

Significant value of hormonal assays as a marker for Male Infertility in Tikrit city.

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Abstract

Background: Infertility is defined as the failure of a couple to conceive after one year of regular unprotected intercourse. Infertility is a problem of global proportions. Several studies with questionable results have focused on the value of serum FSH and AMH to predict the status of spermatogenesis in the testes. Also, possible relationship between seminal plasma inhibin B and spermatogenesis in patients with azoospermia. **Aim:** The aim of the present study is to compare some serum biomarkers of infertile men with normal fertile men to find the significant value of hormonal assay marker in predication of male infertility.

Patients & Methods: A cross sectional study in which samples were obtained from urology clinic in Tikrit teaching hospital, private clinic, from 1-9- 2011 to 1-9-2012. Ninety subjects were participated in the present study (Fifty infertile men & forty fertile subjects). Semen parameters were measured for infertile men & control fertile men. Serum concentrations of FSH, LH, testosterone, AMH & prolactin were measured.

Results: There is significant ($p < 0.01$) reduction in sperm count, motility, viability of infertile men, as compared with same parameters of normal fertile men. Also, there is significant increase in serum MDA ($6.811 \pm 0.53 \mu\text{mol/l}$) in infertile men as compared with fertile men, ($2.454 \pm 0.54^{**}$). However, there is significant reduction in serum glutathione ($p < 0.01$) in infertile patients ($7.1 \pm 1.2 \mu\text{mol/l}$) as compared with fertile men, ($10.123 \pm 1.21^{**}$). Serum FSH, LH, and testosterone levels were found significantly differences between infertile men ($11.35 \pm 2.4 \text{ mIU/ml}$, $9.956 \pm 2.87 \text{ mIU/ml}$ & $5.07 \pm 0.62 \text{ ng/ml}$ respectively) as compared with serum FSH, LH, and testosterone levels of fertile men ($5.53 \pm 2.3^*$, $5.973 \pm 0.92^{**}$ and $9.16 \pm 0.749^{**}$) respectively. The present study show that there is significant reduction in serum AMH in infertile men ($1.40 \pm 0.11 \text{ ng/ml}$), as compare with fertile men, (4.17 ± 0.67). However, regarding serum prolactin, there is no significant differences between infertile men ($4.26 \pm 2.17 \text{ ng/ml}$) as compare with control fertile men (3.91 ± 1.52).

Conclusion & Recommendation: The present study concludes that there is significant reduction in serum testosterone & AMH of infertile men as compare with fertile men. However, there is significant increase in serum FSH & LH in infertile men. There is negative correlation between AMH & serum testosterone in infertile men. The present study recommend the followings;-

Proper selection of patients with respect to hormonal assays which reduces the costs and burden on the infertile patients, & the routine assessment of thyroid hormones and antibodies in infertile men are not recommended.

Key words: Infertile men, semen analysis, FSH, LH, Testosterone, prolactin & AMH.

Introduction

Infertility is defined as the failure of a couple to conceive after one year of regular unprotected intercourse. Infertility is a problem of global proportions. The infertility rates vary between countries and from region to region(1). It is documented that around 15% of married couples are infertile and that approximately 50% of infertility is due to male factor (2).

Evidence now suggests that reactive oxygen species (ROS)-mediated damage to sperm is a significant contributing pathology in 30–80% of cases(3). A few years ago, concern has been expressed about the generation of ROS in the male reproductive tract. This is because ROS, at high levels, are potentially toxic to sperm quality and function(4). Also, additional reports have indicated that high levels of ROS are detected in semen samples of 25% to 40% of male infertility men(5-7).

Several studies with questionable results have focused on the value of serum FSH and AMH to predict the status of spermatogenesis in the testes. Also, possible relationship between seminal plasma inhibin B and spermatogenesis in patients with azoospermia, (8-10). Hyperthyroidism has been found to cause oligozoospermia, asthenozoospermia, abnormal sperm morphology, or occasionally infertility in males, (11). Exposure to excess glucocorticoids either endogenously or exogenously can result in decreased spermatogenesis. Elevated plasma cortisone levels depress LH secretion and induce secondary testis failure, (12).

