Serum Testosterone and Dehydroepiandrosterone-Sulfate (DHEA-S) Level among Oligospermia and Azoospermia Patients

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Abstract: Currently, we aimed to assess serum testosterone and DHEA-S among oligo and azoospermia patients. The study included 60 patients with oligospermia, 60 with azoospermia and 60 normospermia as control. Serum testosterone and DHEA-S levels were measured using competitive ELISA. BMI was calculated from weight/kg divided height/m². The results showed mean serum testosterone levels were lower in oligospermia (p-value 0.081) and azoospermia (p-value 0.034) in comparison with control normospermia, no difference observed in DHEA-S. Pearson's correlation analysis had shown significant negative correlation of testosterone with BMI (r= -0.300, p-value 0.020), DHEA-S had a significant positive correlation with BMI (r= 0.279, p-value 0.031). Present study has shown negative correlation of testosterone with age, although statistically insignificant (r= -0.240, p-value 0.062). The study has concluded that, testosterone levels are lower in oligo and azoospermia compared with normospermia, age and overweight are causative factors of testosterone deficiency, thus could affect spermatogenesis, which may result in infertility especially among oligospermia.

Keywords: Testosterone, DHEA-S, Oligospermia, Azoospermia, Male infertility

I. Introduction

Oligospermia is generally defined as less than 20 million spermatozoa per one ml of ejaculate. While Azoospermia defined as complete absence of sperm from the ejaculate, is present in about 1% of all men and in approximately 15% of infertile men. Oligospermia and azoospermia are one of the main causes of male infertility or sub-fertility [1,2].

Approximately 8–15% of couples are unable to conceive after 1 year of unprotected intercourse. A male factor is solely responsible in 20% of infertile couples and contributes in another 30-40% of couples. According to recent study, In Sudan male factor represents 36.2% of infertile couples. A male infertility factor is often defined by abnormal semen parameters but may be present even when the semen analysis is normal, semen analysis is the cornerstone of the laboratory evaluation of the infertile man and helps to define the severity of the male factor [3-5].

Dehydroepiandrosterone (DHEA) is the main adrenal androgen, which mostly exists in a sulfated version (DHEA-S). Both DHEA and DHEA-S are metabolic intermediates in the biosynthesis of the male sex hormone testosterone [5-7].

Testosterone is the main androgen secreted by the testes, it is essential for normal sperm development, therefore any disorder that result in hypogonadism and hence low testosterone concentration result in infertility [8-10].

Most recent studies indicate that the serum level of testosterone is significantly lower in samples from patients with abnormal sperm characteristics than in men with normozoospermia. Other studies concluded that there is no significant difference in the mean levels of testosterone between patients and control group [12, 13]. Also there are some previous studies demonstrated hormone levels in serum and seminal plasma of men with azoospermia, which concluded that there were no significant differences between azoospermia and control group in the serum concentrations of dehydroepiandrosterone sulfate [14].

Accordingly we hypothesized that, there are an association of androgen hormones and sperm count, age and body weight, thus may contribute to pathogenesis of infertility.

II. Materials and Methods

This is a cross-sectional and hospital based study was carried out in 180 men, they were classified as oligospermia (N=60), azoospermia (N=60) (age group 20-50 years) and normospermia (N=60) as matched control males (age group 24-50 years). All patients had infertility for 2 years and more, they were referred to Al-Khalifa suliman specialized hospital in Khartoum state during period from October to December 2015. DM and Patients received hormonal therapy were excluded from this study.

DOI: 10.9790/0853-1504146265 www.iosrjournals.org 62 | Page
Serum Testosterone And Dehydroepiandrosterone-Sulfate (DHEA-S) Level Among Oligospermia

Samples collection
Semen ejaculate was collected from each subject in sterile plastic specimen container after three days of sexual abstinence. Blood samples were collected from various groups under septic condition (70% alcohol), serum was obtained after centrifugation at 3000 rpm for 5 min and kept at -20°C till used. All patients and controls had consented for the use of blood and semen samples for clinical research, and ethical norms were approved from Al-Neelain University Ethical Committee.

Sperm count
Semen (10 µl) suspension was diluted with sperm-counting diluent (sodium acid carbonate-formaldehyde solution), then examined under microscope using Neubauer counting chamber according to the procedure indicated in the WHO laboratory manual.

Estimation of Testosterone and DHEA-S
According to the manufacture, serum levels of Testosterone and DHEA-S were measured by using TOSOH Bioscience automated immunoassay analyzer AIA-360. Competitive immunoenzymometric assay was used, testosterone and DHEA-S in the test sample compete with enzyme-labeled testosterone and DHEA-S for limited number of binding sites on these hormones specific antibodies immobilized on magnetic beads, then magnetic beads were washed to remove unbound enzyme-labeled testosterone and DHEA-S, and incubated with fluorogenic substrate 4-methylumbelliferyl phosphate (4 MUP). The rate of fluorescence produced by the reaction was measured fluorometricly at 365 and 445 nm. The amount of testosterone and DHEA-S that binds to the beads is inversely proportional to the testosterone and DHEA-S concentration in the sample.

Statistical analysis
Data analysis was performed using SPSS version 20 software. Descriptive statistics on subject demographics were calculated, Mean, SD and Standard error of the mean, t-test and ANOVA were employed to compare mean concentration. Pearson's correlation was applied to correlate between study variables.

