Study of the Immunological State of Patients with Idiopathic Male Infertility

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Abstract

Unexplained infertility (Idiopathic infertility) when the pathogenesis of infertility is not yet elucidated it is often associated to immunologic factors. This condition may be a result of auto immunity of man and woman. Certain criteria have been applied to select the idiopathic infertile males (n=34) enrolled in this study among the total patients attended the infertility center in Babylon. All these patients were with normal couples. Apparently healthy aged matched fertile men were selected as a control sample (n=11). Circulating immunoglobulin concentrations (IgM, IgG, IgA) and complement components C3 and C4 were evaluated by applying Mancini single radial immunodiffusion technique for an idiopathic infertile patients and control involved in this study. Phagocytic activity as well as intracellular killing was done for both infertile patients and control. T-cell % test was also done by E-rosette technique. It was found that infertility was associated with an increase in IgG concentration more than normal. Both IgM and IgG concentrations among azospermic and normospermic patients revealed an increase more than normal level. According to the universal classification of semen abnormalities (variables) it was found that in azospermic group patients a high significant difference in T-cell % and intracellular killing compared to control. In conclusion . The result of immunoglobulin classes and complement components would not be a great assistance in the screening of an idiopathic male infertility although the level of IgG is more than normal level and control. While the result of cell mediated immune parameters such as T-cell % and intracellular activity of neutrophil might be refer to the under line cause of idiopathic infertility, so the further study of cluster of differentiation (CDs), T cell subset and Lymphokines is recommended for idiopathic infertility in combination with Antisperm Antibodies.
Introduction
Infertility is the inability of couples to conceive after a period of 12 months of intercourse without use of contraception [2], this public health problem involves all regions of the world.

Male reproductive failure has become an increasingly important factor in infertility during recent decades, too little is known about the reasons and pathogenesis of deficient spermatogenesis because the intimate mechanisms of normal spermatogenesis have been only partially elucidated.(3). A number of studies have shown that the hormone cycle affects genital tract immunoglobulins (Igs) Levels and T- cell function in both human and rhesus monkey. It was hypothesized that shift in immune cell populations occurring in response to hormone cycles are involved in the observed changes in genital tract immunity [4]. True auto immunity in man may be associated with premature testicular failure, it may be idiopathic or associated with systemic viral infection e.g. mumps [5]. Male infertility has previously been found associated with high titers of circulating antisperm antibodies. Although humoral antibodies may enter the seminal plasma as transudates, total immunoglobulins levels in semen are about one tenth lower than those seen in serum. [6]. Some of the sperm–bound antibodies are associated with complement dependent sperm immobilizing antibodies, indicating that there exist heterogeneity of sperm bound antibodies, this result might be one of the reasons for the controversy about the relationship between ASA and immunological infertility in men [7].

Complement serves as a vital link between innate and acquired immunity by augmenting the humoral response to T- cell dependent antigens and affecting the threshold of B-cell activation [8].

Concentration of immunoglobulins (IgG, IgM, IgA), complement components (C3andC4) in serum has been investigated in this study by single radial immunodiffusion, to indicate humoral response of infertile patients. The percent of T-cells lymphocytes is determined by E-rosette technique, Phagocytic and candidicidal activity of neutrophil also done on patient blood samples, to indicate the cellular immune response.

Many studies have been conducted to detect the immunological factors locally in the semen, but only very rare studies were concerned with the detection of immunological parameters in the peripheral blood among infertile patients .The aim of this study to investigate the immunological state of men with idiopathic male infertility compared with healthy fertile men.

Materials and Methods
Among a group of infertile patients attending Infertility Center /Maternity and Child Care Hospital in Babylon, a 34 male patients (age range 22-45years) were enrolled in this study. All patients were physically examined and their medical records checked. Certain criteria for patients selection were applied to this study as normal hormonal study, no genetic abnormalities, no family history of infertility and no other factors of infertility .All these patients were with normal couples. Whole blood was collected from each patient and immunological examination was determined in all patients. Each blood sample was divided into the following:-

1. Two ml of heparinized blood was transferred into a sterile plastic tube for Phagocytosis test. (20 i.u heparin /ml).
2. Two ml of heparinized blood was transferred into a sterile conical test tube for lymphocyte separation
3. There ml of venous blood was used to provide sufficient serum for immunoglobulins (IgG, IgM, IgA) and complement (C3, C4) .

Single Radial Immunodifusion (SRID) :-
Equal volumes of reference sera and sample were added to wells in agarose gel containing monospecific antisperm, antiserum, the sample diffuse radially through gel and the antigen form precipitin ring with the monospecific antiserum. The result can be calculated easily from the table of diameters provided with plates [9].

