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The using of antisperm antibody assay as a predictive diagnostic test of male infertility

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Abstract

Assessment of male infertility is the first step in resolving this worldwide problem . The aim of the present study is to investigate the predictive value of using ELISA (Enzyme Linked Immuno-Sorbent Assay) test for serum and seminal plasma Antisperm antibodies (ASA) as a complementary tool to assist in diagnosis of infertility . This study was carried out between January 2013 and November 2013 including one hundred (100) selected infertile men who attended to fertility Center in Al-Sadr Medical City . The study also included twenty (20) healthy volunteer fertile as a control . The results showed that the incidence of serum and seminal ASA in infertile men is significantly ($p<0.05$) higher than that in control fertile men. the incidence of serum and seminal ASA is significantly ($p<0.05$) higher in normozoospermic patients than that in control fertile men . There was a high significant negative correlation ($p<0.01$) between the concentration of serum and seminal ASA in infertile men and each of : sperm motility, progressive motility, while a high significant positive correlation ($p<0.01$) is seen between serum and seminal ASA in infertile men and sperm agglutination, and a significant positive correlation ($p<0.05$) with seminal WBC count . The study concluded that ELISA ASA assay can be routinely used as a complementary test to diagnose infertility .

Introduction

Infertility is a worldwide problem which affects approximately 15% of all couples and is defined as inability to conceive after one year of unprotected intercourse during the reproductive age [1] . The Primary infertility affects approximately 15% of couples, with male factor infertility accounting for 50% of cases [2] . In more than 20 % of the cases, the causes of infertility remain unexplained [3] .

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Antisperm antibodies (ASA) are the whole mark of humoral immune infertility. These antibodies are directed to various sperm antigens and implicated in sperm dysfunction. About 10% of infertile men have ASA versus 2% of normal fertile men, while some researchers noticed that the presence of ASA was significantly higher (42.5%) among patients with unexplained and persistent infertility [4] .

A remarkable percentage (about 40%) of infertile couples without any strong etiology for infertility have been shown to possess circulating antibodies capable of agglutinating spermatozoa . These antibodies are found in blood serum, seminal plasma, bound to sperm and also in cervical mucus [5] .

Theoretically, the blood-testis barrier may be breached by a variety of mechanisms resulting in exposure of immunogenic sperm antigens to the immune system which could initiate an immune response, resulting in an inflammatory reaction and ASA formation [6] .

Although not a true measure of fertility, the semen analysis is the cornerstone of male infertility assessment and may predict that the probability of achieving fertility is lower than normal [7] . Apart from the routine semen analysis, specific investigations are required to assess the functional status of sperm [8] .

This study aims to use ELISA antisperm antibody assay for serum and seminal plasma as a complementary test in the evaluation of male infertility .

Materials & Methods

1. **Study subjects** :

The study included one hundred (100) selected infertile men who attended to fertility Center in Al-Sadr Medical City . The study also included twenty (20) healthy volunteer fertile men who have one or more than one child.

2. **Semen and Serum Collection**

Semen samples were collected by masturbation , after 3–5 days of abstinence, in wide mouth disposable plastic container [9] .

The semen was centrifuged at 3000 (rpm) for 10 minutes to obtain the seminal plasma . Also a total of 5 ml of blood was obtained from the patients and control men as well and centrifuged to separate .

3. **Seminal Analysis** :

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Routine seminal analysis was achieved according to the criteria and procedures submitted by WHO [10] .

4. **ASA Assay**

The concentration of serial and seminal ASA was estimated by using ELISA kits equipped by DRG International, Inc., USA . The cutoff point for both serum and seminal plasma was 60 IU/ml (above which the sample was considered positive as fixed on the kit instruction) .

Results

1. Seminal Parameters of Infertile and Fertile Men

The values and statistical difference of seminal parameters between infertile and fertile men is illustrated in table (1) . the table indicates that sperm concentration , motility and progressive motility are significantly lower in the infertile group , while sperm agglutination , abnormal morphology and WBC count are significantly higher in the infertile compared to control fertile group .

2. Incidence of ASA in Infertile and Fertile Men

As shown in tables (2,3) , the incidence of serum and seminal ASA in infertile men is significantly ($p<0.05$) higher than that in control fertile men.

3. Incidence of ASA in Normozoospermic and Fertile Men

As shown in table (4,5) , the incidence of serum and seminal ASA in is significantly ($p<0.05$) higher in normozoospermic patients than that in control fertile men .

