ORIGINAL ARTICLE

ROLE OF ESTIMATING SERUM LUTEINIZING HORMONE AND TESTOSTERONE IN INFERTILE MALES

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ABSTRACT

Background: This study was aimed to determine the levels and ratios of serum LH and Testosterone, among men with history of infertility, having varying sperm counts.

Material & Methods: Two hundred fifty married men, presenting with a complaint of infertility, who had been initially evaluated for their seminal profile, and had been classified into four (04) groups, as azoospermic (50), oligozoospermic (75), asthenozoospermic (50) and normozoospermic (75) were studied for the analysis of serum LH and Testosterone levels using Enzyme Immuno Assay (EIA), along with 50 proven fathers as a control group. The data was compared using student's 't' test.

Results: LH indicated inverse/negative correlation to sperm concentration, while decreased Testosterone levels were associated with depleted sperm concentration. The mean levels of LH (mIU/ml) and Testosterone (nmol/L) for the groups were 13.85±2.33 and 11.86±0.70 (Azoospermia), 10.92±3.79 and 11.88±1.06 (Oligozoospermia), 3.92±1.17 and 16.24±2.05 (Asthenozoospermia), while the levels in normozoospermic men were 7.24±1.02 and 17.29±1.02, respectively. Similarly, the LH/T and T/LH ratios were 1.17(+/-0.28) and 0.86(+/-2.70) respectively in azoospermia, 0.92(+/-0.28) and 1.08(+/-0.17) in oligozoospermia, 0.30(+/-0.10) and 4.14(+/-10.4) in asthenozoospermia 0.42(+/-0.08) and 2.34(+/-0.48) in normozoospermic men. The proven fathers group had 7.74±0.71 mIU/ml LH and 15.88± 1.15 nmol/ml testosterone, while the ratio of LH/T and T/LH was 0.49± 0.28 and 2.05± 0.33, respectively.

Conclusion: The present data indicates that not only the disturbance in LH and Testosterone levels, but also disturbance in their ratios causes infertility, since these hormones act together by maintaining delicate feedback control system.

Key words: Testosterone, Luteinizing hormone, Infertility; Male Factor.

INTRODUCTION

The successful and complete male germ cell development is dependent on the balanced endocrine interplay of hypothalamus, pituitary and the testes. Gonadotrophin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotropins i.e. follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland.[1] FSH binds with receptors in the Sertoli cells and stimulates spermatogenesis, while LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis.[2] The failure of pituitary to secret FSH and LH will result in disruption of testicular function leading to infertility. Testosterone, estradiol and inhibin control the secretion of gonadotrophins through feed back mechanism.[3]

Infertility is a common disorder and nearly one out of every six to eight couples suffers from it at any given time. Infertility among couples in their respective age is more common than hypertension, diabetes, heart diseases and even the common flu.[4]

Globally, it has been estimated that approximately 10-15% couples seek medical help for the problem of infertility. In 20-25% cases the problems are attributable to the male partner, while 30-40% represent female factor. In approximately 30% of cases both partners and in 15% no specific factor can be identified.[5]

In Pakistan where potency is considered a proof of normal fertility, it is usually the wife who bears the blame and is often maltreated for the offence of not bearing children. The husband, who should also be investigated simultaneously either never submits himself for evaluation or is investigated only at the end.[6]

Status of the male can be assessed through simple semen examination, followed by hormonal profile if required.[7] Absence of spermatozoa in the semen ejaculate is called "azoospermia", count less than 20 million/ml "Oligozoospermia" and density of 20 million/ml but progressive motility of less than 25% is called "asthenozoospermia".[8] Male infertility is associated a reduction in the quantity of functional sperms.[9] Decrease in sperm density, eventually leading to azoospermia, has been found to be associated with raised FSH, LH and normal or low testosterone level.[10]

Hormonal disturbances result in almost 1/6 married couples not having any progeny, and the male partner being responsible in nearly half of such cases.[11] Determination of concentration of LH and Testosterone is essential for the evaluation of pituitary gonadal axis and in the evaluation of infertility.[12]

Studies on determination of the gonadotropic hormone, LH and androgenic hormone testosterone are common but their ratios are yet to be reported. The present study, although preliminary in nature, aims to establish the association of the spermiogram with the ratios of the gonadotropic hormone and androgenic hormone.