III. Results
The percentage (%) showed equality of age (<35years and >35years) within oligo and azoospermia. The overweight was more prevalent in both oligospermia (60.0%) and azoospermia (63.3%) is presented in table 3.1. The mean concentrations of testosterone were significantly decreases in both oligospermia (p-value 0.081) and azoospermia (p-value 0.034) in comparison with control group is presented in fig 3.1. While insignificant increases were observed in mean concentration of DHEA-S among oligospermia (p-value .0) and azoospermia (p-value 0.) versus control group is presented in fig 3.2. In present study serum testosterone inversely correlated to the age and BMI (r=-0.240, p-value 0.062) and (r=-0.300, p-value 0.020) respectively, while DHEA-S positively correlated with age (r=0.279, p-value 0.031) all are presented in fig 3.3.

Table 3.1: Frequency of age and BMI among oligo and azoospermia

<table>
<thead>
<tr>
<th>Status</th>
<th>Age/years</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligospermia</td>
<td>&lt;35</td>
<td>46.6%</td>
<td>53.3%</td>
</tr>
<tr>
<td></td>
<td>&gt;35</td>
<td>53.3%</td>
<td>46.6%</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>&lt;35</td>
<td>43.3%</td>
<td>56.6%</td>
</tr>
<tr>
<td></td>
<td>&gt;35</td>
<td>56.6%</td>
<td>43.3%</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligospermia</td>
<td>&lt;26</td>
<td>40.0%</td>
<td>60.0%</td>
</tr>
<tr>
<td></td>
<td>&gt;26</td>
<td>60.0%</td>
<td>40.0%</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>&lt;26</td>
<td>36.6%</td>
<td>63.3%</td>
</tr>
<tr>
<td></td>
<td>&gt;26</td>
<td>63.3%</td>
<td>36.6%</td>
</tr>
</tbody>
</table>

Results expressed as percentage (%)

Fig. 3.1: Mean testosterone level among study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligospermia</td>
<td>377±42.88</td>
<td>0.081</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>355±43.82</td>
<td>0.034</td>
</tr>
<tr>
<td>Control</td>
<td>511±40.53</td>
<td></td>
</tr>
</tbody>
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DOI: 10.9790/0853-1504146265 www.iosrjournals.org 63 | Page
Serum Testosterone And Dehydroepiandrosterone-Sulfate (DHEA-S) Level Among Oligospermia..

Results expressed as Mean±SD, significant difference considered as p-value ≤0.05.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligospermia</td>
<td>193±21.28</td>
<td>0.993</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>213±16.33</td>
<td>0.599</td>
</tr>
<tr>
<td>Control</td>
<td>186±13.13</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed as Mean±SD, significant difference considered as p-value ≤0.05.

Fig. 3.3 Dot plot correlation of Testosterone and DHEA-S with age and BMI

R=positive or negative correlation, p-value indicate strength of correlation

IV. Discussions

Since the testosterone is involved in regulation of germ cell development thus, it becomes necessary to measure testosterone level for management of male infertility [13]. The spermatogenesis requires the presence of gonadotropins and testosterone [13, 16]. Accordingly the aim of this study was to assess testosterone and DHEA-S among patients with oligospermia and azoospermia and their correlation with study variables.

In this study the percentage (%) showed equality of age (<35 years and >35 years) within oligo and azoospermia. The overweight was more prevalent in oligospermia (60.0%) and azoospermia (63.3%), which indicated oligospermia and azoospermia patients were comparatively over weight than normospermia.

The results provide experimental evidence that, there was significant decrease of mean testosterone level in azoospermia versus normospermia p-value 0.034, while insignificant decrease was observed among oligospermia with p-value 0.081. In fact that, spermatogenesis is associated with higher intratesticular levels of testosterone, our result was in agreement with Masanori finding, therefore it not only explain impaired spermatogenesis but also decreased Leydig cells function and pretesticular causes of azoospermia were also suspected [12, 17].

The present study revealed insignificant differences of mean concentration of serum DHEA-S in both oligo and azoospermia in comparison with normospermia p-value 0.993 and 0.599 respectively. Our finding in agreement with previous study by Garcia who reported that there was no direct association between serum DHEA-S level and sperm count (14). Independent secretion of DHEA-S from adrenal gland and its metabolism in peripheral tissues may explain this finding (18).

The results showed negative correlation between BMI and testosterone level with (R=0.300 and p-value 0.020). In fact that obesity is associated with low level of sex hormone binding globulin [20], obese men have been shown to exhibit higher levels of circulating estradiol and/or elevated estradiol per testosterone ratios in multiple studies, which proposed the concept that there is inappropriate suppression of the hypothalamic-pituitary-gonadal axis by elevated estrogens derived from peripheral aromatization (conversion of androgen to estrogen in adipocytes), thus resulting in decreased testosterone production reflected in low levels of circulating testosterone and intratesticular testosterone, this finding may also explain some of the altered semen parameters seen in obese men [19].

Many studies have now demonstrated that as men aging, their serum testosterone concentrations fall. These studies include both cross-sectional and longitudinal studies. In this study insignificant negative correlation was found between serum testosterone and age of patients (R= 0.24, p-value 0.06) this finding was in
agreement with recent studies which proposed this result due to fact that the incidence of testosterone deficiency increased with age due to decline in testosterone production especially after age 30 [20].

Significant positive correlation between serum DHEA-S and BMI was found among patients group (R=0.279, p-value 0.03) which contradict the results of most recent studies conducted about the hormone level in different population, accordingly we recommend conducting further similar studies with larger sample size to clarify this association. Pearson’s correlation revealed no correlation between testosterone, DHEA-S, and other study variables.

V. Conclusion

The study has concluded that, testosterone levels are lower in oligospermia and azoospermia in comparison with control normospermia, age and overweight are causative factors of testosterone deficiency, thus could affect spermatogenesis, which may result in infertility especially among oligospermia.

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DOI: 10.9790/0853-1504146265  www.iosrjournals.org  65 | Page