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Serum and Seminal Plasma concentrations of Inhibin B and FSH: A Case-Control Comparison Study between Fertile and Infertile Males

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Abstract

Background: Infertility is among the most severe medical difficulties universally. Most of the men infertilities are resulted from testicular failure that cause absent spermatocytes from ejaculate (Azoospermia). Sperms can be retrieved invasively from the testicles by TESA or TESE. Inhibin B and FSH are known to be indicators of spermiogenesis and Sertoli cell activity and have been proposed to replace evaluating semen quality in clinical studies. **Objectives:** To inspect the potential variations between the values of seminal plasma and serum inhibin B and compare their levels between fertile and infertile males and evaluate their predictability for positive TESE outcomes. **Methodology:** This study was case-control and included 50 fertile males and 50 azoospermic subjects, who presented with the primary complaint of infertility. All applicants submitted to complete medical history, physical examination, and biochemical assays for Testosterone, FSH, LH, Prolactin, and Inhibin-B in the plasma and semen and were referred for TESE. Statistical study was completed by SPSS. ROC curve analyses were tested for sensitivity and specificity of serum and seminal inhibin to predict positive TESE results. **Results:** The ages of participants ranged from 32.0 to 39.0 years with a history of infertility ranging from 4.0 to 7.0 years. The incidence of diabetes and hypertension was very low. The mean levels of seminal and serum inhibin B measures were higher among fertile subjects significantly (0.001) compared to azoospermic patients. Significant differences in the means of serum inhibin B (p-0.019), but not seminal inhibin B (p-0.6) were detected according to TESE results. Inhibin B and FSH revealed a nonsignificant correlation in serum and seminal plasma of both azoospermic and fertile groups (p>0.05). Serum inhibin B showed a better sensitivity, specificity, accuracy, and predictability for positive TESE results as follows: 73.7%, 67.7%, 70.0%, and ≥41.25, respectively compared to seminal inhibin B: 73.7%, 29.0%, 46.0%, and ≥ 13.60 one-to-one. **Conclusion:** Both seminal plasma and serum inhibin B mean levels were significantly higher among fertile compared to azoospermic male groups. Inhibin B and FSH revealed a non-significant correlation in serum and seminal plasma of both azoospermic and fertile groups. There were significant differences in the means of serum inhibin B, but not seminal inhibin B, according to TESE results. This study does not support the assumption that in vivo seminal and serum inhibin B can be dedicated as a substitute in the investigations of infertile men.

Keywords
Non-obstructive Azoospermia (NOA), Seminal, Plasma, Inhibin B, FSH, Infertility, Testicular sperm extraction or (TESE), Sperm retrieval.

Infertility is among the most severe medical difficulties universally. The incidence of

infertility is increasing and it is reported that one out of every 6-7 couples suffers conceptual difficulties worldwide (Al-Bdairi, Al-Hindy, & Al-Shalah, 2021; Azarbarz et al., 2019). Infertility distresses individuals both medically and psychologically. Males constitute nearly 40% of all infertility couples (Al-Bdairi et al., 2021). Most men infertilities are resulted from testicular failure, which leads to a decline in seminal features (Soudabeh et al., 2010) that ranged from normal semen analyses to totally absent spermatozoa from the ejaculate (Azoospermia).

Azoospermia is due to severe testicular failure is either non-obstructive (NOA) or obstructive azoospermia (OA), which may be detected in less than 1% of normal men, and up to 15% of infertile men (Jarow, Espeland, & Lipshultz, 1989). In OA, sperms can be retrieved by "testicular sperm aspiration (TESA)" or "testicular sperm extraction (TESE)". In males with NOA, scarce areas of spermiogenesis may exist in the testis in about 30% of cases (Al-Bdairi et al., 2021). Hence, any alternative, noninvasive test with highly sensitive, specific, and predictive value has a broad clinical significance and can reduce more than 70% of unnecessary surgical biopsies of the testicles.