Elevated prolactin usually results in decreased FSH, LH, and testosterone levels and causes infertility. Associated symptoms include loss of libido, impotence, galactorrhea and gynecomastia, (12-13).

The aim of the present study is to compare some serum biomarkers of infertile men with normal fertile men to find the significant value of hormonal assay as a marker in predication of male infertility.

Patients and methods

A cross sectional study in which samples were obtained from infertility clinic in Tikrit teaching hospital, private clinic, from 1-9- 2011 to 1-9-2012. Information from the infertile men was obtained before semen analysis.

Ninety subjects were participated in the present study (Fifty infertile men & forty fertile subjects). Fifty infertile men with a mean of age is 30.56 ± 5.41 years. Forty normal men their mean age 28.32 ± 5.26 years were used as control. All men were given clear instructions regarding the accurate semen collection to minimize error. They were asked to avoid sexual intercourse for 3 days. Semen parameters were measured for infertile men & control fertile men.

Blood samples were obtained from the patients and control. Then blood in the plain tubes was allowed to clot at room temperature (25°C) for 1 hour. After that centrifugation was done at (3000) rpm for 3 minutes to separate the serum. The serum was transferred by micropipette and divided into 5 equal fractions in 5 test tubes, one fraction for each hormonal assay. The sera were stored at -20°C until the assay was done.

All subjects were questioned about IVF trials, testicular biopsy, mumps, venereal disease, varicocele, drugs which may interfere with fertility and all underwent complete clinical and physical examination. The subjects considered infertile according to WHO criteria, (14).

Serum concentrations of FSH, LH & prolactin were measured using ELISA. The ELIZA kit that was used was produced by Monobind, Inc. company (U.S.A.). We used test procedure and protocol recommended by the kit manufacturer which was given in details in the kit's insert for FSH, LH & prolactin.

The level of serum testosterone for male patients with infertility and control was measured using enzyme-linked

immunosorbent assay (ELIZA) method. The ELIZA kit that was used was manufactured by Bio-Check, Inc. Company (USA). The test procedure and protocol recommended by the kit manufacturer was adopted which was given in details in the kit's insert.

Serum AMH concentrations were measured using AMH/MIS enzyme linked immunosorbant assay kit (Immunotest material USA).

MDA determination is based on the colorimetric reaction with thiobarbituric acid (TBA) at 90-100°C and pH 2-3 for 15 minutes to form pink color product, which can be measured by spectrophotometer, (15). Semen or serum (GSH) was determined by the modified method and depended procedure on used Elleman's solution. Serum GSH were performed by standard procedure, (16).

Statistical analysis done by using unpaired student T test. All data are present as a mean & standard deviation (SD). Pearson correlation and unpaired T-test was used. P- Value ≤ 0.05 was considered significant throughout the study.

Results

There is significant ($p < 0.01$) reduction in sperm count, motility, viability of infertile men, (51.75 ± 8.5 , 35 ± 5.4 , 40 ± 9.4 respectively) as compared with same parameters of normal fertile men, ($70.76 \pm 5.6^{**}$, $67.1 \pm 10.4^{**}$, $63.5 \pm 13.7^{**}$ respectively) (Table 1).

The study observes a significant reduction in ejaculate volume of infertile men, (2.25 ± 0.42 ml) as compared with ejaculate volume of fertile men, (3.56 ± 0.48 ml^{**}), table 1.

There is significant increase in serum MDA (6.811 ± 0.53 $\mu\text{mol/l}$) in infertile men as compared with fertile men, ($2.454 \pm 0.54^{**}$). Meanwhile

there is significant reduction in serum glutathione ($p < 0.01$) in infertile patients (7.1 ± 1.2 $\mu\text{mol/l}$) as compared with fertile men, ($10.123 \pm 1.21^{**}$), table (2).

