DOI Number: 10.5958/0976-5506.2019.01171.9

Study the Relationship between TEX101 Protein, Inhibin B and Testosterone Hormone in Azoospermia and Severe Oligospermia in Infertile Patients

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Abstract

Background: Infertility remains a global health challenge with devastating psycho-social consequences in many Iraqi communities and an underlying long-term risk for separation of couples. Therefore, this study was aimed to determine the effect of a biochemical marker; Testis-expressed sequence 101 protein(TEX101) and relation with Inhibin hormone and testosterone on infertile couples of (Middle Euphrates center) in Iraqi population. **Method**: The study involved analysis of semen parameters in accordance with the criteria set by World Health Organization in the Fertility Centre in Al-Sadder Teaching Hospital in Al-Najaf/ Iraq and postgraduate Laboratory at Biology Department/ College of Science, University of Kufa during the period from 1st, April 2018 to 30th, August 2018 of (53)Azoospermia infertile patients samples, (50) severeoligospermia samples and (50) samples of fertile subjects (control group). The biochemical marker was achieved by ELISA method. **Results**: The result of current study of biochemical markers showed decrease ($P<0.05$) in TEX101,Testosterone hormone and inhibin B in azoospermia. Also, the study showed that TEX101, Inhibin B and Testosterone were positively associated with sperm concentration, sperm motility and normal sperm morphology. **Conclusion**: TEX101 positively related with Inhibin B and testosterone hormone and semen normal parameters.

Keywords: *TEX101, Inhibin B, Testosterone, Azoospermia, infertility.*

Introduction

TEX101 protein (encoded by testis expressed 101 gene, *Tex101*) was originally identified by [(1)]. TEX101 is a testicular germ cell-specific protein predominantly located on the plasma membrane of germ cells during gametogenesis. TEX101 is not expressed in any other human tissue or cell type, including Sertoli and Leydig cells of the testicular tissue [(2)]. TEX101 is present on the cell surface during all stages of spermatogenesis. Moreover, [(3)] intended to examine the fate of mouse TEX101 during sperm transport through the male reproductive tract. They reported that TEX101 is eventually cleaved and released from the cell surface of epididymal sperm while it passes through the caput epididymis. Spermatozoa are, inarguably, the most highly differentiated cell type of the human body, numerous distinct processes need to be completed for generating mature and functional spermatozoa that have the ability to fertilize the oocyte based on the fact that TEX101 accompanies sperm, either anchored to its membrane, or shed into seminal plasma (SP) [(4)]. The SP proteome of healthy fertile men before and after vasectomy. Furthermore, [(5)] identified a TEX101, had the top candidates for developing biomarkers of vasectomy success. In the follow-up study, 30 of those biomarker candidates were verified in pre- and post-vasectomy SP samples as well as SP from patients with non-obstructive azoospermia (NOA), several testis-specific proteins, such as TEX101, were identified as key male infertility biomarkers [(2)].

Germ cells development is dependent on the balanced endocrine interplay of the hypothalamus, the failure of

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pituitary to secret Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) will result in disruption of testicular function to secrete testosterone leading to imbalance of spermatogenesis [6]. In addition, serum inhibin B has emerged as a sensitive marker of male fertility [7]. FSH induces the Sertoli cells to secrete inhibin and androgen-binding protein and plays a major role in initiation and progression of spermatogenesis. Inhibin acts as an FSH inhibitor secreted from the Sertoli cells, while activin is secreted by the Sertoli cells and the pituitary gland and stimulates FSH secretion [8].