Physiologically, the hormones play a vibrant role in preserving the reproductive functions of the male, however, it is not clear how inconsistency in the concentrations of some hormones affects seminal quality (Meeker, Godfrey-Bailey, & Hauser, 2007). Earlier studies have stated that plasma levels of definite male hormones are closely linked with parameters of semen quality (Mahmoud, Comhaire, & Depuydt, 1998; Subhan et al., 1995). In general, inhibin B and FSH are known be biomarkers of spermiogenesis (Al-Bdairi et al., 2021) and Sertoli cells activity. As well, it has been proposed that evaluating their plasma levels could replace evaluating semen quality in clinical studies (Mabeck et al., 2005). Modern studies revealed that the levels of serum and seminal inhibin B may reflect testicular spermiogenesis status (Corinne, Anatole, & Jeanne, 2020; Turgut et al., 2008). The measures of blood and seminal inhibin B could be significantly different among infertile patients (both NOA and OA) (Laith Amer Al-Anbary et al., 2017). Contrarily, a Nigerian study exposed that the profile of the blood and seminal hormones were correlated non-significantly, and therefore not a useful substitute in male infertility studies (Akinloye et al., 2006).

Inhibin B is formed by Sertoli cells of the testicles and triggered by the follicle-stimulating hormone (FSH). The resultant Inhibin B applies negative feedback on FSH release. Inhibin B measures are commonly correlated with FSH levels (Corinne et al., 2020). Nevertheless, the analytic precision of FSH is restricted because some disorders do not alter FSH secretion (Barbotin et al., 2015). Both inhibin B and FSH act complementarily in male disorders and andrological analysis (Mitchell et al., 2011).

Objectives: To inspect the potential variations between the concentrations of inhibin B between serum and seminal plasma and compare their levels between fertile and infertile males, and evaluate their predictability for positive TESE outcomes.

Materials and methods

This study was an observational case-control, accomplished during the period from February to June 2021, and included 50 fertile males and 50 azoospermia subjects. The two groups presented with the primary complaining of infertility and referred to "Teba Center", Babylon City-Iraq. All applicants submitted to complete medical history, physical examination, and biochemical assays for Testosterone, FSH, LH, Prolactin, and Inhibin-B in the plasma and semen. Those who fulfill the study criteria were selected and referred for TESE.

Hormone Analyses

The hormonal evaluates of testosterone, prolactin, FSH, and LH, had been inspected by "Immuno-Enzymometric Assay from TOSOH® USA". Whereas the blood and seminal Inhibin-B measures have been tested followed by the sperm-retrieval with "chemiluminescent immunoassay (CLIA)" iFlash Inhibin-B, YHLO® Company.

Seminal collection

Seminal fluid analyses had achieved after a fresh sample of the semen was gained from the participants, and only the azoospermia subjects were enrolled in this study. Seminal fluid was collected into a sterile tube at the laboratory of the hospital. Patients were educated to withhold sex for no less than 72 hours before the semen sampling. The samples were liquefied for around 20-60 minutes before performing routine semen analyses.

Testicular sperm extraction

Sperm cell retrieval had prepared from multiple testicular spots. Sterilization of the scrotum with appropriate antiseptic and protection of the surrounded areas with a sterilized cloth were performed. Then use local anesthesia to make a minor incision over the scrotal skin, and remove a small section of the testicular tissue followed by a few stitches for the opening of the testicular tissue and the incised skin. The same procedure is repeated for the other testicles if desired.

Ethical consideration:

All candidates accomplished an informed consent, reliable with the study protocol that was allowed by the institution ethical committee. The research was implemented according to the ideologies of the Helsinki Declaration.

Data Analyses

Statistical scrutiny was done by SPSS version25. Categorical parametrs were written as frequency/percentage. The continuous parametrs were written as (Means/SD). Chi-square and Fisher's Exact tests were applied to expose the association between the categorical parameters. Independent samples t-test was applied to compare between any two groups means. Pearson correlation was applied to assess the relationships between the continuous data. A *p*-value below 5% was significant in these analusis.

Results

Table-1 revealed the mean differences in age and duration of infertility according to study groups including (azoospermia and fertile patients). There were significant differences between the average duration of infertility according to the study group.

Table 1: The differences in age and duration of infertility according to the study group

Variables	Groups	N	Mean	Stan. D	Significance
Age (years)	Azoospermia	50	34.20	7.24	0.518
	Fertile patients	50	35.14	6.11	
Duration (years)	Azoospermia	50	6.96	4.59	0.047
	Fertile patients	50	5.24	3.93	

Table 2 shows the association between study variables (smoking, type of infertility, hypertension, and diabetes mellitus) and study groups including (azoospermia and fertile patients). There was significant associations between the infertility type and the study group. The majority of azoospermic males were complaining of primary infertility in this study.

