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The Relationships between Anti-Thyroid Peroxidase (Anti-TPO) and Inhibin-B as Predictor Biomarkers for Female Infertility

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ABSTRACT

Anti-thyroid peroxidase is an autoantibody generated by the immune system, its primary function is target and neutralize the enzyme thyroid peroxidase, is essential for production of thyroid hormones. Inhibin-B is a peptide belonging to TGF-β family produced by granulosa cells of the growing follicle cohort. It reflects the health of follicles, their overall numbers. The objective of this article is evaluation of Anti-TPO, Inhibin-B for female infertility. A case-control study includes 120females between 20-45 years divided into 60 apparently healthy females used as controls and 60 infertile females distributed 37 with Polycystic ovarian syndrome, 23 with Premature ovarian failure. Serum levels of Anti-TPO, Inhibin-B, LH, FSH, Testosterone, Prolactin and Estrogen were all measured using an ELISA assay. Inhibin-B, Anti-TPO higher in patients than in controls. Inhibin-B significantly increases($p \leq 0.0001$) in PCOS above and under 40years (273.55±75.25 pg/mL), (234.91±52.43 pg/mL), but decreases in POF (18.96±1.21 pg/mL) than in controls (35.58±10.48 pg/mL), (42.04±10.04 pg/mL). AntiTPO increases in PCOS and POF above and under 40 years (16.08±2.14 ng/mL), (17.63±1.93 ng/mL), (14.72±1.19 ng/mL) than in controls(5.92±0.99 ng/mL), (5.93±0.84 ng/mL). The levels of FSH, Prolactin, Testosterone, Estrogen and LH were increased ($p \leq 0.0001$) in PCOS, POF as in controls. The results showed a negative correlation ($p \leq 0.0001$) between Inhibin-B, Anti-TPO ($r = -0.520$) in PCOS. The Receiver operating characteristic (ROC) analysis demonstrated 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%; Accuracy: 95.000%. Anti-TPO 95% CI: 0.907-1.000; P-value: 0.001; Cutoff Point: 9.306; AUC: 98.861% with Sensitivity to Specificity 96.676-96.667%; Accuracy: 97.000%. Anti-TPO, Inhibin-B are useful biomarkers for evaluating fertility status and ovarian health, can used as sensitive indicators for early diagnosis of PCOS and POF in women of reproductive age.

1. Introduction

Infertility is known as the failure to become clinically pregnant following a year or more of frequent, unprotected sexual activity, infertility affects 17.5% of adults worldwide or around 1 in every 6, and according to recent reports from the World Health Organization (WHO) indicate that a substantial number of individuals face infertility during their lifetimes (Wang *et al.* 2023). One of the most important causes of infertility is damage to the pituitary-hypothalamic and "gonads" axis, which leads to hypogonadism, delayed sexual development, and amenorrhoea (Al-Fatlawi & AlSafi 2020). The most prevalent dietary issue in the world is obesity, which is linked to high blood pressure and cardiovascular disease. It is considered a significant reason to increase the chance of infertility (Al-Shibli *et al.* 2023). Given the grave implications infertility bears for women, it is important to examine the origins and relative contributions of the various factors that contribute to infertility, globally infertility is categorized into two groups, primary

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infertility refers to a couple's incapacity to become pregnant, while secondary infertility is when the couple is inability to conceive after a previous pregnancy in the absence of contraception or postpartum amenorrhea (Yang *et al.* 2020). This disorder has several causes; endocrine dysfunctions constitute 25% of infertility issues, and hormonal profile evaluation is considered a crucial component of infertility diagnosis (Lancuba *et al.* 2023). Polycystic ovary syndrome (PCOS) is a most important endocrine disorder that leads to infertility (Zhao *et al.* 2023). About 70% of anovulatory subfertility cases are caused by PCOS (Shareef *et al.* 2020). It affects 6-20% of women of reproductive age and is characterized by issues in metabolism, reproduction, and hyperandrogenism; it is also linked to insulin resistance; this case is made worse by obesity but is independent of it (Nori *et al.* 2023). Premature ovarian failure (POF) is a kind of ovulation disorder described as an intermediate or permanent gonadal insufficiency occurring before the age of 40. The occurrence of secondary amenorrhea characterizes POF, and estrogen decreases (Umer *et al.* 2023).