MATERIAL & METHODS

Subjects: A total of 250 subjects with a history of infertility for a duration of more than one year were included. These patients were referred from the clinics of gynecologists, urologists, and general practitioners from different parts of the country, for semen and hormone analysis. The work was carried out at Reproductive Physiology /Health, Public Health Laboratory Division, National Institute of Health Islamabad.

A total of fifty (50) proven fathers belonging to the same socio-economic status were selected as a control group. The last pregnancy was observed 1-3 years back in the control group. Clinical examination of all the subjects was carried out and information regarding age, health problems, duration of marriage, history of infertility in the family and parity was recorded.

Semen analysis: The semen of the Subjects was obtained and analyzed according to WHO recommended procedure[13] and categorized as normozoospermic, azoospermic, oligozoospermic and asthenozoospermic.

Blood sampling for hormone Analysis: A 5-10ml fresh blood sample was collected from the anterior cubital vein, under aseptic conditions for hormone analysis from each subject, which was transferred to a clean plain labeled tube, allowed to clot, then centrifuged at 6000 rpm for 5 minutes in Hittech centrifuge at room temp, and clear serum was separated and kept at –20^{0}C till assay.

Hormonal Assessment: LH and Testosterone assessment was carried out using electrochemiluminescence technology by Elecsys 2010 analyzer.

Quality Control of Assays: Quality control samples representing the normal and pathological levels of the analytes were used for quality control. Results ± 2SD of the target value were considered acceptable. Only the batches with all both controls being within permissible were accepted.

Statistical Analysis:

Data was analyzed statistically, by application of Student's 't' test, as described by Steel and Torrie.[14]

RESULTS

The results of the study are given in Table 1. The LH/T ratio was highest (1.40±0.28) in case of azoospermia and 0.45±0.08 in case of normozoospermia. In conditions of Oligozoospermia, the LH/T ratio was above 1.00. However, the least value was observed in case of asthenozoospermia (0.30±0.10), when compared with the proven father as a control group (0.88±0.28).

Table-1: Serum LH and Testosterone levels, as well as their ratios.

Group	LH (mIU/ml)	Testo (nmol/L)	Ratio of LH/Testo	Ratio of Testo/LH
Azoospermia	13.85±2.33[b]	11.86±0.70[c]	1.17±0.28[a]	0.86±2.70[a]
Oligozoospermia	10.92±1.22[a]	11.88±1.06[d]	0.92±0.28[a]	1.08±0.17[c]
Asthenozoospermia	3.92±1.17[c]	16.24±2.05[a]	0.24±0.10[a]	4.14±10.4[a]
Normozoospermia	7.24±1.02[a]	17.29±1.02[a]	0.42±0.08[a]	2.39±0.48[a]
Proven fathers	7.74±0.71	15.88±1.15	0.49±0.28	2.05±0.33

a = Non-significant ($p>0.05$), b = Significant ($p<0.05$), c = Significant ($p<0.01$), d= Significant ($p<0.001$).

Values are Mean (±SEM). Normal levels of Testo = 5.2-22.9 nmol/L and LH = 2-12 mIU/ml.

It can be seen that in the case of azoospermia and oligozoospermia, testosterone exhibited significant decreases ($p<0.001$) and ($p<0.05$) respectively, while the increase in case of asthenozoospermia was not significant ($p>0.05$) Significant differences in LH levels were also observed in case of azoospermia ($p<0.05$) and asthenozoospermia (($p<0.01$), being raised in case of azoospermia and depressed in case of asthenozoospermia. The LH/T ratios were significant, ($p>0.05$) among the entire sub fertile groups, when compared with the proven fathers. Due to increased LH and decreased Testosterone levels, oligozoospermic subjects exhibited significantly ($p<0.01$) lower ratios of T/LH.

DISCUSSION

FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and Testosterone. FSH acts directly on the seminiferous tubules, whereas lutenizing hormone stimulates spermatogenesis indirectly via testosterone .[15]

FSH, LH and testosterone evaluation is useful in the management of male infertility.[16] De Krester et al [17] reported elevated levels of serum FSH, LH with increasing severity of seminiferous epithelial damage. In the present study, elevated levels of LH were observed in azoospermic and oligozoospermic males when compared with the levels in proven fathers, as well as normozoospermic men.

