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Evaluation of serum Inhibin-B in polycystic ovarian syndrome (PCOs), premature ovarian failure (POF) and related with obese infertility, Case-Control study

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ABSTRACT

Infertility is the failure to become pregnant after 1 year or more of frequent, unprotected sexual intercourse without taking contraceptives. It has two categories primary and secondary infertility. study aimed to use Inhibin-B for the prognosis of polycystic ovarian syndrome (PCOs) and premature ovarian failure (POF) in female infertility patients. The study was designed A case-control study, including (120) females with a range of age (20-40) years, subdivided into two groups (60) healthy apparent females used as control, and (60) infertile females distributed between (37 PCOs), (23 POF). Inhibin-B serum was measured using an enzyme-linked immunosorbent assay (ELISA). The results showed that the BMI level of patients was (30.95 ± 3.42) as compared to control (27.9 ± 1.93). In PCOs according to age above and under (35 years) showed a significant increase ($p \leq 0.0001$) in Inhibin-B (273.55 ± 75.25), (234.91±52.43) respectively, and a decrease in POF (18.96 ±1.21). Inhibin-B (95% CI: 0.907 - 1.000; P-value: 0.001; Cutoff Point: 77.347; AUC: 95.417%) with Sensitivity to Specificity (91.667% - 98.333%); and Accuracy: 95.000%. The study concluded that the increase in the levels of serum Inhibin-B could be considered a useful indicator of the likelihood that a female of reproductive age will have PCOs and POF that are diagnosed by the Rotterdam standards.

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1. INTRODUCTION

Infertility is an issue in the reproductive system characterized by the inability to succeed in pregnancy following a year or more of repeat, unprotected, and regular sexual intercourse, according to the World Health Organization (WHO), infertility is considered a serious public health issue, and about 85% of infertile couples have a known cause, while the important common causes of infertility are ovulation dysfunction, uterine, and tubal causes [1]. This reproductive disease is divided into two categories: primary infertility, which is the inability of a woman to conceive without having previously become pregnant, and secondary infertility, which is the inability to conceive after a prior pregnancy or a successful last pregnancy [2,3]. And because infertility affects women and their health, it is important to study and know the various factors that contribute to causing infertility, endocrine complications, and defects in gonads related to infertility [4]. Obesity may be a cause of many medical diseases, such as increased risk factors for cardiovascular diseases, type2 diabetes mellitus (T2DM), and hypertension, it has a negative impact on ovulation from abnormal hormone signals as a result of excess weight and it eventually leads to infertility [5]. Polycystic Ovarian Syndrome (PCOs) is a chronic endocrinopathy has unknown causes, affecting (8-13%) of reproductive-aged women [6]. There is interdependence among the pathophysiologic factors linked to PCOs, persistent hormonal imbalance in PCOs causes irregular menstrual cycles and the production of many tiny antral follicles, which results in infertility in females [7].

Disturbances in androgens hormones, insulin resistance, cardiovascular defects, obesity in abdominal, psychological disorders, and infertility are considered as the most common symptoms of PCOs [8]. The Rotterdam criteria are used to diagnose PCOs condition, which needs at least two of the following three symptoms: hyperandrogenism, oligo- or anovulation, and cysts in ovaries [9]. Premature Ovarian Failure (POF) is a prevalent endocrine disorder that hinders or prevents pregnancy in females under 40 years, the etiology of POF remains unclear and is associated with numerous complex variables, including genetic defects [10]. The studies suggested primary ovarian insufficiency (POI) as an alternative term for this endocrine disorder to indicate this malfunction associated with very early ovarian aging. This failure is characterized by menstrual disorder, ovarian atrophy, high expression of gonadotropin to FSH hormone, low estrogen expression, and follicular dysplasia [11]. Inhibin-B is a glycoprotein heterodimer among the superfamily TGF-β (transforming growth factor-β) [12]. This non-steroidal hormone is produced by the granulosa cells of developing follicles in the ovaries and is widely recognized for its capacity to inhibit follicle-stimulating hormone (FSH), the pituitary gland is immediately negatively impacted by the elevated Inhibin-B levels in the serum, which causes FSH levels to drop [13]. An increase in the levels of Inhibin-B means an increase in inhibition of FSH and a low level in this hormone means a reduction in the quality and quantity of ovum resulting in problems with ovulation and causing infertility [14]. The object of this study is to measure serum Inhibin-B levels among females in reproductive age to use them as forecaster biomarkers for PCOs and POF and related to infertility.