Anti-thyroid peroxidase (anti-TPO) is a crucial enzyme involved in the production of thyroid hormone, located at the apical membrane of thyroid follicular cells (thyrocytes) (Akane *et al.* 2024). It plays a crucial role in the generation of thyroid hormones, and its biostructure is intricate, as anti-TPO possesses two active sites and requires heme for functionality (Godlewska & Banga 2019). Because of its strong correlation with infertility independent of thyroid hormone levels, anti-TPO expression in the placenta and endometrium may explain why people with thyroid autoimmunity disease have an increased risk of infertility and abortion. This makes anti-TPO a potential screening and identification biomarker for infertility risk factors (Rahnama *et al.* 2021). Inhibin-B is a glycoprotein hormone that belongs to the TGF-β superfamily, produced by the granulosa cells of small antral follicles in females. Inhibin-B's major physiological function is to inhibit FSH secretion by applying negative feedback (Chaudhary *et al.* 2021). The negative feedback of Inhibin-B would inhibit the release of FSH but not LH from the pituitary (Poulsen *et al.* 2020). A high level of Inhibin-B means an increase in inhibition or decrease in the level 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 (Jankowska *et al.* 2022). This study aims to quantify serum Anti-thyroid peroxidase (Anti-TPO) and Inhibin-B levels in females of reproductive age to utilize them as predictive parameters for infertility.

2. Materials and Methods

2.1. Study Design & Setting

A case-control study was carried out to measure the reproductive characteristics and hormone levels among women with Premature Ovarian Failure (POF) and Polycystic Ovarian Syndrome (PCOS) in Kerbala, Iraq. Identifying PCOS and POF using serum Anti-TPO and Inhibin-B value is one of the objectives of this research project. Data were obtained from 2023 to 2024 from the fertility unit of the hospital in Kerbala. A women's disease consultant gynecologist examined all participants in the study. The number of volunteers in the current study was divided into two groups as follows:

Group A: 60 intact females used as controls, Group B: 60 infertile females, subdivided into those with 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.

Inclusion criteria for this research project were women aged 20-45 years, both have intact ovaries, and were newly diagnosed with PCOS or POF by a consultant gynecologist following a trans-vaginal pelvic ultrasound (Toshiba Xario Prime, Crawley, UK). According to the Rotterdam criteria investigation for PCOS confirmation and measured ovarian volume, the antral follicle count on ultrasound is linked to polycystic ovarian morphology (PCOM). Exclusion criteria for females with any chronic disease, such as heart disease and kidney problems, any cancer, infertile women with male factors, females taking contraceptives, and any drug known to have an impact on sex hormones or metabolism, three months before taking part in this research.

2.2. Data Collection

The individuals were clinically evaluated by a consultant gynecologist, who also conducted a thorough reproductive hormone analysis and documented their menstrual cycle history. For statistical analysis, study participants were divided into two groups based on their menstrual cycle history: normal and oligo-/amenorrhea. Important information on age, marriage period, and fertility status, types of infertility (primary or secondary), length of the menstrual cycle, and previous history of treatment, education level, types of delivery, was evaluated by employing a demographic questionnaire after obtaining verbal assent from the individuals. Participants' body weight was measured by wearing regular clothing and standing on a digital weighing scale (Beurer BF 600) without shoes. Serum

levels of luteinizing hormone (LH, mlU/mL), follicle-stimulating hormone (FSH, mlU/mL), prolactin (ng/mL), estrogen hormone, and testosterone hormone were measured during the follicular phase (1-5 days) of the menstrual cycle. Hormone levels were determined using an enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (ELISA Kit Could clone /USA).

From each female, 5 mL of blood samples were taken. They were allowed to coagulate at room temperature and then centrifuged for 10 minutes at 4,000 rpm. The serum was separated and stored in an Eppendorf tube at -18°C and then used for measuring research tests.

2.3. Estimation of Body Mass Index (BMI)

Body mass index (BMI) is calculated by the formula (Karchynskaya *et al.* 2020):

$$BMI = Weight\ (kg) / Height\ (meters^2)$$

2.4. Compliance with Ethical Standards

Before participating in the study, every participant provided their informed consent and was made aware of any possible risks in accordance with the principles of the Declaration of Helsinki (General Assembly of the World Medical Association 2014). The study protocol, subject data, and permission form were reviewed and approved by the local ethics committee. Document number IQ did this.UOK.CAMS.DCL.REC.4.