These results are accordance with earlier studies,[16, 18-20] which showed gonadotrphic elevation in infertile males. In various studies elevated levels of LH in oligozoospermic and azoospermic males, when compared to normal fertile men, were also documented[21.22] which is an agreement with our studies.

In the present study, the mean serum testosterone levels in fertile and subfertile men were within permissible levels. Similar observation has been recorded in earlier studies,[23-25] which have reported normal level of testosterone in infertile men with Sertoli cell syndrome when compared with control group, but in contrast to another study, where decreased level of testosterone was observed in subfertile males,[26] as sometimes there is a loss of germinal epithelium, but Leydig cells of testes remain intact. This condition is usually reflected by normal plasma testosterone level.

In the present study, serum concentration of Testosterone was although within the limit, but was on lower side. In a number of related studies, low levels of serum testosterone have been shown to be associated with infertility. It has been demonstrated that low levels of serum LH and testosterone are found in men with oligoasthenozoospermia.[27)] It has been reported, that decreased secretions of LH and Testosterone in oligozoospermic men are due to prolonged half life of LH, reduced bio-active LH secretary burst amplitude, lower immunoactive ratio for LH burst amplitude, reduced bioactive / immunoactive ratio in the mass of LH secreted per burst and decreased coordinated release of bioactive LH and testosterone.[28] The suppression of testosterone secretion in infertile men could be due to deficiency of hypothalamic Gn RH, resulting in the impairment of Gonadotropin secretion from pituitary. The deficiency of LH and FSH prevents the gonads from either producing sperms or sufficient quality of testosterone[29]

In our investigations we found a small group of subjects in infertile patients with high testosterone concentrations. It has been reported that testicular tumors prior to puberty produced high concentrations of testosterone leading to precocious puberty. After puberty, they tend to produce more estrogens resulting in feminization with characteristic symptoms of gynecomastia, impotence and testicular tumors. In those conditions, hypothalamic/ pituitary function is reduced by estrogen via feedback, and atrophy of the testicular tissue surrounding the tumor and of the contra lateral testis may result, spermatogenesis ceases, and oligozoospermia or azoospermia is found.[29]

The significant rise in plasma LH level in oligozoospermic and azoospermic males in the study, are in contrasts with the findings of saeed et al.[30] whereas, kuku et al[31] observed elevated LH levels in 26.5% subjects and low level in 5% of infertile males, which is an agreement to our study.

In the study under discussion, individual subjects exhibited different combinations with respect to LH and Testosterone levels. Generally LH levels were high in case of azoospermia and oligozoospermia, and the differences in these levels affected the ratios. Low level of LH, and hence subsequently testosterone, are also found in patients with normal spermatic maturation arrest.[32]

In the present study, it has been found that the ratio of LH/T was high in case of decreased sperm concentration, and low in cases of asthenozoospermia and normozoospermia. The elevation of the LH/T ratio to more than 1.0 signifies depletion of sperm concentration, while its depression to less than 0.5 represents decrease in sperm quality.

It can be seen that in the case of azoospermia and oligozoospermia, testosterone exhibited significant decreases, while the increase in case of asthenozoospermia as well as normozoospermia was not significant. Significant differences in LH levels were also observed in case of azoospermia ($p<0.05$) and asthenozoospermia ($p<0.01$), elevated in cases

of azoospermia and decreased in cases of asthenozoospermia. The LH/T ratios were observed as non significant ($p>0.05$) in all groups in comparisons to the proven fathers. Due to increased LH and decreased testosterone levels, significantly ($p<0.01$) lower ratios of T/LH were observed in cases of oligozoospermia.

The overall results clearly indicate significant increase of LH, in case of depleted sperm concentrations, and significant decrease in cases of asthenozoospermia. Although there was a significant decrease in the testosterone levels in azoospermic and oligozoospermic males when compared with the fertile controls, the increase in the levels of LH might have disrupted the spermatogenic process leading to the decline in the sperm count and infertility.

CONCLUSION

The study suggests that elevated levels of LH and low level of testosterone are responsible for decreased function of Leydig cells and imparting diminished secondary sexual characteristics in infertile patients. Therefore it is important to investigate serum levels of LH and Testosterone and their ratios, specially the LH/T ratio, which could be used as tool to investigate the nature of neuroendocrine cause of infertility, as both these hormones act in association.

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