Material and Method

Semen specimens were collected from patients and controls after 3-5 days of sexual abstinence directly in a dry, clean and sterile disposable container by masturbation in a quiet room adjacent to the laboratory of seminal fluid analysis, analysis and classification of infertile patients was performed according to [9]. After analysis the liquefied specimens were examined under microscope device then samples were centrifuged (3000rpm/min) for 5 minutes to separate the plasma from other components of the semen. The seminal plasma was withdrawn by micro pipette and then placed in Eppindorf tubes for making biochemical tests and stored at -20ºC, the blood samples were obtained from persons by withdrawing 3ml of blood by using sterile medical syringes from brachial vein. Samples were centrifuged (3000rpm/min) for 5 minutes to separate the serum, then measured TEX101 in semen and testosterone, Inhibin B in serum by immunological technique (Enzyme-Linked-Imuno-Sorbent- Assay) by using ELISA device.

Statistical Analysis

The well- known statistical system (Graph Pad prism ver. 5) was adopted, and the analysis of variance table one – way anova (by Tukey's multiple comparisons test) was used for the comparison among subdivided groups in the measured parameters. The results were expressed as (Mean±Stander Error). Correlation coefficients were calculated to estimate the correlation between markers and parameters. The descriptive statistics and correlation coefficients were performed by using mega stat (Version v 10.12) for excel 2007 [10].

Results

Seminal fluid analysis of semen specimens showed a significant difference ($P<0.05$) in some parameters (Table 1).The results indicated a significant decrease ($P<0.05$) in the sperm concentration and volume ejaculation in infertile patient Azoospermia and Severe Oligozoospermia compared with control group. In addition, significantl decrease ($P<0.05$) in sperm motility and normal sperm morphology in Azoospermia and severe Oligospermia compared with control group. Also, a significant increase ($P<0.05$) in the round cells in both Azoospermia and severe oligospermia in comparison with controls (Normospermia).

Table (1) Seminal fluid parameters in infertile and fertile males

Semen and Sperms Parameters	Control group N=50	Severe oligo N=50	Azoospermia N=53	P value
Volume (ml)	3.3±0.11 a	2.0±0.13 b	1.6±0.07 C	p < 0.05
PH	7.4±0.02 a	7.8±0.02 A	6.5±0.03 B	p < 0.05
Concentration (Sperm/ml.)×10^6	77.6 ± 3.9 a	4.755±0.5 C	0.0 C	p < 0.05
Sperms Progressive motile (%)	69.0±1.5 a	23.1±1.6 C	0.0±0.0 B	p < 0.05
Sperm normal morphology(%)	62.5±2.5 a	7.7±0.8 b	0.0±0.0 c	p < 0.05
Round cell concentration ×10^6 cells	1.18±0.15 a	2.47±0.23 b	3.16±0.17 c	p < 0.05

Values were mean ±SE. Total number of patients= 103.

The results of statical analysis showed a significant decrease (P<0.05) of TEX101 protein level in infertile men Azoospermia (148.5±13.7) and Severe oligospermia (180±19.1) compared with control group (339.7 ± 11.7 a) figure (1). Also showed a significant decrease (P<0.05) of Inhibin B level in Azoospermia (6.4±0.28) and severe oligospermia (8.3±0.3) compared to Inhibin B level of control group (12.2±0.34) (Figure 2). Significant decrease (P<0.05) of testosterone level in Azoospermia (2.26± 0.22) and severe oligospermia (3.9±0.32) compared to testosterone level of with fertile men controls (7.31±0.14).

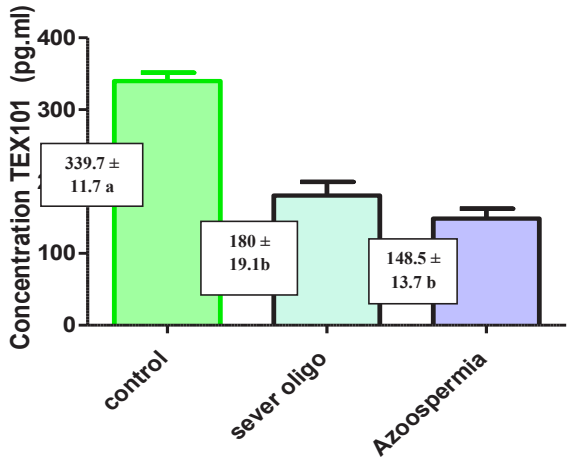


Figure (1) Level TEX101 protein in the seminal plasma of infertile men and fertile men (control)

