Evaluation of Anti-sperm Antibodies in Relevance to Testosterone Levels in Serum and Seminal Plasma in Infertile Men

Maryam H. Khaleefah, Hayder A. L. Mossa, Mohammad O. Selman
High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad-IRAQ.

Abstract:

Background: Immunological infertility is expected to be the reason of infertility in 9-36% of the concerned twosomes. The major cause of immunological infertility is the creation of anti-sperm antibodies (ASA), which influence the capability of insemination of spermatozoa. It has been detected that anti-sperm antibodies (ASA) are present either systemically in blood or locally in seminal plasma of approximately 10% of infertile male patients. Immunity to sperm can cause infertility; humoral antibodies directed against sperm did not necessarily impair fertility unless the circulating antibodies are also present within the reproductive tract and on the living sperm surface. Hormonal imbalance and sperm autoimmunity have been considered as two systems that play in near association and affect each other. Testosterone is the Steroid hormone necessary for the development and maintenance of secondary sexual characteristics as well as initiation and maintenance of Spermatogenesis .It was known that males with abnormal seminal fluids have lower concentrations of the testosterone hormone.

Objectives: To study the serum & seminal plasma antisperm antibodies levels in relevance to levels of testosterone in serum and seminal plasma accordingly to sperm function parameters in different groups of infertile patients.

Subjects,Materials and Methods: Blood and semen samples were collected from (80) subjects (60) infertile patients (20Asthenozoospermic, 20 Oligozoospermic,20Azoospermic) and 20 Normozoospermic subjects .The anti-sperm antibody and testosterone levels were measured by using Enzyme Linked Immunosorbent Assay (ELISA).

Results: The Antisperm antibodies were positive in serum of normozoospermic, asthenozoospermic, oligozoospermic and azoospermic men respectively are about 10%, 5%, 20%,5% and in the total group was 10% positive. The ASA in seminal plasma of normozoospermic men, asthenozoospermic men, oligozoospermic men and azoospermic men respectively are 0%, 0%, 5%, 5% and in the total groups 2.5%.These findings lead to no possibility to make clear correlations with the studied hormones. There was significant increase ($P<0.05$) in the levels of testosterone in serum and seminal plasma in comparison between normozoospermic and infertile men subgroups. There was significant decrease ($P<0.05$) in the levels of progesterone in serum and seminal plasma in comparison between normozoospermic men and infertile men subgroups, the highest levels were observed in asthenozoospermic subgroup. The study showed that 10 patients (12.5%) out of 80 patients showed a positive ASA positive in serum or seminal plasma of all groups. The result was considered positive if the value was ≥60 RU / ml .On these basis and regarding that screening criteria the positive ASA group showed the association with the low levels of testosterone in both types of the samples(serum and seminal plasma), these results was exhibited a high significant difference when compared with negative ASA group.

Conclusion: Determination of the relevance of the levels of testosterone and anti-sperm antibodies in the serum and seminal plasma, which in turn is important to determine the type of infertility especially the immunological type.

Keywords: Anti-sperm antibody, Testosterone,Serum,Seminal plasma,Infertile patients
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Introduction:

Infertility in general affects 10-15% of couples worldwide, in approximately 10% cases the cause of infertility remains unexplained, and the couple is designated as normal infertile couple(1). Male infertility describes a male's inefficiency to cause fertilization in a fertile female over a period of 12 months of consistent and unprotected intercourse. It is estimated that 56% of infertile couples of childbearing age seek medical help. Amidst these couples, 10%-30% of infertility cases are attributed exclusively to a problem of the male and another 15%-30% of cases showed significant anomalies in both partners (2).

Immunological infertility is assumed to be the cause of infertility in 9-36% of the concerned couples. The main cause of immunological infertility is the formation of antisperm antibodies (ASA), which affect the capability of fertilization of spermatozoa(3). Hormonal imbalance and sperm autoimmunity have been considered as two systems that work in close association and affecting each other (4). Testosterone is the Steroid hormone necessary for the development and maintenance of secondary sexual characteristics as well as initiation and maintenance of Spermatogenesis (5). It was known that males with abnormal seminal fluids have lower concentrations of the testosterone hormone (6). Testosterone synthesis is regulated by the hypothalamus and pituitary gland. Gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus, passes through the hypothalamo-hypophyseal portal circulation to the anterior pituitary where it stimulates the production of the glycoprotein hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH and LH are consequently secreted into the circulation to carry stimulatory actions to the testes.

FSH acts on Sertoli cells triggering smatogenesis and hormone synthesis, , primarily inhibin. LH binds to LH receptors on Leydig cells stimulating steroidogenesis and testosterone production(7).