Phagocytosis of Candida:

Phagocytosis involves the ingestion of foreign materials, this ingestion can be determined by incubation of neutrophils with viable *Candida albicans*, then intracellular candida can be seen microscopically, this done by mixing patient sample (Blood) with a suitable amount of fungal suspension, incubate at 37 °C in water bath with shaking intervals for appropriate time, then capillary tube filled with this blood spin by microcentrifuge for constant time. The puffy coat layer was taken, spreaded on a slide and stained with leishman stain to measure the per cent of phagocytic cells. The slides were made from the puffy coat area of first group of capillary tubes while the second group of capillary tubes was used for Candida killing assay. [10-13].

The puffy coat from the second capillary tubes mentioned above were separated and mixed with 1-2 drops of PBS and then put at -20 °C for 2- hours in order to lyse the Neutrophil cells membrane and liberate *Candida* cells by successive freezing and thawing. An equal volume of ice–cold 0.01 % methylene blue is added to the puffy coat prior to centrifugation at 400 g for 5-10 minutes. The supernatant was removed and the cells suspended in the remaining volume and mixed thoroughly. The suspension was examined on a haemocytometer and 300 Candida cells counted and the percentage of killed candida (blue) scored. [11].

**Lymphocytes separation:**

Depending on a density gradient of lymphocyte cells, Lymphoprep is used to separate Lymphocyte cells from whole blood for in vitro testing [32]. Human thymus derived Lymphocytes (T-cells) are detected via attachment of sheep erythrocytes to specific receptors on the cell membrane, a technique known as rosetting quantitation of T- cells in a mononuclear suspension done by Jondal modification of methods employed by some other [10].

**Reporting of results:**

Reporting in both percentage and absolute number of rosette forming cells were applied. Adult normal range is 52–81% [36]. Sheep erythrocytes rosette assay labels virtually all of the T- Lymphocytes in a mononuclear layer and almost no other cells [10,13].

**Results**

**Humoral immune response:**

Humoral immunity including immunoglobulins classes such as IgM, IgG and IgA as well as complement components represented by C3 and C4 were considered as useful parameters in assessing the immunological responses in human. A single radial immunodiffusion method was applied to determine the concentration of each fraction in an infertile patient.

1. **IgM concentration (mg/ml):**

   IgM concentration showed no significant changes in results (P>0.05) compared to control. No significant correlation was calculated between IgM concentration and other immunological parameters.

2. **IgG Concentration (mg/ml):**

   IgG concentration showed a higher level than normal value and no significant difference compared to control. Also no correlation was estimated between IgG concentration and other studied immunological parameters.

3. **IgA Concentration (mg/ml):**

   IgA is considered the first line of defense in reproductive tissues. The vital role of IgA in host defense is reflected by the observation that it is the predominant Ig class produced in the body tissues. The result of IgA concentration showed below...
the control level with no significant results.

Table 1 show the results of immunoglobulins in serum of infertile patients.

<table>
<thead>
<tr>
<th>NO</th>
<th>D.S.</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgA (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>Mean</td>
<td>1908</td>
<td>1408</td>
<td>171.9</td>
</tr>
<tr>
<td>2</td>
<td>N.</td>
<td>34</td>
<td>34</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>S.D.</td>
<td>250.9</td>
<td>520</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>S.E.</td>
<td>430.3</td>
<td>183</td>
<td>13.8</td>
</tr>
<tr>
<td>5</td>
<td>P.value</td>
<td>0.292</td>
<td>0.202</td>
<td>0.313</td>
</tr>
<tr>
<td>6</td>
<td>Sign.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

(( D.S.= Descriptive statistics , N= patients number, S.D. Standard Deviation, Standard Error N.S.= Non Significant.))

Table ( 3 ) show the correlation of C3 with other parameters.

<table>
<thead>
<tr>
<th>Pearson Correlation Sig. 2 tailed Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 (mg/dl)</td>
</tr>
<tr>
<td>C3 Complement (mg/dl)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

The result shows a negative correlation between C3 with C4 and Phagocytosis, and positive correlation with intracellular killing of candida.

B. Cell Mediated Immune Parameters:-
Cell mediated immunity including T cells %, Phagocytosis and intracellular killing were considered as a useful parameter in assessing the immunological response in an infertile patients.

1. **T- Cell Lymphocytes %**: -

Determination of the percent of T-cell lymphocytes by using E- Rosette test to calculate the percentage of positive E-rosette forming cells (ERFC). The result showed a highly significant change compared to control (P<0.01). The study also revealed a highly significant correlation between T-cells % among infertile patients and complement C4 level and Phagocytosis %.

