediators chitotriosidase-1 reflect (10, 11)

The investigation of seminal plasma level of Chitotriosidase-1 as a potential biomarker of silent inflammation, this study revealed that seminal plasma chitotriosidase-1 mean level were 914.40 ± 46.90 . In infertile patients there were significant negative correlation between seminal plasma chit1 and sperm concentration, an evident increase in chitotriosidase-1 level with varicocele compared with those without varicocele, which indicate presence of silent inflammation in association with varicocele due to thermal effect.

Varicocele causes Leydig cell malfunction in several ways, and its degree increases with length of the varicocele. Increased scrotal temperature causes ROS generation and hypoxia; so, inhibition of enzymes involved in sexual steroid biosynthesis reduces intratesticular testosterone production and conversion (12, 13). Hormonal assay reflects the chronicity and high grade of varicocele, as varicocele damaging effect on testicular tissue cause hormonal disturbance, S.FSH, S. LH, and S. Testosterone. Hormones levels abnormality could be even a guideline for proper management of infertile men with and without varicocele, that it might associated with either high S. FSH, low S. Testosterone, which might be the real cause of impaired spermatogenesis, and subsequent infertility. Although there is no correlation between Chitotriosidase-1 level and gonadal hormones serum levels

CONCLUSION

These results point to the presence of inflammation and alteration of the testicular and epididymal microenvironment, and testicular tissue damage accompanying varicocele.

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