2.5. Statistical Analysis

IBM SPSS statistical packages version 23 have been used for statistical data analysis. Descriptive statistics have been used to summarize the analysis's findings. In addition, mean and standard deviation have been calculated, and in order to assess the statistical significance of the experimental results, a p-value threshold of 0.05 was utilized. Furthermore, the Levene test was used to assess homogeneity of variance, while the Shapiro-Wilk test was employed to verify that the data distribution was normal. Nevertheless, to investigate the association between categorical and numerical variables, chi-square and Pearson's correlation analyses were conducted. Using the independent t-test and Mann-Whitney test, statistical differences between the two independent groups have been ascertained.

Additionally, analysis of variance (ANOVA) was employed to make multiple comparisons between groups, followed by Scheffé's and Duncan's post-hoc tests for multiple comparisons within groups. Moreover, for critical patients, receiver operating characteristic (ROC) analyses were used to establish the research parameter cut-off values. The prediction strength was measured using the AUC, and Youden's index was used to determine the optimal cut-off points. Asterisks indicate data having a p-value less than 0.05. Finally, GraphPad Prism 9 was used to create all graphs (Wasserstein *et al.* 2019).

3. Results

Table 1 demonstrates the demographic study of this research and questionnaire details, with significantly increase ($p \leq 0.001$) in BMI groups of overweight controls (100%) compared to the overweight patients (43.44%). Type of causes of infertility showed significant difference ($p \leq 0.001$) between patients and control. Also found a significant difference ($p \leq 0.001$) in medical disease of patients (Diabetes mellitus 1.67%), (Obesity 56.67%), (Hypertension 11.67%), (heart disease 0%), (None 30%) when compared with control groups (0.00%, 8.33%, 6,67%, 1.67% respectively 83.33%). Also found a significant difference ($p \leq 0.001$) in the drug history of patients (100%) when compared to control (100%). With a significant difference in the type of infertility ($p \leq 0.001$) in patients (primary 41.67%), (secondary 58.33%) in compared with the control group (0.00%). The result found a significant difference ($p \leq 0.001$) in the Menstrual cycle of patients (regular 20%), (irregular 80%) when compared with the control (81.67% and 18.33%). Result demonstrated a significant difference ($p \leq 0.001$) in the Job of patients (Housewife 88.33%), (Student 11.67%), (Employee 0.00%) as compared with the control groups (78.33%, 3.33%, respectively 18.33%). And found significant difference ($p \leq 0.001$) in Education level of patients (Illiterate 5.00%), (primary school 28.33%), (secondary school 28.33%), (Institute 3.33%), (University 35%) when compared with the control groups (23.33%, 4%, 1.00%, 13.33%, respectively 13.33%). While no significant difference ($p \leq 0.01$) in Address of patients (Rural 33.33%), (Urban 66.67%) when compared to control groups (23.33% and 76.67%). And no any significant difference ($p \leq 0.01$) in type of delivery of patients (Cesarean delivery 61.70%), (Natural delivery 38.30%) when compared to control groups (53.33% and 46.67%).

Results in Table 2 demonstrated a highly significant increase ($p \leq 0.0001$) in Inhibin-B level (213.24±121.48 pg/mL) and Anti-TPO (15.99±2.10

Table 1. Distribution and characteristics of patients and control according to the study subjects

Parameters		Level	Control		Patients		Total		P-value
			No	%	No	%	No	%	
BMI group		P-Overweight	0	0.00	26	43.33	26	21.67	0.003*
		P-Obese	0	0.00	34	56.67	34	28.33	
		C-Overweight	60	100.00	0	0.00	60	50.00	
Type of cause of infertility	Classification A	Endocrine disorder (PCOS)	0	0.00	47	78.33	47	39.17	0.008*
		Premature ovarian failure (POF)	0	0.00	13	21.67	13	10.83	
		Control	60	100.00	0	0.00	60	50.00	
	Classification B	P-PCOS(<40)	0	0.0	39	65.0	39	32.5	0.005*
		P-PCOS(>40)	0	0.0	8	13.3	8	6.7	
		P-POF(<40)	0	0.0	13	21.7	13	10.8	
		C-Age (<40)	49	81.7	0	0.0	49	40.8	
		C-Age (>40)	11	18.3	0	0.0	11	9.2	
Medical Disease		Diabetes mellitus	0	0.00	1	1.67	1	0.83	0.005*
		Obesity	5	8.33	34	56.67	39	32.50	
		Hypertension	4	6.67	7	11.67	11	9.17	
		Heart disease	1	1.67	0	0.00	1	0.83	
		None	50	83.33	18	30.00	68	56.67	
Drug history		Not receiving	60	100.00	60	100.00	120	100.00	0.001*
		Receiving	0	0.00	0	0.00	0	0.00	
Type of infertility		Primary	0	0.00	25	41.67	25	20.83	0.005*
		Secondary	0	0.00	35	58.33	35	29.17	
		N/A	60	100.00	0	0.00	60	50.00	
Menstrual Cycle		Regular	49	81.67	12	20.00	61	50.83	0.001*
		Irregular	11	18.33	48	80.00	59	49.17	
Job		House Wife	47	78.33	53	88.33	100	83.33	0.001*
		Student	2	3.33	7	11.67	9	7.50	
		Employee	11	18.33	0	0.00	11	9.17	
Education level		Illiterate	14	23.33	3	5.00	17	14.17	0.001*
		Primary schools	24	4.00	17	28.33	41	34.17	
		Secondary school	6	1.00	17	28.33	23	19.17	
		Institute	8	13.33	2	3.33	10	8.33	
		University	8	13.33	21	35.00	29	24.17	
Address		Rural	14	23.33	20	33.33	34	28.33	0.224
		Urban	46	76.67	40	66.67	86	71.67	
Type of delivery		Cesarean delivery	32	53.33	37	61.70	69	57.50	0.356
		Natural delivery	28	46.67	23	38.30	51	42.50	