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Table (1) : **Major seminal parameters of infertile and fertile Men**

Parameter	Infertile Men **(No. = 100)**	Fertile Men **(No. = 100)**	P-Value
Sperm Concentration (million/ml)	40.2 ± 31.8	76.8 ± 34.6	< 0.001
Sperm Motility (%)	45.3 ± 28.8	68.2 ± 28.4	< 0.001
Progressive motility (%)	33.4 ± 26.2	62.1 ± 23.71	< 0.001
Abnormal Morphology (%)	75.65 ± 18.72	51.9 ± 12.13	< 0.001
Agglutination (%)	9.87 ± 8.41	2.31 ± 1.56	< 0.001
WBC Count (million/ml)	2.39 ± 2.75	0.64 ± 0.49	< 0.001

Table (2) : **Incidence of serum ASA of infertile and control group .**

Parameter	**Infertile Men (No. = 100)**	**Control (Fertile) (No. = 20)**	**Chi-Square (P-Value)**
Serum ASA +ve No. (%)	21 (21%)	0 (0%)	χ^2 **= 5.09** (**P < 0.05**)
Serum ASA -ve No. (%)	79 (79%)	20 (100%)	

Table (3) : **Incidence of seminal plasma ASA of infertile and control group**

Parameter	**Infertile Men (No. = 100)**	**Control (Fertile) (No. = 20)**	**Chi-Square (P-Value)**
Serum ASA +ve	23 (23%)	0 (0%)	

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No. (%)			χ^2 **= 5.69** (**P < 0.05**)
Serum ASA -ve No. (%)	77 (77%)	20 (100%)	

Table (4) : **Incidence of serum ASA in normozoospermic infertile (N) and fertile (control) Men**

Parameter	**N (Total No. = 40)**	**Control (Fertile) (Total No. = 20)**	**Chi-Square (P-Value)**
Serum ASA +ve No. (%)	10 (25%)	0 (0%)	χ^2 **= 6.00** (**P < 0.05**)
Serum ASA -ve No. (%)	30 (75%)	20 (100%)	

Table (5) : **Incidence of seminal plasma ASA in normozoospermic infertile (N) and fertile (control) Men**

Parameter	**N (No. = 40)**	**Control (Fertile) (No. = 20)**	**Chi-Square (P-Value)**
Serum ASA +ve No. (%)	11 (27.5 %)	0 (0%)	χ^2 **= 6.73** (**P < 0.01**)
Serum ASA -ve No. (%)	29 (77.5 %)	20 (100%)	

4. Correlation between SFA parameters and concentrations of serum and seminal ASA in infertile Men

As listed in table (6) , there is a high significant negative correlation ($p<0.01$) between the concentration of serum and seminal ASA in infertile men and each of : sperm motility, progressive motility, while a high significant positive correlation

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($p<0.01$) is seen between serum and seminal ASA in infertile men and sperm agglutination, and a significant positive correlation ($p<0.01$) with seminal WBC count .

Table (6) : **Correlation between seminal parameters and serum and seminal plasma ASA of infertile men**

ASA / Parameter	Serum ASA (IU/ml)	Seminal Plasma ASA (%)
Sperm Concentration (Million/ml)	r = - 0.048	r = - 0.004
Sperm Motility (%)	r = - 0.440	r = - 0.415
Progressive Sperms (%)	r = - 0.425	r = - 0.407
Abnormal Morphology (%)	r = 0.155	r = 0.181
Agglutination (%)	r = 0.395	r = 0.405
WBC Count (Million/ml)	r = 0.241	r = 0.267

Critical (r) value at 0.05 = ± 0.197
Critical (r) value at 0.01 = ± 0.256

Discussion

A conventional seminal fluid analysis (SFA) was done for both infertile and fertile groups , the results , as expected , show a high significant difference ($p<0.01$) between the two groups (table 1) .

Although the classical SFA is considered to be the main pillar in male infertility investigation , it allows only a rough estimation of the fertility status because it does not provide fair assessment for the functional status of the sperm [8] . The clinical significance of SFA is further diminished by the heterogeneity of human semen which is large enough to establish subtle variations in semen parameters between men in different geographic areas and between different times or conditions within the same subject [11] .

It was estimated that the predictive value of normal semen testing in anticipation of natural pregnancy is only 60% [12] , so that recent researches seek to

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find new predictor tools by developing and testing specific investigations that can be used to further discriminate between infertile and fertile subjects .

Many previous studies that proved a role for ASA in causing infertility [13,14,15,16] .

However, but this fact still controversial as other researchers did not find a significant difference in the incidence and level of ASA between infertile and fertile men [17,18] .