2. METHOD

2.1 The study design

A case-control study was conducted to calculate levels of sex hormones among women from PCOs and POF in Karbala, Iraq. Diagnosis of PCOs and POF by using the Inhibin-B serum is the primary objective of this project. Data was collected from Oct 2023 to May 2024 from the hospital's fertility unit in Karbala. All the volunteers in the study were examined by a gynecologist. The number of volunteers was divided into two groups:

Group A: (60) intact females used as control.

Group B: (60) infertile females subdivision severing from ovarian disorders used as patients, then categorized into the following:

Group I: (37) infertile female patients with PCOs

Group II: (23) infertile female patients with POF

2.2 Study population

The inclusion criteria for this study were women aged 20–40 years, all of whom have intact ovaries and were newly diagnosed with PCOs and POF by a consultant gynecologist following a trans-vaginal pelvic ultrasound (Toshiba Xario Prime, Crawley, UK). The number of antral follicles appearing in ultrasound is associated with the occurrence of polycystic ovary morphology (PCOM), according to the Rotterdam Criteria Study to confirm PCOs and assess ovarian size [15]. While the exclusion criteria for females with any chronic disease such as heart disease and kidney problems, females with any type of cancer, infertile women with male factors, females taking contraceptives, and any drug that affects sex hormones or metabolism before 3 months of taking part in this research.

2.3 Ethical approval

Before taking part in the study, approval consent was obtained from all participants. They were made aware of any potential dangers before being involved in the study according to the Declaration of Helsinki principles [16]. And by the local ethics committee the study protocol, subject data, and permission form were evaluated and approved. This was done by document number IQ.UOK.CAMS.DCL.REC.4

2.4 Sample Collection

Five ml of blood samples were collected from all participants. Before being centrifuged for 10 minutes at 4000 rpm, they were allowed to coagulate at room temperature. Before being utilized to measure research tests, the serum was separated and kept at -18°C in an Eppendorf tube.

2.5 Estimation of Body mass index (BMI)

Body mass index (BMI) is calculated by [17].

BMI = Weight (kg) / Height (m2)

2.6 Statistical Analysis:

Data analysis has been done statistically by employing IBM SPSS statistical packages version 23. The analysis results have been summarized using descriptive statistics. In addition, Mean and Standard Deviation have been calculated to assess the statistical significance of the experimental results, with a p-value threshold of (0.01). The Mann-Whitney Test has been used for non-parametric data, while the Independent T-test and analysis of variance (ANOVA) have been used for parametric data. Furthermore, assessments of receiver operating characteristics (ROC) were carried out to determine the cut-off point of the research parameters for critical patients. Youden's index was employed to determine the ideal cut-off points, and AUC was utilized to measure prediction strength. Asterisks indicate data with a P value less than 0.05. In the end, all graphs were created by using GraphPad Prism 9.

3. RESULTS AND DISCUSSION

3.1 The Comparison between BMI levels in patients and compared to control

The result was a highly significant increase ($p \leq 0.0001$) in BMI level of total patients, PCOs, POF, Primary and Secondary patients (30.95±3.42), (30.93±3.5), (31±3.24), (30.95±3.85), (30.95±3.13) as compared to control groups (27.9±1.93) table 1.

Table 1: Comparison of Body Mass Index (BMI) for different patient groups as compared to the control

Groups	BMI (Mean)	± Std. Deviation	Value
Total Patients	30.95	3.42	0.003**
Control	27.9	1.93	
PCOs	30.93	3.5	0.001**
Control	27.9	1.93	
POF	31	3.24	0.005**
Control	27.9	1.93	
Primary	30.95	3.85	0.001**
Control	27.9	1.93	
Secondary	30.95	3.13	0.004**
Control	27.9	1.93	

**. The difference is significant in the mean at level $p \leq 0.0001$

In Table 1 measurements of the BMI of individuals enrolled showed an increase in all patients because the majority of patients with infertility have dysfunction of reproductive hormones, which causes abnormal ovulation, leads to irregular menstrual cycles, and increases the chances of infertility, this agreement with [18,19].

3.2 Inhibin-B levels in patients and their controls according to infertility causes

The result recorded a high significant increase ($p \leq 0.05$) in the Inhibin-B level of PCOs patients (266.97 ± 72.88), while a significant decrease ($p \leq 0.05$) in POF patients (18.96 ± 1.21), as compared to the control group (36.76 ± 10.62) fig. 1.