*. Association is significant at p≤ 0.05 level
**. Association is significant at p≤ 0.0001 level
P- Patients, C- Control

Table 2. The level of biomarkers in infertile female patients and compared to the controls

Parameters	Level	Mean	Std. Deviation	P. value
Inhibin-B (pg/ml)	Control	36.76	10.62	0.0002**
	Patients	213.24	121.48	
Anti-TPO (ng/ml)	Control	5.92	0.96	0.0003**
	Patients	15.99	2.10	

**At the p≤0.0001, the mean difference is significant.

ng/mL) in patients compared to the control groups (36.76±10.62 pg/mL; 5.92±0.96 ng/mL).

Based on Table 3, there is a highly significant increase (p≤0.0001) in the Inhibin-B level for both ages <40 years and >40 years (273.55±75.25 pg/

Table 3. Comparison of the level of biomarkers in PCOS and POF patients and control according to age group

Parameters	Aging	Mean	Std. Deviation	P. value
Inhibin-B (pg/ml)	P-PCOS(<40)	273.55[a]	75.25	0.0002**
	P-PCOS(>40)	234.91[a]	52.43	
	P-POF(<40)	18.96[c]	1.21	
	C-Age (<40)	35.58[b]	10.48	
	C-Age (>40)	42.04[b]	10.04	
Inhibin-B (pg/ml)	P-PCOS(<40)	16.08[a]	2.14	0.0009**
	P-PCOS(>40)	17.63[b]	1.93	
	P-POF(<40)	14.72[a]	1.19	
	C-Age (<40)	5.92[c]	0.99	
	C-Age (>40)	5.93[c]	0.84	

*Different letters found that are significantly different in mean,
**The mean difference is significant at level p≤0.0001, P- Patients, C- Control

mL and 234.91±52.43 pg/mL) in PCOS patients. However, in POF patients aged <40 years, it decreases (18.96±1.21 pg/mL), compared to both control age groups (35.58±10.48 pg/mL and 42.04±10.04 pg/mL). A similar result is found in the Anti-TPO levels of both PCOS patient age groups (16.08±2.14 ng/mL and 17.63±1.93 ng/mL), which showed a highly significant increase (p≤0.001) compared to both age groups of the control (5.92±0.99 ng/mL and 5.93±0.84 ng/mL). However, unlike Inhibin-B, Anti-TPO levels in POF patients under 40 years increase (14.72±1.19 ng/mL).

Results showed a highly significant increase (p≤0.0001) in levels of Inhibin-B for both primary and secondary infertility patients (161.26±141.16 pg/mL and 250.36±90.22 pg/mL), compared to the control group (36.76±10.62 pg/mL). A highly significant increase (p≤0.0001) is also found in Anti-TPO levels of both primary and secondary infertility patients (15.40±1.55 ng/mL and 16.41±2.35 ng/mL), compared to the control group (5.92±0.96 ng/mL). This result is summarized in Table 4.

Results showed a high significant increase (p≤0.0001) in Inhibin-B levels of both overweight (208.62±121.82 pg/mL) and obese patients (216.77±122.94 pg/mL), compared to the overweight control (36.76±10.62 pg/mL). As well, a very highly significant increase (p≤0.0001) also was found in Anti-TPO levels of both overweight (15.79±2.37 ng/mL), and obese patients (16.15±1.89 ng/mL), when compared to the overweight control (5.92±0.96 ng/mL), as shown in (Table 5).

