

Seroprevalence of Transforming Growth Factor- β 1 and some hormones on Male Infertility in Nasiriya City, Iraq

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Abstract: Male infertility is a problem that affects 10–15% of males in reproductive age. The current study aimed to detect the role of Transforming Growth Factor- β 1 (TGF- β 1) cytokine and some hormones in infertility outpatients males at Al-Nasiriya Teaching Hospital/Infertility Unit/Thi-Qar Governorate, Iraq. During the period from September 2022 to March 2023, 88 Serum samples from (76 infertility males and 12 control) were collected to assay the levels of TGF- β 1, Testosterone, follicle Stimulating and Prolactin hormones by Sandwich-ELISA technique. Infertility patients were distributed to primary and secondary infertility type with a 66(86.84%) and 10 (13.016%) patient, respectively ($P \leq 0.01$). All infertility patients showed an elevated mean sera levels of TGF- β 1 with (0.734 ± 0.45 ng/ml), as compared with controls (0.670 ± 0.393 ng/ml). No statistical differences were presented between the two groups included in present study regarding this cytokine ($P > 0.05$). The levels of Testosterone, follicle Stimulating Hormone and Prolactin were (3.12 ± 0.76 , 7.69 ± 3.67 , 15.65 ± 2.75), (3.69 ± 1.15 , 5.77 ± 0.89 , 13.23 ± 0.57) in primary and secondary infertility types compared to control was (6.08 ± 1.42 , 7.08 ± 0.73 , 11.37 ± 1.15). The present study results may shed light the role of TGF- β 1 and some hormones as a serological and diagnosis tool in male infertility. This finding may be contributes as a useful tool in disease classification and assessment.

Keywords: Infertility, TGF- β 1, ELISA, Reproductive hormones

I. INTRODUCTION

World Health Organization (WHO) defines infertility as failure to conceive after at least 12 months of regular unprotected sexual intercourse. Infertility is a big health problem worldwide and estimated to affect 8–12% of

couples in reproductive age group [1-2]. The American Society for Reproductive Medical (ASRM) considers infertility as a disease that causes disability such as impairment of function. Diagnostic inference testing should begin without delay when presented in any patient with a medical history, reproductive or sexual history, physical data, and elderly age indicating possible impairment of reproductive function [3].

In male gonads, cytokines are produced physiologically and involved in their normal function; the influence of cytokines in the reproductive system is becoming increasingly important. In consequence, they must also appear as normal components of seminal plasma [4].

TGF- β 1 family contains more than 40 proteins, and their names differ due to the different ways of diagnosing them. This family discovered in humans is 1-3 and encoded by genes that differ from one to another and it is a pleiotropic cytokine which is mainly synthesized and released by regulatory T cells. TGF- β 1 is purified from human platelets, a rich source of this protein. In addition to platelets, TGF- β 1 is also generated in other blood elements from semen plasma and circulating white blood cells. However, more than many years after the initial isolation of TGF- β 1, there is no consensus on the amount of TGF- β 1 present in normal human plasma. The physiological significance of TGF- β 1 in reproductive biology and productivity has been extensively examined in TGF- β 1 null mutant mice, TGF- β 1 found in the somatic cells and germs cells in the testis. These cytokines play an important role in male reproductive system by providing physiological features of the testes [4-6].

Male fertility depends on availability of potent androgen called Testosterone (TES). Testosterone production is regulated by hypothalamic anterior pituitary axis. Two anterior pituitary Follicle Stimulating Hormone (FSH) and



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Luteinizing Hormone (LH) are involved in spermatogenesis and testosterone production [7]. Prolactin (PRL) hormone secreted by pituitary, controls production of LH and FSH by regulation of Gonadotropin releasing hormone (GnRH) through feedback mechanism on the hypothalamus. Ultimately, altered levels of serum hormones may create disturbances in spermatogenesis and cause infertility in males [8].