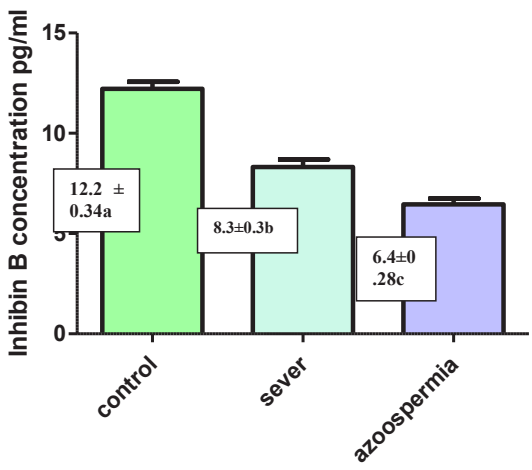


Figure (2) Inhibin B level in serum of infertile men and fertile men (control).

The study showed presence of positive correlation between TEX101 markers with sperm concentration, sperm progressive motile and sperm normal morphology (Figure 3).

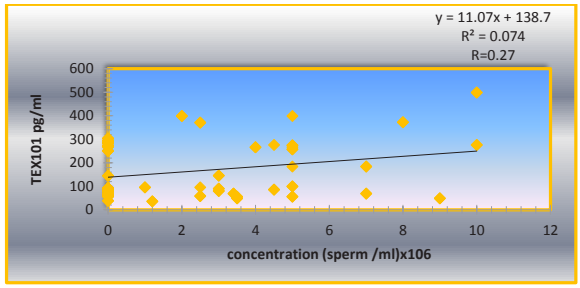


Figure (3) Correlation between TEX101 concentration and sperm concentration.

The study showed positive correlation between Inhibin B markers with sperm concentration, sperm progressive motile and sperm normal morphology, respectively, (Figure 4). Also, the results showed positive correlation between testosteron hormone with sperm concentration, sperm progressive motile and sperm normal morphology (Figure 5).

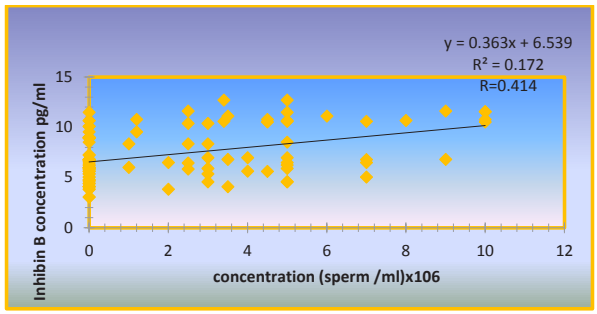
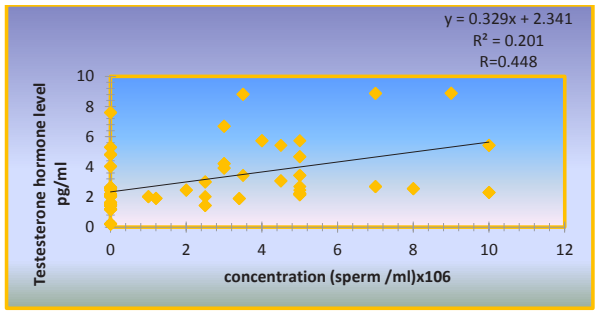


Figure (4) Correlation between Inhibin B and sperm concentration of infertile men.



Figure(5) Correlation of Testosterone and sperm concentration of infertile men.

The study showed the presence of a positive correlation betweenTEX101 proteins, Inhibin B, Testosterone hormone (Figure 6) .

