Relationship of Inhibin-B with Gonadal Hormones Levels in Seminal Plasma of Infertile Patients in Diyala Governorate

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Abstract:

Background:

Over 70 million couples suffer from infertility worldwide and the majority of those couples can be found in developing countries.

Aims of study:

This study aims to determine if there is any significant difference in levels of inhibin B and gonadal hormones in fertile and infertile men.

Subjects, Materials & Methods:

Clinical and hormonal characteristics related to male infertility factor of the infertile patients(n=75)and representative proven fathers as a control(normozoospermic ones)(n=25)were studied. All males attending infertility clinic at Baquba General Hospital, Diyala in Iraq. The study included the measurement of gonadal hormones levels by using (ELISA)method and it was also used in analyzing the inhibin B hormone. The VIDAS instruments were used for enzyme immunoassay measurements Luteininzing Hormone(LH),Testosterone(T) ;and Follicle Stimulating Hormone(FSH). The samples were analyzed and classified according to WHO criteria.

Results:

The results of this study showed that inhibin B hormone and other gonadal hormones in control (representative proven fathers-normozoospermic ones)as inhibin B (240.2±17.4)Pg/ml, LH (1.639±0.103)mIU/ml, Testosterone (0.6253±0.035)ng/ml and FSH (0.469±0.0103)mIU/ml. In asthenozoospermic ones as an example of infertile patients the results showed that inhibin B (76.9±8.99)Pg/ml, LH(1.4311±0.033)mIU/ml, T (0.5483±0.030)ng/ml and FSH (0.495±0.0124)mIU/ml. Except the inhibin B comparisons all of other non significant evaluated parameters & correlations within this study could be ascribed to the role of cascaded role of inhibin B with the gonadal hormones in spermatogenesis.

Conclusions:

It concluded from this study that decreased levels of inhibin B, LH,Testosterone, but increased levels of FSH hormones were shown in asthenozoospermia group as an example of infertile patients,and increased levels of inhibin B,LH and Testosterone but decreased levels of FSH were shown in normozoospermia as control.

Keywords: **Inhibin B, Male infertility, Gonadal Hormones.**

Introduction:

Infertility is defined as failure of a couple to achieve a pregnancy after at least one year of frequent unprotected intercourse. If pregnancy has not occurred within 3years, infertility most likely will persist without medical treatment [(1)] . Male infertility is the sole or contributing factor in almost half of the couples failing to conceive. Semen analysis plays an important role in clinical diagnoses for sterility and infertility treatment including the assessment of sperm concentration, motility and percentage of normal forms are the standard procedure for evaluation the male fertility potential [(2,3)] .Male infertility is a multi-factorial disorder. The functional ability of spermatozoa is primarily determined by their motility. Many factors are responsible for reduction in sperm motility but immunological and hormonal factors are vital. The impact of immunological factor along with hormonal imbalance is reducing sperm motility [(4)]. Seminal plasma in which sperm suspended is considered as an important source for many biochemical markers for functional disorder in male reproductive system [(5)]. The Clinical and hormonal characteristics related to infertility. Seminal characteristic depends on the number of normal physiological and anatomical factors and the immune complex. The hormonal imbalance caused disturbances in the endocrine system, hypothalamus and pituitary, is one of reasons that lead to infertility. Further, concentration of fertility hormones (LH, FSH, Testosterone and Inhibin B [(6)] . Important gonadal hormones include glycoprotein hormones made by the pituitary gland, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH are both glycoprotein with molecular weights of approximately (28 kDa).The release of LH and FSH at the pituitary gland is controlled by pulses of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Those pulses, in turn, are subjected to the estrogen feedback from the gonads[(7)] There are two important hormones secreted from gonads and correlated with FSH and LH. Its Inhibin B and Testosterone, respectively. Inhibin B a (32 kDa) glycoprotein hormone and is a heterodimeric glycoprotein composed of one α-subunit and one β-subunit. The free subunits usually do not have any physiological effects.Therefore, the bioactivity of the inhibin depends on the formation of a dimeric α-β structure, inhibin B is a gonadal dimeric polypeptide hormone that regulates synthesis and secretion of follicle stimulating hormone (FSH) in a negative feedback loop. Inhibin B plays a key role in theregulation of the hypothalamic-pituitary-gonadal hormonal axis during male childhood and pubertal development [(8)] .