Table-2: The association between study variables and study group

Variables	Groups		Total N (%)	P-value
	Azoospermia	Fertile patients		
Smoking				
Yes	33 (66.0)	26 (52.0)	59 (59.0)	
No	17 (34.0)	24 (48.0)	41 (41.0)	
Total	50 (100.0)	50 (100.0)	100 (100.0)	0.155
Type of infertility				
Primary	46 (92.0)	25 (50.0)	71 (71.0)	
Secondary	4 (8.0)	25 (50.0)	29 (29.0)	
Total	50 (100.0)	50 (100.0)	100 (100.0)	<0.001
Diabetes mellitus				
Present	1 (2.0)	2 (4.0)	3 (3.0)	
Absent	49 (98.0)	48 (96.0)	97 (97.0)	
Total	50 (100.0)	50 (100.0)	100 (100.0)	1.000
Hypertension				
Present	0 (0.0)	0 (0.0)	0 (0.0)	
Absent	50 (100.0)	50 (100.0)	100 (100.0)	
Total	50 (100.0)	50 (100.0)	100 (100.0)	-

The study exposed significant differences between the means of inhibin B among seminal and serum between azoospermia and fertile patients (table-3). Both levels were significantly higher among fertile subjects compared to azoospermic patients.

Table 3: The differences between seminal plasma inhibin and serum inhibin according to the study group

Variables	Groups	N	Mean	Stan. D	Significance
Serum inhibin (pg\ml)	Azoospermia	50	69.02	64.29	<0.001
	Fertile patients	50	359.90	168.14	
Seminal inhibin B (pg\ml)	Azoospermia	50	29.20	34.57	<0.001
	Fertile patients	50	396.28	536.37	
	Fertile patients	50	1.25	2.1	

This study revealed non-significant correlation between serum and seminal levels of inhibin B among Azoospermia and the fertile group (table-4).

Table-4: The correlation between seminal and serum levels of inhibin B among the Azoospermia and Normospermia study groups.

		Azoospermic infertile patients	Normospermic fertile patients
Serum and Seminal Inhibin B	*r*	0.161	0.233
	P	0.264	0.104
Serum inhibin B and FSH	*r*	-0.583	0.049
	P	0.001	0.071
Seminal inhibin B and FSH	*r*	-0.380	0.142
	P	0.007	0.151

The mean differences of seminal inhibin and serum inhibin according to TESE results including (positive and negative) among the Azoospermia group of patients were exposed in table-4. There were significant differences in the serum inhibin B, but not seminal inhibin B, according to TESE results.

Table 4: The differences between seminal inhibin and serum inhibin according to TESE results

Study variables	TESE	N	Mean	SD	P-value
Serum inhibin B (pg\ml)	Positive	19	95.84	65.20	0.019
	Negative	31	52.59	58.86	
Seminal inhibin B (pg\ml)	Positive	19	25.93	37.77	0.606
	Negative	31	31.20	32.95	

Table 5: The mean differences in study variables including (FSH, LH, Prolactin, and testosterone) according to TESE results including (positive and negative) among the Azoospermia group of patients. There were significant differences between means of LH and FSH according to TESE results.

Table 5: The mean differences of FSH, LH, Prolactin, and testosterone according to TESE results

Study variables	TESE	N	Mean	SD	P-value
FSH	Positive	19	11.15	9.52	0.009
	Negative	31	20.38	12.59	
LH	Positive	19	4.33	2.41	0.001
	Negative	31	8.25	5.61	
Prolactin level	Positive	19	9.15	4.38	0.836
	Negative	31	9.47	5.78	
Testosterone level	Positive	19	368.11	170.07	0.127
	Negative	31	275.46	222.93	

Figure 3 shows the ROC curve for sensitivity and specificity of serum and seminal inhibin (pg/ml) to predict positive TESE results. Serum inhibin reveals (AUC=0.719, P=0.01), 95% CI (0.576-0.862) and the optimal cut off value to predict positive TESE test was (≥41.25) (sensitivity=73.7%, specificity=67.7% and overall accuracy=70.0%). Seminal inhibin (AUC=0.475, P=0.764), 95% CI

(0.313-0.636) and the optimal cut off value to predict positive TESE test was (≥ 13.60) (sensitivity=73.7%, specificity=29.0% and overall accuracy=46.0%).