Patients,Materials and Methods:

This study was carried out in the High Institute for Infertility diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, through the period from November 2017 to May 2018. The clinical examination was performed by a consultant urologist in charge of the male Infertility Unit in the Institute for all the men attended in this study. It included collection of seminal plasma and serum of 80 patients attended the male infertility outpatient clinic in the institute. The total number of infertile couples included in this study was 60 as study group (GA) which classified into three subgroups as: (Group A1=20) Asthenozoospermia (GA2=20) Oligozoospermia, (GA3=20) Azoospermia , and the group B (GB) twenty normozoospermic men (GB=20).

Blood Collection and Samples Preparation:

The blood was allowed to coagulate for 30 minutes then centrifuged at 3000 rpm for 10 minute to separate the serum. The serum was quickly frozen and stored at -36C° until hormonal assays were performed. After liquefaction step, the seminal plasma was separated by centrifugation of semınal fluıd at 3000 rpm for 10 minutes and stored at -36° C until hormonal analysis. Eighty semen samples were taken by masturbation after 3–7 days of sexual abstinence in a disposable sterile container from the patients who attended to the infertility clinic. Patient gave the specimen within special collection room performed for this purpose. Each sample collectıon was transported to the laboratory immediately and placed in an incubator at 37°C until complete liquefaction time, after that the semen samples were analyzed by check in microscopic by microscopic examination using the standardization of WHO (2010).

Hormonal and Anti-sperm antibody Assay: The testosterone hormone and ASA value in serum and seminal plasma were measured by using ELISA(enzyme-linked immunosorbent assay).

Results:

Comparison between different groups in sperm function parameters:

A comparison was made between the study groups through sperm function parameters where, the mean and SE of the sperm concentration, progressive motile sperm and morphologically normal sperm for the normozoospermic men was significantly higher than infertile subgroup (asthenozoospermic men, oligozoospermic men and azoospermic men). The mean and SE of non-progressive motile sperm, immotile sperm, round cells, agglutination for Asthenozoospermic men was significantly higher than that other infertile subgroups and Normozoospermic men, as shown in the table(1) below.

Comparison between different groups in level of testosterone in serum and seminal plasma:

The comparison between different groups in level of testosterone based on the value of mean and SE and as follows:

the mean and SE of testosterone in serum for normozoospermic men was significantly (P<0.01) higher than infertile subgroups, as shown in table (2). The mean and SE of testosterone in seminal plasma for normozoospermic men was non-significantly higher than infertile subgroups, as shown intable (3).

Comparison between ASA positive and ASA negative groups in levels of testosterone in serum and and serum.

The mean and SE of testosterone in serum for ASA positive was significantly less than ASA negative, while the mean and SE of testosterone in seminal plasma for ASA positive was significantly less than ASA negative, as shown in table (4)

Table(1): Comparison between different groups in sperm function parameters

Groups	Sperm Function Parameters (Mean ± SE)						
	Sperm Concent-ration	Progress-ive motile sperm	Non-Progress-ive motile sperm	Immotile Sperm	Round Cells	Agglutin-ation	Morpho logicall y normal sperm
Normo	63.60 ± 4.43 a	45.05 ± 1.78 a	27.60 ± 1.52 a	27.35 ± 1.63 b	8.15 ± 0.93 ab	0.50 ± 0.25 b	44.05 ± 1.51 a
Astheno	44.60 ± 3.99 b	14.95 ± 1.79 c	30.75 ± 2.74 a	54.30 ± 4.13 a	8.60 ± 1.14 a	7.75 ± 2.25 a	30.50 ± 2.28 b
Oligo	11.10 ± 0.72 c	24.20 ± 2.51 b	28.50 ± 1.10 a	47.30 ± 2.91 a	5.30 ± 0.62 c	1.25 ± 0.87 b	28.50 ± 2.33 b
Azoo	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 c	5.85 ± 0.53 bc	0.00 ±0.00 b	0.00 ±0.00 c
LSD value	8.464 **	5.021 **	4.687 **	7.483 **	2.374 **	3.474 **	3.474 **
P-value	0.0001	0.0001	0.0001	0.0001	0.013	0.0001	0.0001

** (P<0.01)

M±SE=Mean±Standard Error

Means having with the different letters in same column differed significantly.

Table(2): Comparison between difference groups in level of testosterone in serum

Group	No.	Mean ± SE
Normozoospermic men	20	4.04 ± 0.20 a
Asthenozoospermic men	20	2.64 ± 0.19 bc
Oligozoospermic men	20	3.11 ± 0.21 b
Azoosoermic men	20	2.28 ± 0.12 c
LSD value	---	0.526 **
P-value	---	0.0001

** (P<0.01)

M±SE=Mean±Standard Error

Means having with the different letters in same column differed significantly.

