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Evaluation of Inhibin-B Hormone Levels and Their Relationship with Interleukin-2 and Selected Antioxidant Enzymes in Infertile Males in Kirkuk, Iraq

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Abstract

Background: Infertility affects approximately 15% to 20% of couples around the world, male infertility contributing to 20–50% of cases, and azoospermia, which affects 1% of men. Non-obstructive azoospermia and oligospermia are key causes of male infertility. This study evaluated serum Inhibin-B, IL-2, and antioxidant enzyme levels in these patients.

Methods: Serum levels of Inhibin-B, IL-2, Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPX1) were measured using enzyme-linked immunosorbent assay (ELISA), and mean levels were compared between the non-obstructive azoospermic, oligospermic, and control groups.

Result: The findings indicated a notable reduction in Inhibin-B levels ($p < 0.05$). GPx1 and SOD levels also exhibited a significant decrease ($p < 0.05$). IL-2 levels were significantly elevated ($p < 0.01$) in non-obstructive azoospermic patients, whereas no significant difference in IL-2 was observed in oligospermic patients compared to controls.

Conclusion: The study highlights that azoospermic and oligospermic males exhibit reduced levels of Inhibin-B and antioxidant enzymes (SOD, GPX1), with IL-2 elevation restricted to azoospermic patients. These findings point to impaired spermatogenesis linked to Sertoli cell dysfunction, oxidative stress, and inflammation, suggesting their potential as supportive biomarkers; however, their measurement alone is insufficient to accurately assess reproductive function, particularly concerning semen quality as a biomarker of male infertility.

Introduction

Male infertility constitutes a multifaceted condition influenced by environmental, lifestyle, and genetic variables. It affects approximately 7% of the male population worldwide and is estimated to account for 20–50% of infertility cases in couples, with variation according to study populations and diagnostic criteria [1]. The causes of infertility are diverse. It can involve issues with sperm production or quality, as well as hormonal imbalances and environmental factors. With advancements in knowledge and technology, a substantial number of cases of male infertility still lack clear explanations, making it difficult for doctors to diagnose and treat effectively [2].

Various factors can impact infertility, such as issues like Y chromosome microdeletions and chromosomal translocations, as well as mutations in individual genes. The hormonal balance is also crucial. Disruptions in the hypothalamic pituitary gonadal (HPG) axis can worsen the condition by affecting testosterone and hormones like FSH and LH that are essential for sperm production [3,4].

Infertility rates vary by region; the highest incidence is found in areas of Africa and Central and Eastern Europe, where up to 12% of men face fertility challenges. Environmental and social stressors, including the impact of conflict, lifestyle changes, and psychological pressure, may have contributed to a possible rise in infertility rates in Iraq between 2000 and 2016 [5,7]. Additional factors such as employment stress, dietary habits, and genetic predispositions may also play a role. Smoking, in particular, has been shown to negatively affect sperm production and quality, potentially reducing fertility compared with non-smokers [6–8].

Hormonal factors play a role in fertility by influencing processes like spermatogenesis through hormones such as FSH and LH produced by the pituitary gland along with testosterone and Inhibin B - a hormone that has recently gained significance for its vital role in male reproductive health as it acts as the primary form of inhibin regulating spermatogenesis and serves as an essential biomarker, for male fertility status [9, 10].

Azoospermia is a condition characterized by the complete lack of sperm in the ejaculate, which poses a significant challenge in the infertility of male patients. Azoospermia is chiefly classified into two types: obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). This study focuses specifically on non-obstructive azoospermia [11].

NOA results from testicular or endocrine malfunction, leading to pre-testicular failure, whereas OA is caused by post-testicular failure, often due to blocked genitalia or ejaculatory dysfunction [12].

