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Evaluation of Anti-sperm Antibodies and Some Cytokines Profile in Seminal Plasma of Iraqi Infertile Males

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Abstract:

This study aimed to investigate the role of seminal plasma anti-sperm antibody (ASA) and cytokines (IL-2, IL-4, IL-10, IL-13, IL-17A and TNF-α) levels in the aetiopathogenesis of male infertility in a sample of Iraqi patients.

A group of males with primary infertility attending Kamal Al-Samaraie Hospital, Center of Infertility and *in vitro* Fertilization (Baghdad) and Baghdad Teaching Hospital (Infertility Clinic) during the period March-August 2010 to October 2010 were enrolled in this study, in addition, 16 fertile males (control). Based on WHO criteria of 2010 for general seminal fluid analysis, the patients were distributed into three groups: azoospermic (AZO), Oligozoospermic (OLI), and asthenozoospermic (24 patients for each group). Anti-sperm antibody (ASAs) and cytokines levels were assessed in seminal plasma fluid by Enzyme Linked Immuno Sorbant Assay (ELISA).

The mean of seminal plasma ASAs in azoospermia and oligospermia patients, as well as, controls showed no significant difference (38.7, 41.2 and 43.8 U/ml, respectively), but the three means were significantly lower than the mean (55.4 U/ml) of these antibodies in asthenospermia. when patients and control were evaluated in terms of their positivity for ASAs, the highest frequency of positive cases was observed in asthenospermia patients (41.7%) followed by controls (25.5%), azoospermia (20,8%) and finally oligozoospermia (16.7%), but these differences were not significant when each group of infertility was compared with controls.

The mean of IL-2, IL-10, and IL-17A levels in seminal plasma showed no significant difference between the four investigated groups, while the levels of three cytokines (IL-4, IL-13 and TNF-α) showed deviations in infertile patients, but such deviation was subjected to the type of investigated cytokine and type of infertility. Interleukin-4 and TNF-α were more significant in oligozoospermia patients, while IL-13 was exceptionally increased in asthenozoospermia patients, and it seemed that the other investigated cytokines had no effect on azoospermia.

Key Words: *Male infertility, Azoospermia, Oligozoospermia, Asthenozoospermia, Cytokines, Anti-sperm antibodies.*

الخلاصة:

هدفت الدراسة الحالية الى التحري عن دور أضداد النطف ومستوى الحركيات الخلوية (IL-2 و IL-4 و IL-10 و IL-13 و IL-17A و TNF-α)، في السائل المنوي في نشوء وامراضية العقم الذكري في عينة من المرضى العراقيين.

شملت الدراسة مجموعة من الذكور المصابين بالعقم الأولي والمراجعين لمستشفى كمال السامرائي لأمراض العقم وأطفال الأنابيب وبادة العقم في مستشفى بغداد التعليمي خلال المدة آذار- آب 2010 وتم انجاز الفحوصات في تشرين الأول 2010، كما اشتملت الدراسة أيضا 16 من الذكور الخصبين (سيطرة). تم توزيع المرضى الى ثلاث مجاميع استنادا للفحص السريري والتحليل العام للسائل المنوي وكانت كالآتي: اللانطفية Azoospermia (24 مريض) وقلة النطف Oligozoospermia (24 مريض) ووهن النطف Asthenozoospermia (24 مريض)، وقد توصلت الدراسة الى انه لم يظهر فرقا معنويا في أضداد النطف في البلازما المنوية لمرضى اللانطفية وقلة النطف وكذلك في أفراد السيطرة (38.7

و 41.2 و 43.8 وحدة/مل على التوالي)، ولكن حصل انخفاض في هذه المعدلات الثلاث معنويا مقارنة بمعدل هذه الأضداد في مرضى وهن النطف (55.4 وحدة/مل)، وعندما قيم المرضى والسيطرة في ضمن ايجابيتها لأضداد النطف، فقد سجل مرضى وهن النطف أعلى نسبة مئوية (41.7%) وتلاها في ذلك أفراد السيطرة (25.0%) ومرضى اللانطفية (20.8%) وأخيرا مرضى قلة النطف (16.7%)، الا ان هذه الأختلافات لم تكتسب الدلالة المعنوية عند مقارنة كل مجموعة من مجاميع المرضى مع السيطرة.