Table 6 demonstrated no significant difference (p≤0.0001) in the FSH level of PCOS patients (5.83±2.30 mlU/mL), while a high considerable increase (p≤0.0001) in the FSH level of POF patients (33.51±3.61 mlU/mL), as compared to the control (8.10±2.15 mlU/mL). A highly significant increase (p≤0.0001) is also found in the prolactin level for both PCOS (29.24±4.99 ng/mL) and POF patients (26.06±3.04 ng/mL), compared to their control (13.44±3.30 ng/mL). Also, there is a high significant increase (p≤0.0001) in the testosterone levels of PCOS patients (58.53±17.39 pg/mL), but no significant difference in the testosterone levels of POF patients (7.24±2.01 pg/mL), as compared to their control (5.34±0.90 pg/mL). In addition, there is a highly significant increase (p≤0.0001) in the estrogen level of PCOS patients (186.25±48.23 pg/mL), and a significant decrease in the estrogen levels of POF patients (10.07±1.43 pg/mL) compared to the control (24.93±7.23 pg/mL). With a highly significant increase (p≤0.0001) in the levels of LH of PCOS and POF patients (17.15±5.8 mlU/mL), (19.77±2.88 mlU/mL) than in controls (2.93±1.28 mlU/mL).

According to Figure 1, a negative correlation (r = -0.520) existed between inhibin-B and anti-TPO in

Table 4. Comparison of the level of biomarkers in primary and secondary types of infertility as compared to control

Parameters	Level	Mean	Std. Deviation	P. value
Inhibin-B (pg/ml)	Control	36.76^{c}	10.62	0.0009**
	Patients	161.26^{b}	141.16	
	Secondary	250.36^{a}	90.22	
Inhibin-B (pg/ml)	Control	5.92^{c}	0.96	0.0004**
	Patients	15.40^{b}	1.55	
	Secondary	16.41^{a}	2.35	

*Different letters found that are significantly different in mean
**The mean difference is significant at level p≤0.0001

Table 5. Comparison of the level of biomarkers in infertile patients at different BMI as compared to the controls

Parameters	Level	Mean	Std. Deviation	P. value
Inhibin-B (pg/ml)	P-Overweight	208.62^{a}	121.82	0.0003**
	P-Obese	216.77^{a}	122.94	
	C-Overweight	36.76^{b}	10.62	
Inhibin-B (pg/ml)	P-Overweight	15.79^{a}	2.37	0.0005**
	P-Obese	16.15^{a}	1.89	
	C-Overweight	5.92^{b}	0.96	

*Different letters found that are significantly different in mean,
**The mean difference is significant at level p≤0.0001, P- Patients, C- Control

Table 6. Comparison between Research Parameters in patients, and their controls stratified by infertility status

Parameters	Level	Mean	Std. Deviation	P. value
FSH (mlU/ml)	PCOS	5.83^{cb}	2.3	0.0002**
	POF	33.51^{a}	3.61	
	Control	8.10^{b}	2.15	
Prolactin (ng/ml)	PCOS	29.24^{a}	4.99	0.0007**
	POF	26.06^{b}	3.04	
	Control	13.44^{c}	3.3	
Prolactin (ng/ml)	PCOS	58.53^{a}	17.39	0.0004**
	POF	7.24^{b}	2.01	
	Control	5.34^{b}	0.9	
Estrogen (pg/ml)	PCOS	186.25^{a}	48.23	0.0001**
	POF	10.07^{b}	1.43	
	Control	24.93^{b}	7.23	
LH (mlU/ml)	PCOS	17.15^{c}	5.81	0.0001**
	POF	19.77^{a}	2.88	
	Control	2.93^{b}	1.28	

*Different letters found that are significantly different in mean
**The mean difference is significant at level p≤0.0001