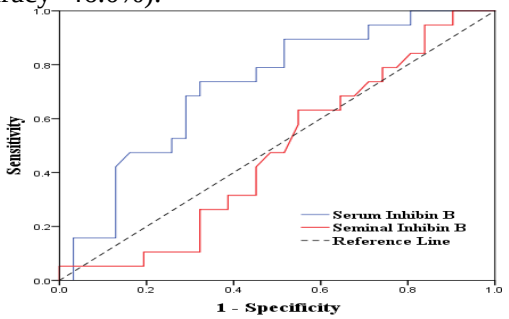

Figure 3: ROC curve for specificity and sensitivity of seminal plasma and serum inhibin B to predict positive TESE

Discussion

The etiology and developmental particularities of azoospermia, and the mechanisms of its progress, remain essential issues for inspection to enhance the diagnosis, prevention, and therapy of male infertility. The present study investigates serum Inhibin B values and their associations with seminal inhibin B among patients with azoospermia compared with fertile subjects as well as evaluates their predictability for positive TESE outcomes. The main outcome of this study was that both seminal and serum inhibin B mean levels were significantly higher among fertile compared to azoospermia patients. Moreover, there was non-significant correlations between the serum and seminal inhibin B among azoospermia and fertile groups.

Consistent with our findings were the results published by a previous Chinese study though their sample size was small (Hu et al., 2003), and Japanese investigators reported lower serum levels of inhibin B compared to seminal levels (Saito et al., 2007). A Nigerian study also displayed a non-significant correlated hormonal profile of serum and semen among infertile males (Akinloye et al., 2006).

In the meantime, a previous Iraqi study revealed a highly significant difference between seminal and serum inhibin B in azoospermia and significant differences in normozoospermia and no significant differences in patients with oligozoospermia (Laith Amer Al-Anbary et al., 2017). On the other extreme, Katsuyuki et al. (2007) in a survey that included 81 infertile patients, revealed a strong correlation between serum and seminal inhibin B (Saito et al., 2007).

To our knowledge, this is the first survey in Iraq, which compares serum measures of Inhibin B and their association with seminal levels among patients with azoospermia compared with fertile subjects and evaluates their results with TESE outcomes. In this study, there were significant differences in the means of serum inhibin B, but not seminal inhibin B, according to TESE results. Comparable outcomes had been reported by other scholars (Tsujimura et al., 2004). Similarly, a recent German review reported the same findings and concluded that before performing TESE, defining the hormone and genetic profile is crucial (Rosellen, Steffens, & Kranz, 2021).

Inhibin B belongs to the same family of the "transforming growth factor-β", which is a multifunctional cytokine controlling proliferation, differentiation, and additional functions in various cells (Al-Hindy et al., 2020; Dleikh et al., 2020; Mousa, Al Saffar, & Al-Hindy, 2020). Spermiogenesis is a complex physiological process that necessitates several growth factors (Griswold, 2016), hypophysial gonadotropins and testosterone, which indirectly normalize sperm cells' genesis and functions of testicles in different manners (Lee et al., 2016).

Harmonious to previous studies, negative associations between plasma Inhibin B and FSH had been reported in more than a few studies in Cameron (Katsuyuki et al., 2007), Japan (Katsuyuki et al., 2007), and China (Hu et al., 2003). Inhibin B release is triggered by the FSH secretions, which causes a negative feedbacking on FSH release and a paracrine testicular effect (Corinne et al., 2020).

Equally, Inhibin B and FSH levels are sensitive more than individually as predictors for testicular histology and the existence of spermatozoa cells (Barbotin et al., 2015). Seminal inhibin-B reflects spermiogenesis but has less sensitivity to estimate spermiogenesis than serum inhibin-B (Saito et al., 2007). Huang et al. (2012) reported that the combined measurement of seminal and serum inhibin B does not increase the analytical precision of TESE outcomes.

In contrast, significant alterations were detected between positive and negative TESE results of seminal inhibin B readings by other scientists (El Garem et al., 2002; Tunc et al., 2006). However, two previous studies revealed that plasma inhibin B levels are predictive of successful TESE among males with NOA (Ballescá et al., 2000; Soudabeh et al., 2010).

Based on the aforementioned findings, we had concluded that the hormonal profiles of inhibin B and FSH revealed a non-significant correlation in seminal plasma and serum; and henceforth cannot be applied as a dedicated substitute in the

investigations of infertile men

Conclusion

Inhibin B levels in the serum and seminal plasma were higher significantly among fertile compared to azoospermia groups. The levels of Inhibin B and FSH revealed non-significant correlations in serum and seminal plasma of both azoospermia and fertile groups. There were significant alterations in the means of serum inhibin B levels, but not seminal inhibin B, according to TESE results. This study does not support the assumption that in vivo seminal and serum inhibin B can be dedicated as a substitute in the investigations of infertile men.

Conflict of interest

The authors declared that there were no conflicts of interest.

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