2. **Phagocytosis (Phagocytic activity of neutrophil)**:-

Phagocytic activity of neutrophil was studied by using *Candida albicans* and calculated the percent of positive Phagocytosis after incubation of viable neutrophil with Candida for 1 hour. The result was showed no significant change after compared to control samples.

3. **Intracellular Killing (Candidcidal Activity Of Neutrophil)**: -

Determination the percent of intracellular killing of *Candida albicans* ingested by viable neutrophil showed no significant change (P>0.05) of result in comparison with control samples.

<table>
<thead>
<tr>
<th>NO</th>
<th>D.S.</th>
<th>T- Cells %</th>
<th>Phagocytosis%</th>
<th>Intra.Kill. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>Mean</td>
<td>50.28</td>
<td>63</td>
<td>73.5</td>
</tr>
<tr>
<td>2</td>
<td>N.</td>
<td>32</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>S.D</td>
<td>17.4</td>
<td>12.9</td>
<td>13.4</td>
</tr>
<tr>
<td>4</td>
<td>S.E</td>
<td>3.08</td>
<td>2.23</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>P.value</td>
<td>0.01</td>
<td>0.304</td>
<td>0.21</td>
</tr>
<tr>
<td>6</td>
<td>Sign.</td>
<td>H.S</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 4 show the results of ( T- cell%, phagocytosis and intracellular killing).

<table>
<thead>
<tr>
<th>Pearson Correlation Sig. 2 tailed</th>
<th>Ig A ( mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T- cells %</td>
<td>0.578</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>

The result show highly significant difference in T-cell %and no significant in other parameters and the correlation only between T-cell % and IgA (tables 4 and 5).

**Semen variables (Nomenclatures):** -

Since it is often difficult to describe all deviations for normal semen variables with words and numbers, a nomenclature was introduced to indicate the kind of alteration. It is important to recognize that this nomenclature describes only some semen variables and does not imply any causal relationship [15]. According to this system division the obtained data of this study, statistical analysis of immunological parameters were done for each group. To compare the results of oligospermic group with the results of other semen variable groups (Nomenclatures). All these results mentioned below. The per cent of each nomenclature among all the patients involved in this study are as follows: -

1. Oligospermia = 29.4 %. (11 patient from 34)
2. Teratospermia = 26.5 %. (8 patient from 34).
3- Azoospermia = 17.6 %. (6 patient from 34).
4- Asthenospermia = 14.7 %. (5 patient from 34).
5- Normospermia = 11.8 %. (4 patient from 34).

According to this classification the results of immunological parameters appear as follow:

This table show the summarization of all studied parameters among semen variable groups (nomenclatures.). There is a highly significant result of T-cell % among all group of semen variable, also there is a significant in result of intracellular activity of neutrophil among Oligo., Terato. And Azospermia while no significant result in other group of semen variable.

**Humoral immune parameters:-**

The results of IgG and IgM in Azoospermia and Normospermia are more than normal value while the other groups are within the normal level. While the result of IgA in Azoospermia and Normospermia appeared as greater than other groups of semen variable but still with normal range.

The results of Complement Component (C3 and C4) showed that the result of C3 complement in Teratospermia and Asthenospermia appeared as below normal level while no difference in result of C4 concentration.

**Cellular immune parameters:-**

The result of T- cells % among teratospermia, Asthenospermia and oligospermia showed below the normal level and showed highly significant difference compared to control samples, while no significant difference in phagocytic activity of neutrophil among all groups of semen variables and there is significant in result of intracellular killing in Teratospermia, oligospermia and azospermia and no significant difference in result among other groups of semen variables. After compared the results with control samples.

**Discussion**

1. **Immunoglobulins in serum:-**
   
   IgG concentration showed a higher level in patients compared to control with non significant difference (P> 0.05). Also it was found that the infertility was associated with an increase in IgM concentration compared with control. The result of IgA level among infertile patients showed a slight lower compared to control group. Stanislavov et al [16] by using the seminal plasma and serum sample among selective group of infertile patients suffering from chronic nonspecific prostatitis at reproductive age, their results were matched with the results of this study, keeping in mind the present study was not specific for prostatitis. Hiroki et al [17] showed that there is no significant difference observed on albumin, alpha-1, alpha-2, Beta and Gamma, and Immunoglobulins (IgA, IgG and IgM) concentration besides no specific relationship was found between the concentration of these investigated parameters and the etiology of infertility or fertilization revealed by in vitro fertilization (IVF). Other workers Bruce et
al [18] showed that Immunoglobulins are present in a greater percentage of infertile men with varicocele than infertile men without varicocele. Munuce [19] showed that there is no association between antibodies concentration and semen quality among normospermic samples with immunological infertility, so the study suggested that antibodies would not be of great assistance in the screening of an immunological male infertility. Most agglutinating antibodies to sperm in seminal plasma are of the IgA class, an immobilizing antibodies are predominantly IgG.