This study aimed to compare serum levels of Inhibin-B, Interleukin-2 (IL-2), and antioxidant enzymes (SOD and GPX1) among infertile men with non-obstructive azoospermia, oligospermia, and healthy controls. The goal is to improve our understanding of the underlying mechanisms of male infertility and to support the development of more effective diagnostic and therapeutic approaches by analyzing these endocrine and biochemical factors [13,14].

Methods

The research involved a group of one hundred men who were unable to conceive. Among them were 30 men with azoospermia and 70 men with oligospermia. All of the patients had aberrant semen analysis. Furthermore, a control group of 20 healthy individuals was incorporated, which was obtained from the infertility consultant centre at Azadi Teaching Hospital and private laboratories in Kirkuk, Iraq.

Data was obtained from individuals between the ages of 20 and 50. The sperm count categories of patients have been used to classify them into two distinct groups.

Patients were classified based on sperm concentration according to the World Health Organization (WHO) 2010 guidelines: oligospermic patients had sperm counts < 15 million/mL, while non-obstructive azoospermic patients had a complete absence of sperm in the ejaculate [15]. This classification ensures reproducibility and standardization of patient grouping for the study.

Informed Consent and Ethical Clearance

After obtaining Institutional ethical clearance from Tikrit University's Central Scientific Ethics Committee, participants in the present study were recruited. All participants provided written informed consent prior to inclusion in the study.

Study Participants and Criteria

Inclusion Criteria

1-The individual is a male suffering from primary or secondary infertility.
2-Patients who provided written informed consent and agreed to take part in this study.

Exclusion Criteria

1-Couples were excluded if a female factor for infertility was identified.
2-Exclusion of individuals with normal fertility (i.e., control participants with known male or female factor infertility were excluded from the infertile groups).
3-Individuals with other known factors that could affect the measured analytes (e.g., severe systemic illness, recent medications, etc.) were excluded.

Sampling

The study participants provided a total of five milliliters of blood. The blood samples were subsequently transferred to gel containers and left undisturbed for 20 minutes to promote clotting. Subsequently, the gel containers were centrifuged at 4,000 rpm (depending on rotor radius) for 15 minutes to separate serum from other blood components. Serum was separated and stored in a freezer maintained at -20°C until analysis. Prior to assays, samples were thawed to room temperature and mixed gently.

Evaluation of Inhibin-B Hormone level and Interleukin-2, Superoxide dismutase (SOD), GPX1 (Glutathione peroxidase) serum concentrations

Commercially available reagents were used to measure the concentrations of Inhibin B, Interleukin-2, Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPX1). To achieve this goal, in identifying infertility issues in patients, the enzyme-linked immunosorbent assay (ELISA) method was utilized. The diagnosis of infertility involved collecting history from patients as well as performing a range of diagnostic assessments, such as semen analysis, alongside hormone evaluations. For antioxidant enzymes (SOD and GPX1), concentrations were reported in ng/mL according to the ELISA kit instructions, representing protein concentration rather than enzymatic activity.

Demographic Characteristics

The study collected information primarily related to the demographic characteristics of the individuals involved. This included details like their age and smoking behaviours as documented in their records.

Statistical evaluation

The findings underwent analysis through ANOVA testing in Minitab software. Post-hoc comparisons were performed using Duncan's multiple range test; however, this test is less robust than alternatives such as Tukey's HSD, especially with unequal group sizes. Differences were considered statistically significant at $p < 0.05$ and $p < 0.01$.

Results

In contrast to the other groups, the serum concentration of Inhibin-B in male patients with infertility identified as having azoospermia and oligospermia decreased statistically significantly, as illustrated in Figure 1 and Table 1. This discovery is corroborated by a probability level ($P < 0.05$).

Figure 2 and Table 1 demonstrate a considerable rise in serum levels of IL-2 in patients with azoospermia. The statistical analysis indicated significant discrepancies across the different groups, as demonstrated by a probability level ($P < 0.01$).

