Estimation of IL-1 Beta and IL-18 Level in Sera and Seminal Plasma in Infertile Men in Baghdad

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ABSTRACT

Background: To evaluate the role of Interlukin-1 beta(IL-1 beta) & Interlukin-18 (IL-18) concentration in sera and seminal plasma of patients compared to fertile men.

Methods: Sixty Six male (infertile group), and 25 fertile male (control group).

Patients and control group’s seminal plasma & sera were analyzed using ELISA technique to determine IL-1 beta and IL-18.

Results: The mean of IL-1 beta levels in control group (normospermia) in sera and seminal plasma is (92.1 & 31.7) pg/mL, respectively, while the mean of IL-18 in control group (normospermia in sera & seminal plasma is (410.8 & 141.3) pg/mL.

Whereas, sera IL-1 beta in patients group is (13.2, 41.6 & 59.8), it is in seminal plasma (5.1, 13.9 & 22.4) pg/mL in azoospermia, oligozoospermia, and asthenozoospermia, respectively.

Sera IL-18 in patients group is (131.8, 267.3 & 226.6), while in seminal plasma it is (50.1, 92.7 & 81.8) pg/mL in azoospermia, oligozoospermia, and asthenozoospermia, respectively.

Conclusion: The cytokines IL-1 beta & IL-18 may play an important role in pathogenesis of male factor infertility.

INTRODUCTION

Clinical studies frequently use a one year period to exposure for defining infertility[1].

Male infertility is a multifactorial syndrome encompassing a wide variety of disorders and more than 50% of infertile males, the cause of their infertility is unknown (idiopathic) and can be congenital or acquired, however, they include several factors: genetic, immunological, and environmental, as well as hormonal imbalance [2].

Also, the most common causes of male infertility as cleared by [3]are:

1. Incomplete sperm collection and use of condom, which contain spermicidal agents.
2. Varicocele.
3. Cryptorchidism: failure of descent of the testes in the scrotum during fetal development.
4. Infection with sexually transmitted diseases.
5. Occupational and environmental factors, exposure to radiant heat, radiotherapy, irradiation, and chemotherapy.

6. Diseases of the testes such as tuberculosis and syphilis.
7. Endocrine cancers such as hypogonadotropic, hypogonadism, hyperproluctinaemin, and hyperthyroidism.

The 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[4, 5].

The cytokines play a multifactorial role in the reproduction physiology of men and women [6], and those produced by Th1& Th2 cells can influence sperm fertility potential [7].

IL-1 beta plays an important role in differentiation ofcells, it is also involved in apoptosis, as well as it takes part in physiologic control of reproductive system especially in...
MATERIALS AND METHODS
A total of a 91 individuals (66 infertile patients and 25 apparently healthy males as control group), whose ages ranged between (20-59) years, were studied. The semen specimen and sera of patients and control group were collected during (March-June) 2014. The semen specimens were collected through masturbation after 3 days abstinence in a sterile container, the samples were incubated for 30 min at 37°C for liquefaction. A routine semen analysis was performed upon liquefaction according to (WHO) [11]. The remaining of semen sample was centrifuged at 1000 rpm, then seminal plasma was separated in sterilized tubes, stored at -20°C until further analysis. Concentration motility of spermatozoa, morphology, sperms, agglutination level and leucocytes (pus cells in semen specimens) in counted in one mL of semen were evaluated.

After examining of patients and control groups’ seminal plasma, 2-3 mL of their blood also collected in plain tubes, centrifuged, and then stored at (-20°C).

Statistical Analysis:
The results were presented as mean ± standard deviation for better comparison with relevant data. Also, frequency percentage was used to describe the study variables.

Assay Procedure of IL-1 Beta by ELISA Technique:
1. All reagents and samples bring to room temperature before use.
2. 100µL of each standard and samples was added into appropriate wells, which covered and incubated at 37°C for (2.5)hrs.
3. The solution discarded and washed 4 times by multichannel pipette.
4. 100µL of the prepared biotinylated antibody was added to each well, which incubated for 1 hr. at 37°C.
5. The solution discarded and washed once again.
6. 100µL of the prepared streptavidin solution was added to each well, which incubated for 45min. with gentle shaking.
7. The solution was discarded and the washing repeated.
8. 100µL of TMB was added to each well, which incubated for 30 min.
9. 50 µL of stop solution was added to each well, and read at 450nm immediately.

Assay Procedure of IL-18 by ELISA:
1. All reagents and samples bring to room temperature before use.
2. 100µL of each standard and samples was added into appropriate wells, which covered and incubated at 37°C for (2.5)hrs.
3. The solution discarded and washed 4 times by multichannel pipette.
4. 100µLof the prepared biotinylated antibody was added to each well, which incubated for 1 hr. at 37°C.
5. The solution discarded and washed once again.
6. 100µL of the prepared streptavidin solution was added to each well, which incubated for 45min. with gentle shaking.
7. The solution was discarded and the washing repeated.
8. 100µL of TMB was added to each well, which incubated for 30 min.
9. 50 µL of stop solution was added to each well, and read at 450nm immediately.

RESULTS AND DISCUSSION
The spermatozoan count in control group was 69.1×10^6/mL, while it was significantly (P<0.05) decreased in asthenozoospermia 47.4×10^6/mL, and for oligozoospermia is 6.3×10^6/mL. The azoospermia patients showed no spermatozoa in their specimens, table (1).