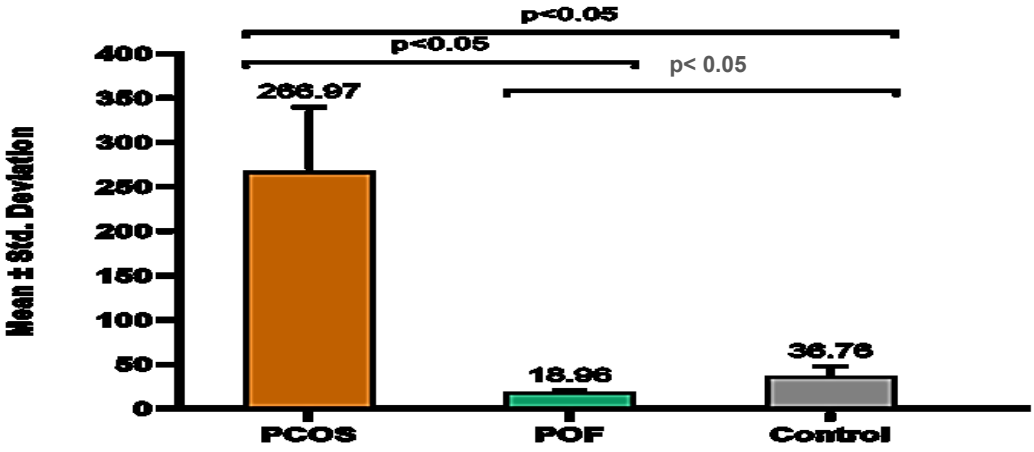
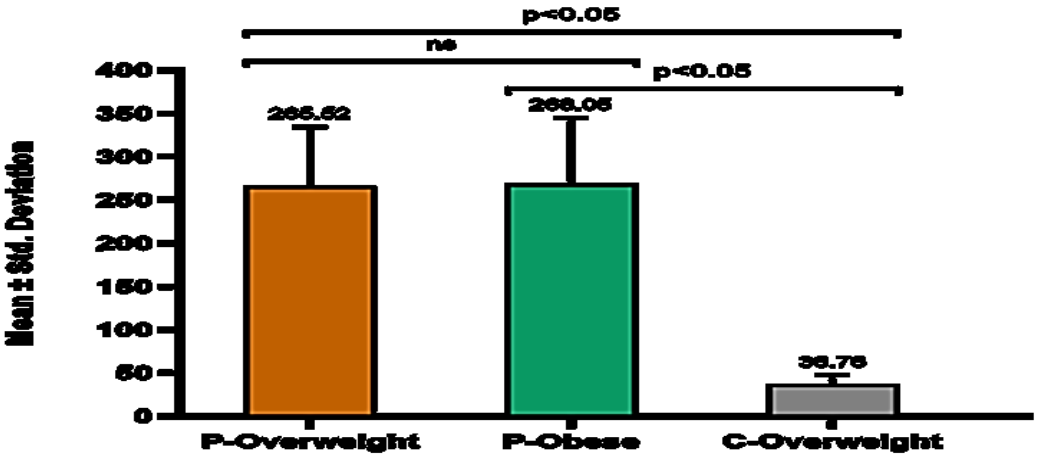


Figure 1 Comparison of the level of Inhibin-B in PCOS and POF infertile patients and compared to control

In figure (3-1) elevated levels in PCOs patients, Inhibin-B is a significant factor of ovarian reserve assessment in PCOs patients, the reason for its rise is that in PCOs patients the fat rises which in turn contributes to increased production of androgens and ovarian hormones including Inhibin-B which inhibits the hormone FSH, and that agreement with [20,21]. While in POF patients result showed a decrease in the Inhibin-B, one of the main causes of the gradual rise in blood FSH levels in POF patients is that Inhibin-B gradually diminishes with a decline in ovarian follicle quantity and quality, weakening its inhibitory impact on FSH, so can consider Inhibin-B as an indication of ovarian inefficiency, that agreement with [22,23].

3.3 Inhibin-B levels in PCOs patients and their controls according to the BMI Groups.

The study showed high significant increase ($p \leq 0.05$) in Inhibin-B level of the overweight and obese PCOs patients (265.52 ± 69.00), (268.05 ± 76.91), as compared to the overweight control group (36.76 ± 10.62) fig. 2.