Furthermore, the current investigation demonstrated, as depicted in Figure 3, Figure 4, and Table 1, that a statistically significant decrease was observed at a probability level ($P < 0.05$) in the serum level of Glutathione peroxidase or Superoxide dismutase among infertile patients.

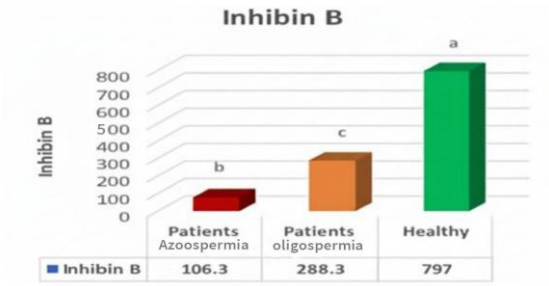

Figure 1: Assessment of Serum Inhibin B levels (pg/mL) in studied groups.

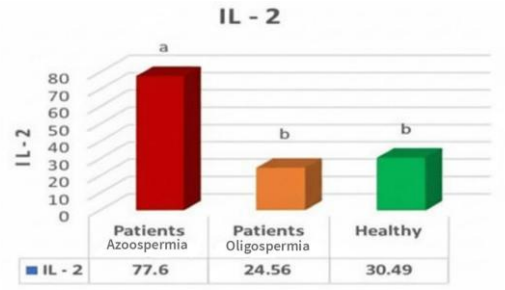

Figure 2: Assessment of Serum IL-2 levels (pg/mL) in studied groups.

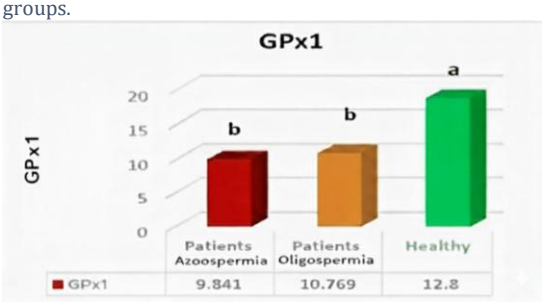

Figure 3: Assessment of Serum Glutathione peroxidase levels (ng/mL) in studied groups.

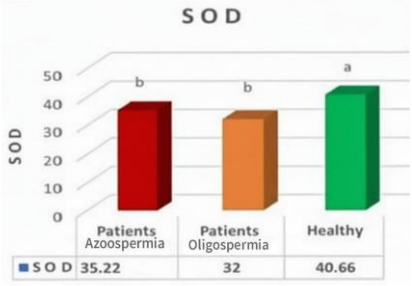

Figure 4: Assessment of Serum Superoxide dismutase levels (ng/mL) in the studied groups.

NO.	Groups	N	Inhibin-B (pg/ml)	GPX1 (ng/ml)	SOD (ng/ml)	IL-2 (Pg/ml)
1	Azoospermia	30	$106.3^{b} \pm 20.8$	$9.841^{b} \pm 2.056$	$35.22^{b} \pm 4.28$	$77.60^{a} \pm 7.02$
2	Oligospermia	70	$288.3^{c} \pm 27.3$	$10.769^{b} \pm 2.111$	$32.00^{b} \pm 4.05$	$24.56^{b} \pm 3.75$
3	Control	20	$797.0^{a} \pm 37.2$	$12.80^{a} \pm 3.360$	$40.66^{a} \pm 7.17$	$30.49^{b} \pm 3.81$
4	Overall P-value		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.01$

Table 1: Hormone and Enzyme Levels in Infertile and Control Males Based on Infertility Type
Results are presented as Mean ± Standard Deviation (SD). Groups include Azoospermia (group 1, N=30), Oligospermia (group 2, N=70), Control (group 3, N=70).
*Different superscript letters indicate significant differences between groups for that analyte ($p<0.05$ or $p<0.01$).
$P<0.05$: a,b,c indicate significant differences between groups at $p<0.05$.