بالنسبة لمعدل مستويات الحركيات الخلوية IL-2 وIL-10 وIL-17A في البلازما المنوية للمجاميع الأربعة المدروسة، فلم يظهر أية اختلافات معنوية، في حين أظهرت الحركيات الخلوية IL-4 و IL-13 و TNF- α تغيرات معنوية، حيث ارتفع معنويا معدل مستوى IL-4 في البلازما المنوية لمرضى قلة النطف ووهن النطف (24.5 و 22.3 بيكوغرام/مل، على التوالي) مقارنة بمرضى اللانطفية والسيطرة (15.6 و 17.1 بيكوغرام/مل، على التوالي).

بالنسبة للحركي الخلوي IL-13 ، فقد ارتفع مستواه معنويا في مرضى وهن النطف (31.3 بيكوغرام/مل) مقارنة بمعدله في مرضى اللانطفية (14.0 بيكوغرام/مل)، قلة النطف (10.2 بيكوغرام/مل) أو السيطرة (13.3 بيكوغرام/مل). أظهر TNF-α أعلى معدل في مرضى قلة النطف (106.3 بيكوغرام/مل) مقارنة بمرضى اللانطفية و وهن النطف (102.0 و 100.1 بيكوغرام/مل) أو السيطرة (98.6 بيكوغرام/مل). ومن خلال دراسة نسب الحركيات الخلوية، فقد تبين ان أكثر التغايرات وضوحا هي تلك االنسب التي شملت IL-13 وتحديدا في مرضى وهن النطف وهذا ما يؤكد السبب المناعي للعقم في هؤلاء المرضى والتي ربما يؤدي فيها IL-13 دورا مهما.

Introduction:

It has been estimated that infertility affects 13% to 15% of couple ̧s worldwide, with both male and female factors being present in many of these cases and in roughly half of these cases, the defect can be traced to the males [1].

Male infertility is a multifactorial syndrome encompassing a wide variety of disorders, and in more than 50% of infertile males, the cause of their infertility is unknown (i.e. idiopathic) and can be congenital or acquired; however, several factors are involved, and they include genetics, immunological and environmental factors, in addition to hormonal imbalance [2].

Immunological factors have been suggested to be involved in the etiology of male infertility, and much concern has been focused on anti-sperm antibodies (ASAs) and cytokines, and their effects on semen quality have been questioned [3- 4].

Immune privilege in the testis is essential to maintain immunological tolerance to male germ cells during their development into spermatozoa, but immunity to sperm through the production of ASAs is thought to contribute to infertility, with 9-36% of infertility in couples being attributed to an immunological mechanism [5]. The immune privilege in the testis is maintained by blood-testis barrier, constitutive expression of anti-inflammatory cytokines and by testicular macrophages. In addition, testicular macrophages present in the interstitial tissue are thought to be essential for male reproductive function by regulating testosterone production by leydig cells [6].

It has been understood that proteins of human spermatozoa may act as auto antigens in human males. They are produced during spermatogenesis in the testis or may be attached to the sperm membrane during the passage through the seminal tract (sperm-coating antigens) [7]. The main risk factor for the development of ASAs is disruption of vas deferens, as well as, traumata of the genital tract may also induce ASAs. They can occur in blood and seminal fluid, and then attach to the sperm surface[8]. Anti-sperm antibodies may influence pre-fertilization stages of the reproduction process (sperm agglutination and/or immobilization and sperm-oocyte interactions) and they can inhibit the development of the post-fertilization zygotes[9]. They can also impair spermato-

zoal function at different stages: sperm motility, cervical mucus penetration and oocyte penetration, acrosome reaction, capacitation, and migration in the fallopian tube. Accordingly, the fertilizing capacity of human spermatozoa is impaired [10].

Cytokines are low-molecular-weight soluble protein messengers that are involved in all aspects of innate and adaptive immune response, including cellular growth and differentiation, inflammation, and repair. They act in an antigen-nonspecific manner and are involved in a wide array of biological activities ranging from chemotaxis to activation of specific cells to induction of broad physiologic changes [11].