Table (3): Comparison between different groups in level of testosterone in seminal plasma

Group	No.	Mean ± SE ( )
Normozoospermic men	20	0.903 ± 0.03 a
Asthenozoospermic men	20	0.809 ± 0.05 ab
Oligozoospermic men	20	0.853 ± 0.05 ab
Azoosoermic men	20	0.765 ± 0.04 b
LSD value	---	0.133 NS
P-value	---	0.2058

NS: Non-Significant,Means having with the different letters in same column differed significantly.

Table (4): Comparison between ASA positive and ASA negative groups in levels of testosterone in serum and and serum

Hormones	ASA positive (Mean ± SE)	ASA negative (Mean ± SE)	P-value
testosterone in serum	1.771±0.17	3.007±0.13	0.0005
testosterone in seminal plasma	0.523±0.03	0.875±0.02	0.00001

Discussion :

Infertility affects 8-12% of couples worldwide, (8). There are many studies published regarding low sperm quality (9,10,11). The present study, take the comparisons between study groups (normozoospermic, oligozoospermic, asthenozoospermic and azoospermic males) with sperm function parameters like (sperm concentration, progressive motile sperm, non-progressive motile sperm, immotile sperm , round cells, sperm agglutination, morphologically normal sperm) . The results of this study showed the sperm concentration, progressive motile sperm in normozoospermic men are significantly (P<0.01) higher than other infertile subgroups but in both the non-progressive motile sperm and immotile sperm in infertile groups they were exhibited significantly (P<0.01) higher values than that of normozoospermic men. The summary of this part of the study is identical to each of WHO (1999) and WHO (2010) manuals (12, 13), in addition to what was listed in the above, regarding round cells this study agree with I. Ilieva, *et al* 2015 which found that the increase in the round cells in the ejaculates can be correlated with the higher percent of the abnormal gametes (14).

The current study was comparable to what was stated by former literatures in some parameters where demonstrated by both Canellada A. *et al* 2002 and Atig, F. *et al* 2012, which reported that sperm motility and sperm concentration were the worst in infertile subgroups (15, 16).

Spermatogenesis depend mainly on androgen, especially testosterone, which is produced in the empty space of the testicle by Leydig cells (17). Testosterone hormone is synthesised enzymatic transformations in Leydig cell from cholesterol which stored in an ester form in lipid droplets by LH activation that are hydrolysed of cholesterol ester hydrolase. Cholesterol by cytochrome P450scc converted to pregnenolone when LH stimulates the labile regulatory protein StAR (18, 19). In the current study the result of testosterone in serum and seminal plasma for normozoospermic men was significantly higher than other three infertile subgroups, in addition to that the testosterone comprise non-significant differences and were observed between infertility subgroups but the difference in normozoospermic men from other infertility subgroups was significant.

In view of the different levels of testosterone among the study groups, studies like Alaa S. Al-Nahi (2015), Al-Daghistani, H. I. *et al* (2010) and Omer, E.-F. E. (1985) and Luboshitzky, R. *et al.* (2002) had concluded that decrease testosterone level with the infertile groups because the abnormal spermatogenesis is often associated with altered level of gonadotropins and testosterone (20-23).

On the other hand with regard to the correlation between study groups and testosterone, Wei, T.-C. *et al* 2013 noted that a significant correlation between the serum testosterone and morphologically normal sperm as shown in present study (24), and in relation to seminal plasma testosterone , Zalata, A. *et al* 2013 found that the sperm count, sperm motility and morphologically normal sperm percentage demonstrated significant positive correlation with seminal testosterone, this study is so close with present study (25).

There are another studies support the present study like Meeker, J. D. *et al* 2006,Ismael *et al* 2017 which demonstrated that testosterone was in an association with sperm motility (26,27) .

With regard to immunological infertility (ASA), it adversely affects sperm motility (28). The ASA is determined to be positive according to a numerical value ≥60 RU / ml, the proportion of men with positive ASA from 80 men as much as was 12.5% that was detected in all study subgroups, regarding that these findings was in accordance to the findings of studies of R. Kiran *et al* 2007, Amjad H. *et al* 2007, Mohammed R.K. *et al* 2016 and Fauzia I. *et al* 2012 (4, 29- 31).

In this study which discussed the comparison between positive and negative ASA cases according to testosterone levels. Although there is one study of Rangari, K. *et al* 2013 that deals with these comparisons, it was agreed on the one hand and differ on the other hand with the current study, in the testosterone it was agreed in the trend of seminal plasma while it was not agreed in the trend of serum (32). the risk factors for developing ASA including a history of testicular injury, torsion and infection that could be relevant to the alteration of steroid hormone levels such as testosterone in the seminal plasma of males with ASA(positive ASA) that suggest the relationship between the testicular steroid hormone levels and autoimmunity to sperm antibodies(4,20).

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