Discussion

This study confirms that infertile men with azoospermia and oligospermia have significantly lower serum Inhibin-B levels compared with healthy males, which reflects Sertoli cell dysfunction. Inhibin B, which is released by Sertoli cells in response to FSH, plays a crucial role in regulating FSH secretion in humans. The evaluation of serum inhibin B concentrations has become a dependable indicator of Sertoli cell activity and spermatogenesis [16]. Reactive oxygen species levels can rise as a result of tobacco use, both internally and externally. The main cause of this is the high concentration of lead or reproductive toxicants in tobacco products, which lowers inhibin B levels [17-19]. The current study demonstrated a substantial rise in the serum levels of interleukin-2, which is considered a pro-inflammatory cytokine, in individuals with azoospermia in comparison to fertile males, and is consistent with the previously conducted research by Gu et al. (2020) [20].

Cytokines significantly influence the control of spermatogenesis. Interleukin-2 (IL-2) is a type of cytokine that serves as a messenger within the body's immune system, playing a role in regulating the function of blood cells like lymphocytes, which are essential for the immune response [21].

The body's natural response to infections depends on interleukin-2 (IL-2). An important characteristic of the system is its capacity to differentiate between what belongs to the body and what doesn't [22]. Lymphocytes express IL-2 receptors, which mediate their actions [23].

T cells from the thymus require IL-2 to grow, proliferate, and become 'effector' T cells [24]. T cells typically produce interleukin-2 (IL-2) during an immunological response. The immunological response, which is aided by T cells, is the key driver of the inflammatory process in the testis [25]. Antigens attaching to the T cell receptor (TCR) release interleukin-2 and induce IL-2 receptor synthesis. The presence of IL-2 receptors in human testicular germ line and ejaculated sperm indicates IL-2 could play a role in testicular function [26].

The researchers found that oligoasthenozoospermic men had lower superoxide dismutase levels than those with normal sperm [27]. Although previous studies investigated oligoasthenozoospermic men, our study focused solely on oligospermic patients, which may account for differences in findings and limits direct comparability.

Our finding that patients with oligospermia and azoospermia have lower GPX1 levels is consistent with previous research [28]. An imbalance of reactive oxygen species (ROS) and antioxidants causes oxidative stress, which contributes to many human diseases. Strong evidence links oxidative stress to male infertility, as sperm DNA breakage and aberrant semen parameters increase. Antioxidants may improve semen quality and minimize ROS [29].

Glutathione Peroxidase (GPX1) plays a crucial role in protecting spermatozoa from oxidative damage. Adequate levels of GPX1 help maintain the integrity of sperm cell membranes by reducing reactive oxygen species (ROS) and preventing lipid peroxidation. This antioxidative defense supports sperm motility and overall sperm quality, potentially reducing the incidence of idiopathic infertility [30]. The oxidative markers GPX1 and SOD showed notable differences between the infertile and control groups. The research cited above highlights how seminal plasma antioxidants and blood plasma antioxidants may both play a role in determining male fertility [31].

In conclusion, this study confirms that infertile men with azoospermia and oligospermia have significantly lower serum Inhibin-B levels compared with healthy males, which reflects Sertoli cell dysfunction. Furthermore, antioxidant enzymes (SOD and GPX1) levels were markedly reduced in both patient groups, supporting the role of oxidative stress in impaired sperm function. Importantly, IL-2 was significantly elevated only in azoospermic patients, suggesting a possible inflammatory contribution to severe male infertility. While these biomarkers provide insight into male infertility, their measurement alone is insufficient to fully assess reproductive function, particularly semen quality.

Notably, a limitation of the study is the unequal sample sizes among the groups, particularly the small control group (n=20), which may reduce statistical power and affect the reliability of comparisons. Future studies with larger, balanced groups and additional

biomarkers are recommended to strengthen diagnostic accuracy and clarify mechanistic pathways.

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Author Contributions

The authors participated equally in the study.

Competing Interest

There are no competing interests.

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