Inhibin B measurement is a better marker of infertile status than FSH and LH. Concentration of inhibin B in patients with infertility may provide useful information on spermatogenesis and possibly serve as a more direct marker of spermatogenesis than FSH. Inhibin B is produced by Sertoli cells, provides negative feedback on FSH secretion, and may prove to be an important marker of the function of seminiferous tubules [(9)] Testosterone is a steroid hormone

from the androgen group and is found in mammals, reptiles, birds, and other vertebrates. In mammals, testosterone is primarily secreted in the testes (Sertoli cells) of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. Androgens. included dihydroepiandrosterone (DHEA), androstenedione and testosterone. In the adrenal gland, DHEA is also produced de novo by the testes or the ovaries. DHEA is the precursor of androstenedione, which is the precursor of testosterone and estrogen. Sperm are produced in the seminiferous tubules within the testes (Production is controlled by two hormones from the pituitary gland). Sperm are transported into the epididymus which stores and improves the fertility of sperm [(10)] . The hormonal regulation of testicular function is a complex system controlled by the hypothamus-gonadal axis. The gonadotropins luteinizing hormone (LH)and Follicle-stimulating homone(FSH)are produced and secreted by the gonadotropic cells of the pituitary gland. The secretion of the pituitary glands is regulated by the hypothalamic gonadotropin-releasing hormone, and its function is in turn the control of gonadal steroids and peptides that influence its activity.LH and FSH exercise their tasks via specific receptors located in the Leydig cells and Sertoli cells respectively. LH stimulates testosterone synthesis of the Leydig Cells, whereas FSH controls spermatogenesis via the Sertoli cells and is regulated through a negative feedback by inhibin B, produced by the Sertoli cells. The effect of T (which also has autocrine and paracrine action in the testis) is important for spermatogenesis and control of the secretion of LH**.**

Sertoli cells are somatic cells located within the germinal epithelium. In adulthood these cells are inactive. These cells are located on the basal membrane and extend to the lumen of the tubules seminiferous. The intact testis with complete spermatogenesis contain 800-1200×106 Sertoli cells or approximately 25×106 Sertoli cells per gram testis. These cells are stimulated by FSH, and are responsible for the production of inhibin B. Sertoli cells are the major source of inhibin B. Inhibin correlates inversely with FSH in adult men. It is widely believed that inhibin B is the physiological endocrine regulator of FSH in men [(13)] **.**

Subjects,Materials & Methods:

The present study was conducted at Baquba Medical Hospital and a few private pathological laboratories in Diyala, and in the College of Medicine at Diyala University. The patients attended the clinic with the complaint of infertility and were within the male partner cause of married couples. The study was conducted from April 2011 to July 2012, and the infertility clinics were visited regularly to collect samples and relevant information of infertile patients over the period at this time.75 patients and 25 male(as control) who fulfilled the inclusion criteria were selected for the study. Male subjects ranged from (18-40.3) years. Another twenty five (25) fertile Iraqi men within a similar age group were studied as control. The control samples were acquainted similarly as the study group samples.

Study design and Subjects:

A case-control design was used in which patients were compared with age matched healthy controls(Normozoospermia Group),. Male patients were recruited Al-Shames teaching and Ebtehaal Laboratory who had been unable to initiate pregnancy during a period of at least one year of unprotected sexual intercourse. All participants had through clinical workups that included a clinical histo-physical examination, endocrinologic studies and laboratory testing of ejaculates. Results of these examinations pointed to different types of male infertility.The inclusion criteria were:-[1] male infertility for at least one year with intact female partner;[2]a minimum of two pathological spermiograms at interval of six weeks showing asthenozoospermia according to WHO criteria;[3] a minimum of 3 months without having received andrologically effective treatment or cholesterol lowering (statin) drugs;[4] no excessive dietary meat or dairy products intake for the last 3 days. Exclusion criteria were:-[1] thyroid dysfunction, adrenal disorder,hyperprolactinemia;[2]any case of infertility after systemic physical examination and laboratory testing, including genetic disorders;[3] infectious disease or immunologic-associated disease, or presence of any major systemic disease;[4] smoking;[5] alcohol abuse and[6] abnormal psychological stresses.

The subjects comprised: Group I (n=25), fertile healthy men as controls. Group II (n=75), Asthenozoospermia as an example of infertile group.

The sample of the seminal fluid was collected after three days of abstinence directly to enhance optimal quality and quantity of semen. Seminal fluids collected into a clean, dry, sterile, disposable Petridis made of glass or plastic in a room near the laboratory or at home in the morning and brought into the hospital within 1 hour of collection. The container labeled with the necessary information included name and age of a couple, file number, abstinence period and time of sample collection. The freshly ejaculated samples were allowed to liquefy for 30 minutes at room temperature before evaluation for sperm characteristics according guidelines laid down by WHO, 2005. And according to guidelines classification of subject into normozoospermic and infertile men was depended on WHO criteria of normal semen values presented in manual of NAFA-ESHRE[14,15] .

Seminal fluid analysis: Initial macroscopic examination: Included assessment of Liquefaction, Appearance, pH and Volume, Semen samples were analyzed according to the World health Organization Laboratory Manual and consistency to WHO [13] .

Preparation of seminal plasma:Briefly, semen was allowed to liquefy at room temperature for 30 minutes and centrifuged at 2000×g for 10 minutes at the same temperature to obtain the seminal plasma. Cloudy samples of seminal plasma were clarified by centrifugation at 1200×g for 10 minutes and the pellets were discarded. The seminal plasma was separated as a clear solution and frozen at-80 ºC until the time of analysis.