Regarding serum FSH, LH, and testosterone levels, there were significant differences between infertile men (11.35 ± 2.4 mIU/ml, 9.956 ± 2.87 mIU/ml & 5.07 ± 0.62 ng/ml respectively) as compared with serum FSH, LH, and testosterone levels of fertile men ($5.53 \pm 2.3^*$, $5.973 \pm 0.92^{**}$ and $9.16 \pm 0.749^{**}$, respectively), table (2). Furthermore, there is a positive correlation between serum Testosterone and sperm motility, ($r=0.54$).

The present study show as in table 2, that there is significant reduction in serum AMH in infertile men (1.40 ± 0.11 ng/ml), as compare with fertile men, (4.17 ± 0.67).

However, regarding serum prolactin, there is no significant differences between infertile men (4.26 ± 2.17 ng/ml) as compare with control fertile men (3.91 ± 1.52).

Discussion

In the present study, there is significant ($p < 0.01$) reduction in sperm count, motility, viability of infertile men, as compared with same parameters of normal fertile men. These semen parameters reflects the presence of male infertility, because all the above value are below the normal accepted values (13).

In present study, the sperm motility was significantly lower ($P < 0.01$) in infertile patients than control fertile men. The results of present study were in accordance with previous workers who stated that sperm motility was lower in infertile men than fertile population under study. The percentages of sperm motility in their infertile groups were $31.9 \pm 19.2\%$ & $49 \pm 25.5\%$ (6, 8).

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In the present study, it was observed that the percentage of normal sperm morphology was significantly lower ($P < 0.01$) in infertile patients as compared to control fertile men. Many researchers reported similar results in infertile versus fertile population (2, 4, 6, 8). They pointed out that the percentages of normal morphology sperms in infertile were (54.37 ± 13.2 , 29.22 ± 10.08 ; 40.1 ± 14.1 & $21.7 \pm 10.9\%$ respectively).

The difference between present results and other researchers reports is probably due to different exposure to the above risk factors and of course different genetic, racial and environmental backgrounds, (17-19).

A significant increase in serum MDA & a decrease in serum glutathione levels in infertile patients as compared with fertile men. This finding gave an indication of the presence of oxidative stress in infertile men. Also, it is reflected by the normalization of antioxidant activities & concurrent decrease of MDA in fertile men (2, 4, 7).

Previous retrospective studies have indicated that semen quality has been declining in the past several decades, & semen quality is affected by various factors other than physical environments, such as age, occupation, cigarette smoking and other lifestyle factors (20). The WHO manual provides guidelines for assessing the various semen variables, (14); however, it is still difficult to compare the values between different laboratories. Furthermore, several studies have indicated geographical differences in semen quality, probably related to environmental factors; however, ethnic or genetic differences cannot be excluded. A common set of reference values may therefore not be appropriate to use worldwide, (1, 8, 20, 21).

For all the above reasons, in the present study a control group to compare with the SFA findings of patients was included, rather than comparing present

SFA results with the reference values of the WHO manual, (14).

Elevated prolactin usually results in decreased FSH, LH, and testosterone levels and causes infertility, (13). In the present study, regarding serum prolactin, there is no significant difference between infertile men (4.26 ± 2.17 ng/ml) as compared with control fertile men (3.91 ± 1.52).

In the present study, there is high statistically significant difference of serum FSH & LH, & testosterone hormones ($p < 0.01$) in all fertile parameters as compared with infertile men.

In other studies that had compared the level of serum hormones in fertile against infertile men, many of these papers pointed out that serum FSH and serum LH was higher in men with infertility than fertile group, (2, 4).

In the present study, serum testosterone was markedly lower ($P < 0.05$) in infertile patients than fertile control men ($P < 0.05$).

The pattern of hormonal abnormalities that was found in the present study runs in line with that of hypogonadism. In primary hypogonadism, there is reduction in serum testosterone level and elevation of serum FSH and serum LH values as a result of the negative feedback mechanism of the hypothalamic-pituitary-gonadal axis, (14).