A large number of cytokines have been identified, and many are crucial in regulating lymphocyte development and in determining the types of immune responses evoked by specific responses [11- 12].

Cytokines play a multifaceted role in the reproductive physiology of men and women [13].and those produced by T_H1 and T_H2 cells can influence sperm fertility potential [3]. In this respect, various immunologic factors, including immunoglobulins, cytokines and growth factors, have been demonstrated in human semen [14].

Increased levels of several of these factors in semen from men with genital infections suggest their involvement in immune defense of the male genital tract [15]. Furthermore, there is increasing evidence that cytokines can adversely affect spermatogenesis and steroidogenesis and there is also evidence that cytokines and other immune factors are intrinsically involved in normal reproductive physiology, and that local or systemic perturbation of these factors due to inflammation or infection can negatively affect testicular function, and as a result affect fertility [16-17].

Furthermore, they exert a significant role in reproductive processes influencing sperm cells, oocytes, sperm oocyte fusion, nidation, and implantation of early embryo [18]. In addition, cytokines have been implicated as regulators of gonadal steroid secretion, corpus luteum function, embryo development and sertoli cell seminiferous tubular dysfunction [19-20]. The present study focused on ASAs and six cytokines belong to T helper (H) 1 (IL-2 and TNF- α), T H 2 (IL-4 and IL-13), T H 17 (IL-17A) and T regulatory (reg; IL-10) cells.

Materials and methods:

Subjects:

A total of 72 males with primary infertility attending Kamal Al-Samaraie Hospital, Centre of Infertility and in vitro Fertilization (Baghdad) and Baghdad Teaching Hospital (Infertility Clinic) during the period (March - August 2010) were enrolled in this study.

They were clinically examined and evaluated by the consultant medical staff at the two hospitals, and under the supervision of this staff, information sheet (Appendix) was filled. The patients were Iraqi Arabs and their age mean ± S.E. was (30.3 ± 0.7 years). In addition, 16 fertile males (controls), matched patients for ethnicity and age (31.4 ± 1.1 years), were also included in the study, and they were husbands of wives who had fertility complications.

Examination of Anti-sperm antibodies and Cytokines:

Anti-sperm antibodies (ASAs.) samples were examined by using ELISA kit from DRG (Germany), while cytokines examined by using ELISA kits from Biovendor (Germany).

The seminal plasma level of cytokines and ASAs was analyzed using the computer programme SPSS (Statistical Package for Social Sciences) version 13.

Table-1: Seminal plasma level of anti-sperm antibodies and Cytokines in infertile patients (azoospermia, oligozoospermia and asthenozoospermia) and controls.

Groups	Azoospermia	Oligozoospermia	Asthenozoospermia	Controls
No.	**24**	**24**	**24**	**16**
Anti-sperm Ab.	38.7 ± 4.3^{B}	41.2 ± 2.4^{B}	55.4 ± 4.4^{A}	43.8 ± 4.1^{B}
IL-2	21.8 ± 4.2^{A}	22.7 ± 2.2^{A}	18.1 ± 0.9^{A}	19.4 ± 1.4^{A}
IL-4	15.6 ± 4.2^{B}	24.5 ± 2.2^{A}	22.3 ± 0.9^{A}	17.1 ± 1.4^{B}
IL-10	6.9 ± 0.1^{A}	7.0 ± 0.2^{A}	7.5 ± 0.3^{A}	8.0 ± 0.6^{A}
IL-13	14.0 ± 2.5^{B}	10.2 ± 1.9^{B}	31.3 ± 3.8^{A}	13.3 ± 3.8^{B}
IL-17	4.1 ± 0.1^{A}	3.9 ± 0.1^{A}	4.4 ± 0.1^{A}	4.3 ± 0.1^{A}
TNF-α	102.0 ± 1.5^{B}	106.3 ± 2.1^{A}	100.1 ± 0.8^{B}	98.6 ± 0.9^{B}

*Different letters: Significant difference ($P \leq 0.05$) between means.