II. PATIENTS AND METHODS

A total of (n=88) serum samples obtained from 76 Iraqi infertility patients and 12 apparently healthy fertile males were considered as a control whom consulting Al-Nasiriya Educational Hospital / Infertility unit, Southern Iraq, during the period from September 2022 to March 2023. All samples were secured for TGF- β 1 by Sandwich-ELISA assay. TGF- β 1 in biological samples usually exist in inactivated forms and activated before testing. The Hormones Testosterone, Follicle Stimulating Hormone and Prolactin (FSH, Testosterone, and PRL) are Measured by fully automated quantitative test for use with Auto analyzer cobas e411 instruments system as which used for the determination of hormones in human serum using Electrochemiluminescence technology. Statistical analysis proceeded in all groups of the study, descriptive statistics were analyzed by (Chi-square), independent t-test were performed using mean and standard deviation (SDs) for continuous variables and *p* values of 0.05 and 0.01 were considered to be significant. All analysis was performed with statistical package for Social Sciences (SPSS) (version 23.0 SPSS Inc., Chicago, 111).

The study was gained the ethical permission from Thi-Qar Health Directorate via their agreement code No. 336/2022.

III. RESULTS AND DISCUSSION

Most of Infertility patients, with highly significant differences, were diagnosed as primary infertility with 66(86.84%) and only 10(13.016%) patients were with secondary infertility ($P \leq 0.01$) (Fig. 1). These results may be due to management of sexually Transmitted Infections (STI) and infections following abortion or first pregnancy[9]. Primary infertility is inability to become pregnant, and secondary infertility is occurrence of pregnancy or the birth of at least one child. Primary and secondary infertility resulted from the intersection of many different demographic features and factors, including duration of marriage, and socioeconomic status, the advanced age of the couple can affect the chances of having a new child. In primary infertility, most men with high to medium economic status and social status can contribute to solving infertility problems as this can affect quick access to many diagnostic methods and use of various methods for infertility treatment [10].

The dominance of primary infertility in the results of the present study were in compatible with other similar local studies whom founded that the most infertile male had primary type with 153 (76.5%), 36(78.3%) and 84%; in

contrary to whom suffering from secondary infertility type with an occurrences of 47 (23.5%), 16(21.7%) and 16%, respectively [11-13].

As seen in Table (1), all infertility patients had shown elevated mean sera levels of TGF- β 1 (0.734 \pm 0.45 ng/ml), as compared with the controls (0.670 \pm 0.393 ng/ml). No statistical differences were shown between two groups included in current study regarding this cytokine ($P > 0.05$).

TGF- β 1 is a peptide growth factor distributed in germ cells at different levels in the testis, regulating function of testes and maintaining the stability of testis environment by acting on several cells. Its elevated levels were found in infertile individuals, and in male partners in couples with recurrent involuntary abortion. TGF- β 1 in normal range regulates a variety of cellular processes, including the secretory function of Leydig and Sertoli cells, testis development and spermatogenesis. TGF- β 1 plays a role in directing germ cells to apoptosis in high concentration [14-15]. TGF- β 1 can lead to testicular disease and can lead to reduced fertility in men because signaling is indispensable for testicular formation [16-18]. Sertoli cells form blood testis barrier (BTB) known as Sertoli cell barrier, which functions to provide nutrient, structural support for developing germ cells and protect from attack by adaptive immune cells [19-20]. It is that tight junctions formed by adjacent Sertoli cells are a major component of the blood testis barrier and it plays an important role in maintaining the microenvironment of spermatogenesis in the testes. The testis is the main male reproductive organ (spermatogenesis) and synthetic androgen, which produces sperm, mainly testis. Sertoli cells are described as key cells for spermatogenesis [21-22]. Target cells for the action of TGF- β 1 are Sertoli cells [23]. TGF- β 1 regulates the secretion of immature testicular cells, suggesting TGF- β 1 involved in natural spermatogenic epithelial development [24]. TGF- β 1 expression was observed in normal sperm cells and Sertoli cells. The testicular stroma mainly includes Leydig, macrophages and other immune cells [25]. This cytokine was mainly expressed in Leydig cells these cells can regulate of testicular immune microenvironment and natural spermatogenesis by secreting TGF- β 1 [26-27]. TGF- β 1 expression can be seen in tight junctions between Sertoli cells, suggesting play an important role in maintaining structural stability of fine tubules, cytokines exert profound effects on lymphocytes and macrophages [28-29], but depend on phase of the inflammatory response and micro environmental context, TGF- β exerts proinflammatory activity, acting to promote resolution phase [30]. The present study results regarding TGF- β 1 levels are also supported by other local study in Kufa, Iraq [31], whom found a TGF- β 1 levels were in infertile men (n=150) (71.24 \pm 1.19) more than levels in fertile men (n=26)(68.3 \pm 0.52). Outside Iraq, the present results also in agreement with study, in Ukraine, whom founded elevated TGF- β 1 levels in infertile males (n = 55) compared to health males (n=27) [32].