Serum AMH was found to be significantly lower in infertile men as compared with fertile control men. This is in accordance with results of a previous study, (22, 23). The regulation of AMH after birth is complex; basal levels of AMH are independent of gonadotropin regulation, for example, during childhood and in patients with hypogonadotropic hypogonadism. (24).

Several studies with questionable results have focused on the value of serum

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FSH, LH, testosterone and AMH to predict the status of spermatogenesis in the testes. The present study compares some serum biomarkers in infertile men & control fertile men. The results showed significant difference in FSH, testosterone and AMH concentrations in infertile men as compared to normospermic men. This is an indication of defective spermatogenesis and as a result of feedback control probably by inhibin B or may be a direct involvement of AMH.(19).

AMH was found to be negatively correlated with serum testosterone this in agreement with other studies. (25). The negative correlation between AMH and FSH this in lines with previous studies, (26, 27) either might reflect an involvement in the signaling and regulation of FSH or most probably to be a symptom of impaired or immature Sertoli cells. (28).

In conclusion, AMH should be carefully evaluated in oligospermic and azospermic men. As anti-Mullerian hormone is a marker of both Sertoli cell proliferation and protein synthesis activity in response to FSH before puberty and also a useful marker of FSH action in the assessment of testicular function in the prepubertal boys, (29)

Also, no significant difference in serum Prolactin was found among infertile & control men. This point out to an important findings that serum hormones (FSH, LH, Testosterone and Prolactin) should not be requested routinely for every infertile men. Instead, they should be sent semen analysis for many times, then only in cases of azospermia, moderate to severe oligozoospermia (< 10 million/ml) or when there is clinical indication.

In present time & in Iraqi community, hormonal assays are not always available in every hospital and clinicians usually send infertile men to private laboratories in which the hormonal assays are expensive & inaccurate due to the presence of different methods of measurements.

So, this study stresses on proper selection of patients with respect to hormonal assays which reduces the costs and burden on the infertile patients.

This observation may indicates that serum Testosterone and serum FSH are the best two hormones to be requested for initial evaluation of male infertility in Iraqi men and possibly for all infertile men. This selectivity again reduces the hormonal assays costs on the patients. If these two hormones are abnormal, then the full endocrine evaluation could be started.

The present study concludes that there is significant reduction in serum testosterone & AMH in fertile men as compare with fertile men. However, there is significant increase in serum FSH & LH in infertile men. There is negative correlation between AMH & serum testosterone in infertile men.

The present study recommend the followings;-

1- Proper selection of patients with respect to hormonal assays which reduces the costs and burden on the infertile patients.

2-The routine assessment of thyroid hormones and antibodies in infertile men is not recommended.

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Table (1) Show the mean & standard deviation of semen parameters of infertile men & control fertile men.

Semen Parameters	Infertile men	Fertile subjects
Count (Million/ml)	51.75 ± 8.5	70.76 ± 5.6**
Motility (%)	35 ± 5.4	67.1 ± 10.4**
Viability (%)	40±9.4	63.5 ± 13.7**
Morphology (%)	58.88±6.5	76.5 ± 7.41**
Volume (ml)	2.25±0.42	3.56 ± 0.48**

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Table (2): Show the mean & standard deviation (S.D) of serum parameters of infertile & control fertile men.

Parameters	Infertile men	Fertile subjects
MDA ($\mu\text{mol/l}$)	6.811 \pm 0.53	2.454 \pm 0.54**
glutathione ($\mu\text{mol/l}$)	7.1 \pm 1.2	10.123 \pm 1.21**
FSH mIU/ml	11.35 \pm 2.4	5.53 \pm 2.3*
LH mIU/ml	9.956 \pm 2.87	5.973 \pm 0.92**
Testosterone (ng/ml)	5.07 \pm 0.62	9.16 \pm 0.749**
AMH (ng/ml)	1.40 \pm 0.11	4.17 \pm 0.67 **
Prolactin (ng/ml)	4.26 \pm 2.17	3.91 \pm 1.52 NS

SD: standard deviation
NS: Non significant

****:** p< 0.01

***:** p< 0.05