Table 1: Spermatozoa Count in Infertile patients & Control Group(10^6/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Spermatozoa Count ± S.E E×10^6/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>22</td>
<td>6.3±0.79</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>26</td>
<td>47.4±1.33</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>18</td>
<td>69.1±2.02</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>54.1±2.02</td>
</tr>
</tbody>
</table>

Different letters significantly difference (P≤0.05) between means.

Table 2: Spermatozoa Motility in Infertile Groups & Control Group

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Motility Mean ± S.E %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Progressive</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>25</td>
<td>54.1±3.74</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>22</td>
<td>47.1±3.98</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>18</td>
<td>31.9±4.17</td>
</tr>
<tr>
<td>Control</td>
<td>26</td>
<td>23.1±0.85</td>
</tr>
</tbody>
</table>

Different letters: significant difference (P<0.05) between means of patients & control groups.

The number & percentage of infertile patients aging (30-39) years is 59.09% of infertile population of the studied groups(table(3)) which was found to be the highest among other age groups, with high significant differences (P<0.01).
In this study, our focus was the role of microglial cells. It indicated that an abnormal sequence of ejaculation can cause decreased sperm function. Reduced sperm motility can therefore be a symptom of disorders related to male accessory sex gland secretion and to the sequential emptying of these glands [15].

Effect of age on male infertility indicated in age group (30-39) years, which was (59.09%) of total infertile patients, because the percentage of married men are much higher in this age group than other ages, this could be because of low financial status resulted by continuous wars that taken place in our country but does not correlate to a known hypothesis, due to the fact that Iraqi families consider this age suitable for marriage, these results agree with other studies which revealed that 76% of infertile couples aging (25-34) years [16].

The present finding of progressive motility in oligozoospermia, asthenozoospermia and control group differ from the results agree with [11] guide that stated the lower reference limit for semen analysis in control groups. Therefore, the observed significant differences in progressive motility in oligozoospermia and asthenozoospermia groups compared to azoospermia and normospermia groups strongly suggests that sperm motility is an important factor related to male fertility. Furthermore, it has been suggested that sperm motility assessment can be used more directly to address problems affecting the man and his reproductive organs, the presence of inflammatory cells suggests an ongoing inflammatory reaction and in addition, the spermatzoa, which are exposed to seminal resicular fluid show decreased motility, survival & production of the sperm chromatin [14]. So, it indicates that an abnormal

Level of IL-1 beta is (P<0.05) significantly lower in azoospermia sera than normospermia, asthenozoospermia, and oligozoospermia sera (P<0.01), while the level of this interleukin was lower in oligozoospermia seminal plasma than normospermia, asthenozoospermia, and oligozoospermia seminal plasma, table (4).

Different Letters: significant difference (P≤0.01)& (P≤0.05) between means of sera and seminal fluid. Table (5) shows that IL-18 level is lower in azoospermia sera than normospermia, asthenozoospermia, and oligozoospermia sera (P<0.01), whereas, the level of this interleukin is lower in azoospermia seminal plasma than normospermia, asthenozoospermia, and oligozoospermia seminal plasma (P<0.01).

Different Letters: significant difference (P<0.01) between means of fertile and infertile.

The spermatzoan concentration correlates with pregnancy rate and time of pregnancy [12], and is a predictor of conception [13]. The observed significant differences between asthenozoospermia, oligozoospermia and control group agree with [11] guide that stated the lower reference limit in each group, as well as the control fits the obligation of such guide. Therefore, the observed significant differences in spermatzoan concentration between oligozoospermia, asthenozoospermia, and control group are expected.

The present finding of progressive motility in oligozoospermia and asthenozoospermia patients strongly suggests that sperm motility is an important factor related to male fertility. Furthermore, it has been suggested that sperm motility assessment can be used more directly to address problems affecting the man and his reproductive organs, the presence of inflammatory cells suggests an ongoing inflammatory reaction and in addition, the spermatzoa, which are exposed to seminal resicular fluid show decreased motility, survival & production of the sperm chromatin [14]. So, it indicates that an abnormal

### Table 3: Distribution of Infertility According to Age

<table>
<thead>
<tr>
<th>Age Range Group/Year</th>
<th>No.</th>
<th>%</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>5</td>
<td>7.57</td>
<td>0.01 Significant differences</td>
</tr>
<tr>
<td>30-39</td>
<td>9</td>
<td>13.63</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>13</td>
<td>16.69</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>9</td>
<td>13.63</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: IL-1 Beta Level in patient and Control Groups’ Sera and Seminal Plasma

<table>
<thead>
<tr>
<th>Studied Cytokine</th>
<th>Studied Groups Mean±S.D pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Normospermia</td>
</tr>
<tr>
<td>IL-1 Beta Serum</td>
<td>92.1±6.3 S</td>
</tr>
<tr>
<td>IL-1 Beta Seminal Plasma</td>
<td>31.7±1.7 S</td>
</tr>
</tbody>
</table>

### Table 5: IL-18 Level in patient and Control Groups’ Sera and Seminal Plasma

<table>
<thead>
<tr>
<th>Studied Cytokine</th>
<th>Studied Groups Mean±S.D pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Normospermia</td>
</tr>
<tr>
<td>IL-18 Serum</td>
<td>410.8±11.9 S</td>
</tr>
<tr>
<td>IL-18 Seminal Plasma</td>
<td>141.3±7.9 S</td>
</tr>
</tbody>
</table>
REFERENCES