The current study recorded a relatively high rate of incidence of serum ASA (21%) among infertile population (Table 2) , this result is closely similar to the percentage of positive serial ASA (21.42%) reported in Mosul city by Shaya and Al-Dabbagh who used Tray Agglutination test (TAT) [19] , this percentage is also very similar to the results submitted by Abdulla who indicated a percentage of (20.1%) for positive serum ASA [20] , similar result obtained by TAT methods indicated that about (22.3%) of infertile male subjects have positive serial ASA [21] .

The current study also reported a high incidence rate (25%) of seminal ASA in the men infertile population (table 3), this result comes along with many previous studies that used different methods to detect seminal ASA : Al-Daghistani *et al*. performed a study on 150 Jordanian infertile men indicating about (25.34%) of them are positive for seminal ASA [22] , Abdulla reported a slight higher percentage (29.94%) [20] , while a slight lower percentage (21.42%) was reported by Shayaa and Al-Dabbagh [19] .

The result in table (4) showed that the percentage of positive serum ASA for normozoospermic patients was (25%) with a significant increase over fertile group (0%) , this result agrees with similar studies that reported a relatively similar percentage (32.1%) in the serum of unexplained infertile men [23] .

Accordingly, the results of the current study indicated that ASA differences between normozoospermic and infertile patients supports the idea of using ASA assays as predictive tools to diagnose infertility since they can further discriminate between infertile and fertile subjects .

One of the aims of the present study is to investigate the relationship between conventional SFA and some immunological procedures which are simple and non-expensive , so that they can be used as complementary specific diagnostic tests for infertility investigation .

Table (6) shows the correlation between serum and seminal ASA and each of sperm concentration, sperm morphology, ejaculate volume total sperm motility, progressive sperm motility and sperm agglutination , and finally seminal WBC count .

The effect of ASA on seminal parameters is still a conflict issue, some studies reported strong correlation between the two [24, 25] , but other researchers reported that there is little evidence that suggests a cause/effect relationship between ASA and the abnormality of semen parameters [4] .

ASA may decrease the motility of spermatozoa through agglutination and immobilization or interfering with sperm mucus interaction , thereby inhibiting sperm migration through the female genetic tract [26] .

It was found that both infection and sperm antigen sensitization can trigger the immune system to the formation of leucocytes, thereby forming ASA . Inflammation may lead potentially to genital tract disruption and ASA formation, while infections in the genital tract were found to be correlated with production of ASA [27] .

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الخلاصة

تعد عملية تقييم العقم عند الرجال الخطوة الأولى في طريق حل هذه المشكلة العالمية . إن الهدف من هذه الدراسة هو البحث عن الأهمية التنبؤية لفحص الإليزا (الامتزاز المناعي المقترن بالإنزيم) للأجسام المضادة للنطف في كل من المصل والبلازما المنوية باعتباره من الفحوصات المساعدة في تشخيص العقم . تم إجراء هذه الدراسة للمدة من كانون الثاني 2013 إلى تشرين الأول 2013 ، إذ تضمنت الدراسة إجراء فحص السائل المنوي والفحوصات المناعية لعينة مكونة من (100) من الرجال العقيمين الذين يراجعون مركز الخصوبة في مدينة الصدر الطبية في محافظة النجف الأشرف ، كما شملت الدراسة (20) شخصاً من الرجال الخصبين (مجموعة السيطرة) .

أظهرت النتائج وجود ارتفاع معنوي ($p<0.05$) في نسبة الأجسام المضادة وتركيزها في كل من المصل والبلازما المنوية في الرجال العقيمين مقارنة مع الرجال الخصبين ، كما بينت الدراسة أن نسبة الأجسام المضادة في مجموعة (أسوياء النطف) مرتفعة ارتفاعاً معنوياً ($p<0.05$) مقارنة مع الرجال الخصبين .

كما بينت النتائج وجود علاقة عكسية ذات دلالة إحصائية عالية المعنوية ($p<0.01$) بين كل من تركيز الأجسام المضادة في المصل والمني وبين كل من تركيز النطف وحركة النطف والحركة التقدمية للنطف ، كما أظهرت النتائج وجود علاقة طردية ذات دلالة إحصائية عالية المعنوية ($p<0.01$) بين تركيز الأجسام المضادة في المصل والمني وبين نسبة التلازن في النطف ، وأوضحت النتائج أيضاً وجود علاقة طردية معنوية ($p<0.05$) بين تركيز الأجسام المضادة في المصل والمني وبين عدد خلايا الدم البيض في المني .

استنتجت الدراسة أن فحص الإليزا (الامتزاز المناعي المقترن بالإنزيم) يمكن أن يستعمل في المختبرات كأحد الفحوصات المساعدة في تشخيص العقم عند الرجال .

استعمال فحص الأجسام المضادة للنطف كأحد الاختبارات التشخيصية التنبؤية لحالة العقم عند الرجال

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