Anti-sperm antibodies (ASAs.) samples were examined by using ELISA kit from DRG (Germany), while cytokines examined by using ELISA kits from Biovendor (Germany).The seminal plasma level of cytokines and ASAs was analyzed using the computer programme SPSS (Statistical Package for Social Sciences) version 13.

Table-2: Observed numbers and percentage frequencies of anti-sperm antibodies positive and negative in infertile patients (azoospermia, oligozoospermia and asthenozoospermia) and controls.

Groups	No.	Anti-sperm antibody status				*P≤
		Positive		Nigative		
		No.	%	No.	%	
Azoospermia	24	5	20.8	19	79.2	N.S.
Oligozoospermia	24	4	16.7	20	83.3	N.S.
Asthenozoospermia	24	10	41.7	14	58.3	N.S.
Controls	16	4	25.0	12	75.0	-

*P: probability of comparison with control; N.S. Not significant (P>0.05).

The means of seminal plasma anti-sperm antibodies (ASAs) in azoospermia and oligozoospermia patients, as well as, controls showed no significant difference (38.7, 41.2 and 43.8 U/ml, respectively), but the three means were significantly lower than the mean (55.4 U/ml) of these antibodies in asthenozoospermia (Table-1).

Results and Discussions:

The means of seminal plasma anti-sperm antibodies (ASAs) in azoospermia and oligozoospermia.The results of ASAs level in the seminal plasma of the investigate groups may high light their potential in conferring infertility in asthenozoospermia patients, and although their positivity status revealed no significant difference, there is still 41.7% of such patients were ASA-positive, and the difference might have been affected by the low sample size that has an effect on Chi-square analysis, which may lead to an underestimation of ASAs impact in infertile patients presented as

asthenozoospermia. However, the question is to what extent ASAs can cause infertility.

Antibody activity to spermatozoa has been demonstrated in males, but their clinical significance remained controversial, although considerable data from both human and animal experiences suggest that ASAs can affect fertility in a variety of ways. Mechanisms that can affect the fertility potential of a male include an effect on sperm transport, sperm capacitation and acrosome reaction, sperm–egg interaction and, lastly, a possible systemic effect under which the presence of ASAs is only reflective of a general immune activation and thus represents only one amongst many, possibly non-specific, responses [21].

With regard to sperm transport, investigations have suggested that the spermatozoon's capability to pass through the cervical mucus is affected if spermatozoa are coated by sperm antibodies. Both IgA and IgG antibody isotypes appear capable of this inhibition. Complement-mediated sperm immobilization, in contrast, appears to be mediated by IgG, IgA and IgM ASAs [22].

Spermatozoa must undergo capacitation and acrosome reaction before being able to fuse with the zona pellucida of the oocyte in the first step of the fertilization process. It has been suggested that some ASAs can induce a premature acrosome reaction, thus shortening the life span of available spermatozoa for fertilization. In contrast, other ASAs, especially in various animal models, have been shown to block the acrosome reaction and thus prevent fertilization [23-24].

Sperm-egg interaction has in recent years been quite well investigated. In various animal models sperm antibodies have been able to prevent sperm binding to zona pellucida-based receptors. In the human model the data have remained controversial. While some authors have suggested that fertilization is impaired in the presence of ASAs, many have demonstrated exactly the opposite. Human data are primarily derived from clinical *in vitro* fertilization (IVF) experiences which cannot always be equated with *in vivo* conditions[10].

Polyclonal autoimmune activation may also result in production of ASAs, and it was demonstrated indirectly that individuals with significant ASA levels concomitantly demonstrated a significant degree of non-organ-specific autoantibody abnormalities. More importantly, however, it was demonstrated that mouse ASAs, which were able to inhibit fertilization, were completely cross-reactive with some non-organ-specific autoantibodies.

This observation has led to the conclusion that some ASAs and at least some classical non-organ-specific auto antibodies can inhibit the fertilization process in identical fashion [25].

An incidence of 8.9-48% [26]. of infertile couples was quoted. Among the male patients of infertile couples, 2.5-19% was described to present ASAs in serum [27]. and 7.8–20.1% presented ASAs in seminal plasma [28]. In fertile men, ASAs were found in serum and semen, but in definitely lower frequencies [29]. and they were frequently found to bind to intracellular sperm antigens, while in the living spermatozoa, ASAs obviously bind only to cell membrane proteins [30].