Measurement of inhibin B in seminal plasma:In normozoospermic subjects as control, the measurement of inhibin B in

undiluted seminal plasma revealed values in excess of the maximum levels of the standard curve. Therefore, the sample was diluted up to 1:4 to obtain a reading. A possible effect of a matrix factor was excluded by assessing serial dilutions. The lower detection value of the method is 15pg/ml [16].Measurement of Follicle Stimulating Hormone (FSH), Luteinizing hormone (LH) in Seminal Plasma, The assay principle combines on enzyme immunoassay sandwich method with a final fluorescent detection (ELIFA) [16].

Results:

In the present study,(Inhibin B, LH,T and FSH) hormones tests were measured for 100 individuals (25control and 75 infertile patients) divided into two main groups as shown in table 1 .

The results of this study showed that inhibin B hormone and other gonadal hormones in control (representative proven fathers-normozoospermic ones)as inhibin B (240.2±17.4)Pg/ml,LH (1.639±0.103) mIU/ml, Testosterone (0.6253±0.035)ng/ml and FSH (0.469±0.0103)mIU/ml. In asthenozoospermic ones as an example of infertile patients the results showed that inhibin B (76.9±8.99)Pg/ml, LH(1.4311±0.033)mIU/ml, T (0.5483±0.030) ng/ml and FSH (0.495±0.0124) mIU/ml.(Table 1).

Plasma inhibin B Hormone Levels in control and infertile patients in seminal plasma. Inhibin B hormone was measured in seminal plasma of all groups as mean±SD of inhibin B hormone for these groups ,respectively.In Normozoospermia as a control group , there was negative correlation between inhibin B and(FSH, Testosterone) (r=-0.016,-0.031), respectively and positive correlation between inhibin B and LH, (r=0.102) respectively .

In Asthenozoospermia patients, there was negative correlation between inhibin B and(FSH, LH and Testosterone, (r=-0.039, -0.039, -0.027),respectively(Table 2).

Table (1) Plasma inhibin B and Gonadal Hormones Levels in Control and Infertile Patients expressed as mean±SD

Parameters	Normozoospermia	Asthenozoospermia
Inhibin B(pg/ml)	240.2±17.4	76.9±8.99
LH(mIU/ml)	1.639±0.103	1.431±0.033
FSH(mIU/ml)	0.469±0.010	0.495±0.0124
Testosterone(ng/ml)	0.625±0.035	0.5483±0.030

The testosterone level of male control and Asthenozoospermia patients were found to be 0.6250 ng/ml and 0.5483ng/ml respectively, these results showed that testosterone was increased in male Asthenozoospermia patients rather than control. All the comparisons were within non significance degrees except the comparisons of levels inhibin B.

Table (2): The correlation coefficients of inhibin B with gonadal hormones in fertile and infertile groups

Parameter	LH		Testosterone		FSH	
	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile
Inhibin B	r=0.102	r=-0.039	r=-0.031	r=-0.027	r=-0.016	r=-0.039

Discussion:

For initiation of spermatogenesis and maturation of spermatozoa, FSH is necessary. In the infertile men, higher

concentration of FSH is considered to be a reliable indicator of germinal epithelial damage[17]. That could be agreed with the present study FSH level were elevated in infertile males when compared with the levels in proven fertile controls but with non significance degrees.. As mentioned above the non significance degrees of comparisons of gonadal hormones could be ascribed to the cascading criteria of the inhibin B and gonadal hormones which can be interpreted as the main role of inhibin B within the evaluated correlations which appear to be the physiological feedback signal for FSH during spermatogenesis. As the study of Bohring *et al.* [18] that agreed with our study which conducted with the determination of serum levels of inhibin B, FSH, LH and testosterone in infertile & fertile men.

In addition to the major playing role of FSH in the induction and maintenance of spermatogenesis and can be a useful marker of the histological condition of the testis[10]. However, the diagnostic accuracy of FSH was questioned due to a wide overlap of FSH levels in regular and reduced spermatogenesis states this observation led researchers to reach more specific and direct markers of spermatogenesis and there is a growing interest regarding the role of inhibin B in evaluation of infertility[8] . So evaluation of hormonal profile ascribed to hypothalamic-pituitary-testis axis and has been repeatedly considered an important diagnostic tool for infertility[9] .

Conclusions:

It concluded from this study that decreased levels of inhibin B, LH,Testosterone, but increased levels of FSH hormones were shown in asthenozoospermia group as an example of infertile patients,and increased levels of inhibin B,LH and Testosterone but decreased levels of FSH were shown in normozoospermia as control.

Except the significant degree of comparison in inhibin B,the non significant comparisons within gonadal hormones could be ascribed to the physiological feedback signal during spermatogenesis.

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