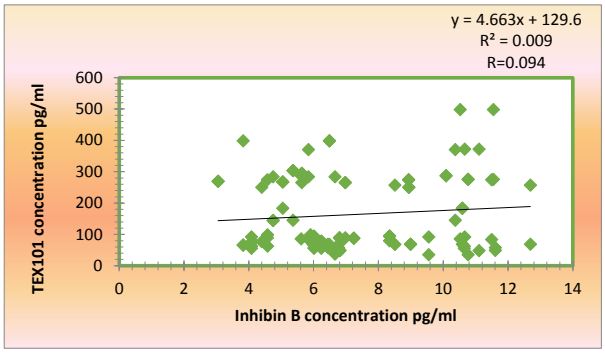


Figure (6) The correlation between TEX101 and Inhibin B hormone.

Discussion

The results showed a significant decrease (P<0.05) of TEX101 protein level in infertile men azoospermia and severe oligospermia compared with fertile control group and showed positive correlation between sperms concentration, progressive motile, sperms normal morphology and TEX101 level these results may be effected in the protein synthesis that comes from a defect in the gene expression which is responsible for the protein production . Studies have confirmed that the TEX101 is essential for human fertility. Spermatogensis processes need to be completed for generating mature and functional spermatozoa that have the ability to capacitating processes, based on the fact that TEX101 accompanies sperm, either anchored to its membrane, or shed into seminal plasma [3].

A study by [11] showed that in different patients, Azoospermia, Oligospermia men infertility was diagnosed and found to alter or lack the TEX101 gene, this provided a proof that TEX101 is a germ cell marker glycoprotein involving in gametogenesis [12]. It is later found on spermatocytes, spermatids and testicular sperms after the onset of puberty and is shed as sperms that pass the caput epididymis [3].

The results agreed with a study by [13] showed a positive correlation between TEX101 protein expression and semen and sperm parameter TEX101 protein expression which was positively significantly correlated with sperm concentration, sperm progressive motility in addition to sperm normal morphology. This fact suggested that TEX101 expression per germ cell may vary in different individuals, the fraction of TEX101 cleaved from the surface may vary or TEX101 was released into SP not only by epididymal spermatozoa, but also by testicular germ cells .

Normal expression of the TEX101 is associated with progressive motility and acrosome reaction, while abnormal channel expression may be involved in the pathogenesis of Azoospermia ,specifically, disruption of the TEX101genes in mice causes male infertility with findings of immotile spermatozoa and failed hyperactivated motility in unexplained infertile and asthenospermia. These studies found that TEX101 protein has significant decrease (P<0.05) in infertility patients especially in azoospermia compared with healthy men [12,14].

The present study agreed with [15] revealed that inhibin B levels are significantly reduced in men with infertility problems, irrespective of etiology, compared with fertile men. So that, the present study also agreed with them when said that inhibin B levels are more sensitive markers of male factor infertility than other available hormones. These results may be due to the differences in the etiology of infertility such as external factors or internal histological or physiological factors.

The present study showed positive correlation between Testosterone hormone with TEX101 marker. Proteins deficiencies are the most promising molecules to develop infertile biomarkers. Alternations in protein abundance and activity in different physiological states reflect dynamic alternations which may hardly be predicted at the genome level [16]. LH stimulates the production of testosterone by the Leydig cells, and in turn acts on the sertoli and peritubular cells of the seminiferous tubules to stimulate spermatogenesis. Testosterone is essential for growth and division of germinal cells in forming spermatozoa [17].

TEX101 mutations of the X chromosome were recently noted in array comparative genomic hybridization study, affecting germ cells in testis, caused meiotic arrest, disruption of hormone function and azoospermia [18]. TEX101 codes for a testis-specific meiotic protein that regulates DNA double-strand break repair and has proven essential to normal spermatogenesis in mouse models [19].

Conclusions and Recommendations

The results concluded that TEX101 related positively with testosterone and Inhibin B hormone and semen normal parameters included sperm concentration, motility and normal sperm morphology. It is recommended to search for genetic study of TEX101

mutation and histological and physiological effects on sexual hormone imbalance in males.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding.

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