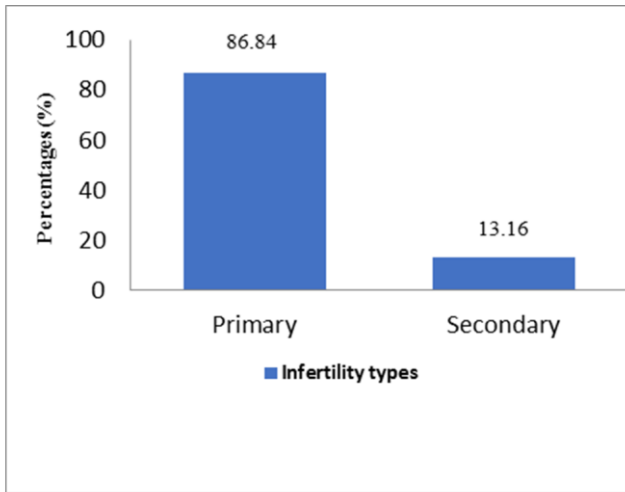


Figure. 1: Percentages of infertility types.

Table (1) : Level of TGF-β1 in infertility patients.

Group	No.	TGF-β1 Mean (ng/ml) ± SD
Patient	76	0.734 ± 0.45
Control	12	0.670 ± 0.393
T-value 0.18 p-value 0.85		

The current study data showed a significant decrease in the level of Testosterone (TES) levels in primary and secondary infertility were (3.12 ± 0.76), (3.69 ± 1.15) compared with control (6.08 ± 1.42), whereas the levels of FSH and PRL increased significant in primary infertility were (7.69 ± 3.67), (15.65 ± 2.75) compared to secondary patient were (5.77 ± 0.89 and 13.23 ± 0.57) as shown in Table 2 ($p \leq 0.05$).

Table (2): Hormonal changes parameter between primary and secondary infertility (mean ± SD).

Hormone Group	TES (ng/ml)	FSH(IU/ml)	PRL (ng/ml)
Primary	$3.12 \pm 0.76c$	$7.69 \pm 3.67a$	$15.65 \pm 2.75a$
Secondary	$3.69 \pm 1.15b$	$5.77 \pm 0.89b$	$13.23 \pm 0.57b$
Control	$6.08 \pm 1.42a$	$7.08 \pm 0.73ab$	$11.37 \pm 1.15c$
L.S.D	0.39	1.39	1.05

*Results represent mean ± Standard deviation (S.D)

* Means having different letters in the same column differed significantly ($P \leq 0.05$).

* (L.S.D) least significant difference.

Increase levels of serum PRL detrimental effect on male reproduction by inhibiting of pulsatile release of gonadotropins from anterior pituitary gland and direct effect on spermatogenesis [33].

The current results are relatively agreement with two local studies that found hormone levels vary with different types of infertility, noted that males with primary infertility had hormone levels (FSH 10.1 ± 3.50 , 4.01 ± 1.73 and testosterone 2.62 ± 0.6 , 4.01 ± 1.49), while secondary infertility were (FSH 6.53 ± 1.80 , 4.29 ± 1.6 and TES (2.70 ± 0.7 , 4.29 ± 1.09) [12, 13]. Other local study in Nasiriya, Iraq founded that TES decrease in total patients that reach (1.38 ± 0.85) and (7.79 ± 0.62) in control. But level of PRL increased in patient (37.28 ± 3.50) and (2.99 ± 0.65) [34-38].

IV. CONCLUSIONS

Infertility continues to be one the most socio health problems which need a lot of diagnosis and treatment. TGF-β1 cytokine and hormone like TES, ProL and FSH may contribute as a diagnostic tool in disease classification and assessment.

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