2. Complement Components in serum:—

The complement levels in this study showed a slight difference in both (C3 and C4 level) compared to the control samples. D’cruz and Haas[20] suggest that human seminal plasma contain inhibitor for both the initial and the terminal portions of the complement cascade thereby protecting sperm from complement mediated injury in the male reproductive tract. An antibody effect on semen quality should involve a complement mediated sperm cytotoxicity occurring within the male genital tract, however, anticomplementary activity has been reported in human semen and it was recovered in the low M.W. of the seminal plasma (20,000-60,000 Dalton) using gel filtration chromatography. This fraction inhibited total complement activity as well as the activity of the early complement components C1 and C3, afterwards a potent inhibitor of C5b-7 complexes was identified in human seminal plasma where it was found in 5-10 fold higher concentration than in serum. Expression of CD46 (membrane cofactor of complement) on their spermatozoa confers resistance to complement mediated injury on host cells, the loss of CD46 is sperm specific, probably due to testicular germ cell specific regulation of CD46 production [21].

3. Phagocytosis of candida and intracellular killing:—

There is no significant results were showed of Phagocytosis and intracellular killing among infertile patients. There are other related studies on selected patients who revealed the importance of phagocytic cells among reproductive tract and its effect on infertility or immunological response of reproductive system. One of these related studies showed that the very few cell mediated immunity assays have been introduced, this is due to the complexity of the cellular immune system and to the fact that cellular components mediating infertility are still being defined and elaborated.[1].

I. T—cells % :-

The result of T-cell % showed a highly significant result compared to control samples. (P <0.01), and give good correlation between T-cells% and IgA level. From the other studies Reinherz et al [22] showed that there is a low correlation between T cells % and monoclonal antibodies, T cells sub populations having receptor for FC portion of IgM, IgG antibody in the interstitial spaces between seminiferous tubules exists on immunocomptent tissue (Macrophages and Lymphocytes), the existence of down regulation of cell mediated immune system at this level by the T cells suppress any immune reaction, presentation of sperm antigens to lymphocytes and small constant antigen leak through these area service to desensitize the immune system to sperm antigens. Waltraud [23] showed that marked relationship of proinflammatory cytokines with semen quality, the significant association with seminal leukocytes and other potential inflammation markers suggests that IL-8 might be used as sensitive marker for silent male genital tract infection. The reproductive tract of both men and women contain amyraid of immune response cells, activation of these cells stimulate them to secrete Lymphokines and Monokines these act at least in part locally to regulate immunological reactions but also affect tissues outside the immune system.
2. **Semen variable Nomenclatures:**

The result of immunoglobulins and Complement component among all groups of semen variable nomenclatures showed no significant changes in compared with control samples. The result of both IgG and IgM among Normospermia and azoospermia is greater than normal value and control but with no significant difference. While there is a highly significant changes in T-cell % among all groups of nomenclatures and significant difference in intracellular killing in Teratospermia, Oligospermia and azoospermia. Bruce et al [18] showed that no lymphocytes were identified in the peripheral testis, more recent studies have demonstrated in patients with oligospermia and obstructive azoospermia, T-cells of the helper/ inducer phenotype predominated in patients with unilateral testicular obstruction and in post vasectomy patients. Measurement of sperm Ab. in serum and seminal plasma correlated with these finding. Also showed that following vasovasostomy a decreased level of T-suppressor /cytotoxic cells was associated with the presence of antisperm antibody. Cellular immunity alterations were detected early and persisted for many years, while humoral antisperm antibodies were not detected during more than two years. Azoospermic patients showed auto sensitization in 50% of the cases [24].

**Conclusion**

The result of immunoglobulin classes and complement component would not be a great assistance in the screening of an idiopathic male infertility although the level of IgG is more than normal and control. The presence or absence of antispermatozoaal antibody is more recommended studying among idiopathic infertility. While the result of cell mediated immune parameters such as T-cell % and intracellular activity of neutrophil might be refer to the under line cause of idiopathic infertility, so the further study of cluster of differentiation (CDs), T cell subset and Lymphokines is recommended for idiopathic infertility in combination with ASA.

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