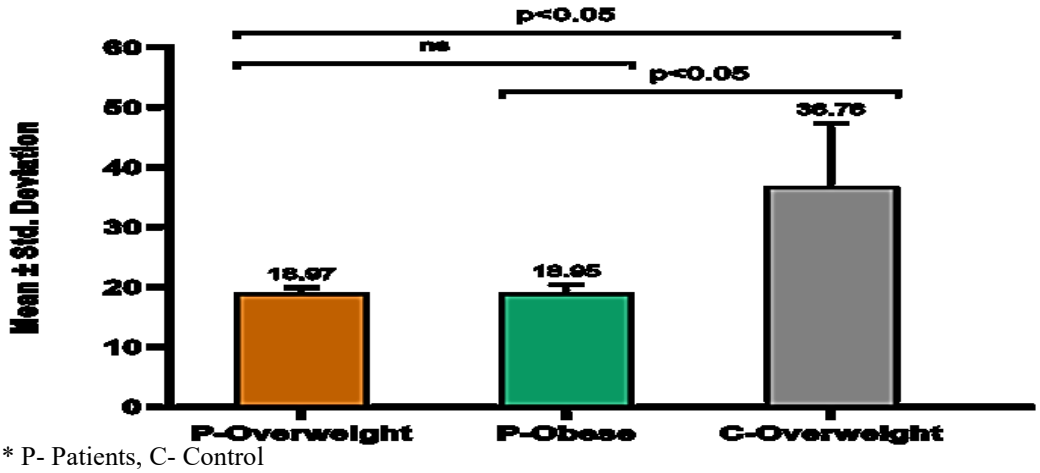
*** P- Patients, C- Control**

Figure 2: The Comparison of the Inhibin-B levels in PCOS patients, according to the BMI Group and their controls

Inhibin-B levels showed an increase in both overweight and obese PCOs patients because of the over-activity of ovaries produced by high androgens, Inhibin-B production increases, that agreement with [24].

3.4 Inhibin-B levels in POF patients and their controls according to the BMI Group

Found in results a high significant decrease ($p \leq 0.05$) in Inhibin-B level of the overweight POF patients (18.97 ± 0.97), and obese POF patients (18.95 ± 1.46), as compared to the overweight control group (36.76 ± 10.62) Fig. 3.



* P- Patients, C- Control

Figure 3: Comparison of the level of Inhibin-B in POF patients at different classes of BMI and compared to the control.

The results found significant decrease in levels of Inhibin-B in both overweight and obese POF patients. This low is due to the failure of ovarian cells responsible for the release of Inhibin-B, and that agreement with [25].

3.5 Inhibin-B levels of females in patients and their controls according to age group

The result in table (3-2) showed a high significant increase ($p \leq 0.0001$) in the Inhibin-B level of the (20-25) age group of patients (158.57 ± 132.00), as compared to the control groups (33.44 ± 8.90), while a high significant ($p \leq 0.0001$) in (26-30) age group of patients (249.68 ± 104.04), as compared to the control groups (37.79 ± 12.75), and a high significant ($p \leq 0.0001$) in (31-35) age group of patients (246.55 ± 122.21), as compared to the control groups (38.94 ± 7.29), and ($p \leq 0.0001$) in (36-40) age group of patients (203.01 ± 97.66), as compared to the control groups (41.83 ± 11.64).

Table 2: The Comparison of Inhibin-B levels in patients and their control according to the age group

Parameter	Level	Control		Patients		P. Value
		Mean	± Std. Deviation	Mean	± Std. Deviation	
Inhibin-B (pg/ml)	20-25	33.44	8.9	158.57	132	0.004**
	26-30	37.79	12.75	249.68	104.04	0.008**
	31-35	38.94	7.29	246.55	122.21	0.001**
	36-40	41.83	11.64	203.01	97.66	0.005**
**. The mean difference is significant at p≤0.0001.						

Studies found a relationship between the production of dysfunctional glands with age and fertility [26]. In general, the variation trend of Inhibin-B in healthy women of all ages rapidly decreases after the age of 40, but in infertility women the increased high levels of Inhibin-B lead to low levels of FSH and secondary amenorrhea, therefore causing infertility, that agreement with [27].

3.6 Inhibin-B levels in different types of female infertile patients and their controls according to Aging

Result in table 3 found high significant increase (p≤0.0001) in the Inhibin-B level of both (<35) (273.55 ± 75.25), (>35) (234.91 ± 52.43) PCOs patients, while a no significant in the Inhibin-B level of POF patients (<35) (18.96 ± 1.21), as compared to both group of control (35.58 ± 10.48, 42.04 ± 10.04).