Therefore, the present results shared these findings, and ASAs continue as an important immunological profile of male infertility, but the type of ASAs has to be defined in order to reach a better understanding of their role in infertility, because ASAs from both the man and woman, may be targeted against different antigens involved in various steps of human fertilization, such as acrosome reaction,

capacitation, migration in the fallopian tube, and motility [31].

Furthermore, the impairment of sperm function by ASAs in males constitutes an autoimmune disease, and in this respect, it is of relevance whether the cognate antigens are expressed specifically on spermatozoa or if they are general cell antigens, i.e. whether the autoimmunity is restricted to spermatozoa or if it may also affect other organs [32].

In a more recent investigation, a significant correlation was observed between ASA positive seminal plasma and testosterone concentration among infertile Jordanian cases, and the results suggested a relationship between testicular steroid hormone levels with autoimmunity and sperm antibodies which may influence the motility of ejaculated spermatozoa among infertile males [33].

The results of cytokine seminal plasma level demonstrated that IL-4, IL-13 and TNF-α revealed significant variation between infertile patients and controls, while no significant differences were observed in the levels of IL-2, IL-10 and IL-17A. Interleukin-4 and IL-13 are T_H2 cytokines[34].

therefore their role in humoral immunity is expected, especially in asthenozoospermia patients because such patients demonstrated an increased level of ASAs in the seminal plasma, However, the cellular source of T_H2 cytokines in the testis is unknown, and is not likely T_H2 cells, since they are absent from the testis. The likely source is from mast cells present in the interstitial space and such cells are known to produce IL-13 [34- 35].

In addition, testicular macrophages present in the interstitial tissue are thought to be also essential for male reproductive function by regulating testosterone production by Leydig cells[5] and the testosterone has been suggested to be positively associated with an increased level of ASAs in the seminal plasma of infertile males [33].

However, activation of macrophages by IL-4 was also described as the up-regulation of both the mannose receptor and MHC class II expression, and a reduction in pro-inflammatory cytokine secretion[37]. Interleukin-13, a cytokine that shares the IL-4R subunit in its receptor [38].was also shown to alternatively activate macrophages [39]. Such findings, together with the results of present study, suggest that IL-4 and IL-13 are produced in the testis and they may impact infertility in asthenozoospermia patients, especially through their effect on ASA production, because the immune privilege in the testis is maintained by the blood–testis barrier, constitutive expression of anti-inflammatory cytokins and by testicular macrophages [6].

Interleukin-4 also showed a significant increased level in the seminal plasma of oligozoospermia patients, and therefore this cytokine might be involved in reducing sperm concentration, which is an important feature in these patients. There is no direct evidence to support such finding and conclusion, but it has been suggested that the content of IL-4 in seminal plasma is closely related to male reproduction, and the increase of contents reflects the state of immunity and infection of the reproductive system, and influences sperm functions [40]. However, in a more recent investigation, cytokine levels defined as 'high' (based on the 75 percentile for each cytokine in oligoasthenospermatic men) were obtained especially for IL-8, IL-5, IL-6 and IL-10, but not IL-4 [3].

The oligozoospermia patients were further characterized by a significant increased level of TNF-α in their seminal plasma. Tumour necrosis factor-α is a pro-inflammatory cytokine that is mainly produced by macrophages in response to

foreign antigens or infection and is released as signals among cells at the early stage of innate immunity [41] and its level was demonstrated to be significantly elevated in leukocytospermic patients [42].

It has also been associated with poor semen quality and male infertility, and there is increasing evidence that TNF-α can adversely affect spermatogenesis and steroidogenesis. Furthermore, a relation between seminal polymorphonuclear cells and TNF-α, has been reported in infertile men with some effects on sperm motility, viability, and membrane integrity, that may contribute to poor fertilizing capacity of spermatozoa during inflammatory conditions[16, 19, 43,44].

These findings, together with the present results, suggest some role of TNF-α in reducing male fertility possibly through its inflammatory potentials.

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