Table 3: The Comparison of Inhibin-B levels in patients and control according to Aging

Parameter	Level	Mean	± Std. Deviation	P. Value
Inhibin-B(pg/ml)	P-PCOs(<35)	273.55	75.25 a	0.0002**
	P-PCOs(>35)	234.91	52.43 b	
	P-POF(<35)	18.96	1.21 c	
	C-Aging(<35)	35.58	10.48 c	
	C-Aging(>35)	42.04	10.04 c	
** Mean difference is significant at the p≤0.0001. *. Different letters found that are significantly different in mean P-Patient, C-Control				

In PCOs patients. Inhibin B has been found to increase due to the increased number of small growing follicles that are characteristic of PCOs due to that excess in Inhibin-B production inhibits the over-excess of FSH levels, contributing to the hormonal irregularities seen in PCOs, that agreement with [28]. While patients with POF showed a decrease in levels of Inhibin-B, Inhibin-B is thought to be one of the predicted indicators for ovarian function to resume, recent research has demonstrated that as POF progresses, there is a markedly ongoing decrease in Inhibin-B, so when the level of Inhibin-B decreases, the excretion of the hormone FSH will increase, and this hormonal imbalance indicates a failure of ovarian function, which agrees with [29].

3.7 Receiver Operative Characteristic Curve (ROC) for research parameter

The result in Table 4 and Fig. 4 suggested that Inhibin-B (95% CI: 0.907-1.000; P-value: 0.001; Cutoff Point: 77.347; AUC: 95.417%) with Sensitivity to Specificity 91.667%-98.333%; and Accuracy: 95.000% in identifying infertility.

Table (3-4): Receiver Operative Characteristic Curve (ROC) for Inhibin-B

Metrics		Inhibin-B(pg/ml)
Std. Error		0.024
Asymptotic Sig.		0.009
Asymptotic 95% Confidence Interval	Lower Bound	0.907
	Upper Bound	1.000
Cutoff Point		77.347
Area Under Curve (AUC)		95.417%
Sensitivity		91.667%
Specificity		98.333%
Accuracy		95.000%
Positive Predictive Value		98.210%
Negative Predictive Value		92.190%

Comparable outcomes were recorded in earlier research that revealed that the AUC, sensitivity, and specificity of both Inhibin-B were (82.84%, 36.8 %, and 86.6 %) [30]. Another previous study showed sensitivity and specificity for Inhibin-B (95%, 86%) [31]. And the differences in sensitivity and specificity results could be explained by differences in the kits and procedures used, the patients and control people chosen for these studies, and other factors.

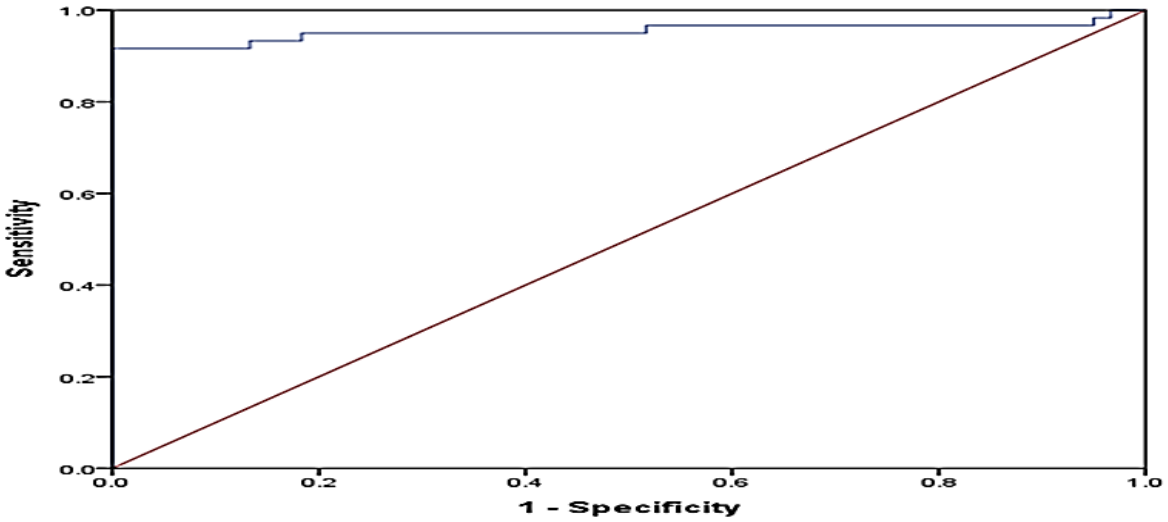


Figure 4): ROC Curve Demonstrated the sensitivity and specificity values for the Inhibin-B parameter.

4. CONCLUSION

Conclude from the current study that the serum concentration of Inhibin-B was significantly higher in PCOs patient groups, and it had lower levels in POF patients than in healthy control. This is useful to indicate the role of this parameter in the early identification and prediction of the type of female infertility and their causes.

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