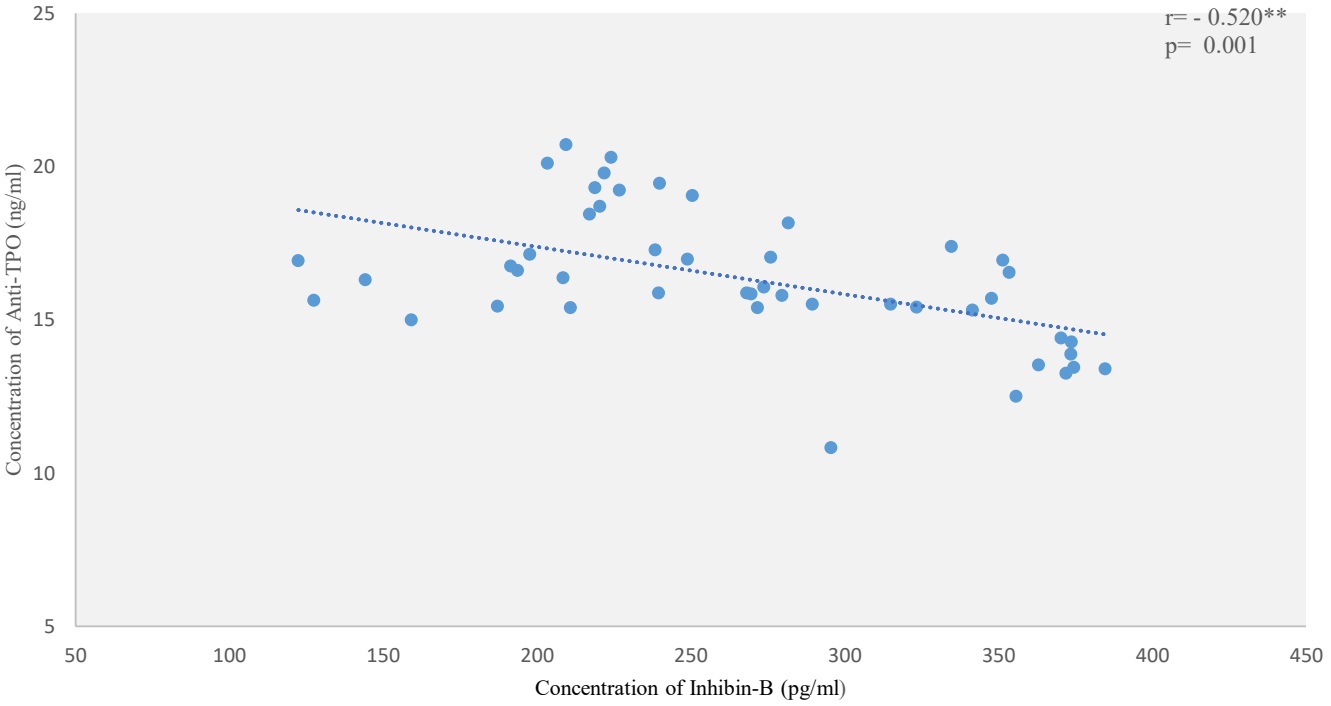

Figure 1. Correlation coefficient of Inhibin-B concentration and Anti-TPO concentration based on PCOS patients

PCOS patients, which was statistically significant at p = 0.001. Inhibin B may be decreased in PCOS patients due to decreased ovarian and thecal cell volume, a decrease or normal number of small follicles, and since their FSH levels have not increased, there is no need to use more Inhibin B to suppress FSH. And when these abnormal processes and hormonal imbalances occur, the thyroid gland releases anti-TPO as an immune response to these defects. Due to an increase in these autoantibodies, anti-TPO may be able to cross the blood follicle barrier during the maturation stage, creating a cytotoxic environment that affects oocyte maturation. Thus, it is proven that the rise in these antibodies can lead to impaired fertility and miscarriage.

The result in Table 7 and Figure 2 suggested that the Receiver Operative Characteristic Curve (ROC) demonstrated 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%. And for Anti-TPO (95% CI: 0.970-1.000; P-value: 0.001; Cutoff Point: 9.306 AUC: 98.861%) with Sensitivity to Specificity 96.676-96.667%; and Accuracy: 97.000% in identifying infertility.

The result in Table 8 and Figure 3 suggested that prolactin (95% CI: 0.907-0.982; P-value: 0.001; Cut-off Point: 19.373; AUC: 94.472%) with Sensitivity to Specificity 93.333%-85.000%; and Accuracy: 89.167%. And for Testosterone (95% CI: 0.900-0.997; P-value: 0.001; Cutoff Point: 7.254; AUC: 94.875%) with Sensitivity to Specificity 88.333%-98.333%; and Accuracy: 93.330%. Also for Estrogen (95% CI: 0.668-0.871; P-value: 0.001; Cutoff Point: 56.945; AUC: 76.917%) with Sensitivity to Specificity 76.667%-93.333 %; and Accuracy: 85.000%. And for LH (95% CI: 0.891-0.985; P-value: 0.001; Cutoff Point: 11.325; AUC: 93.778%) with Sensitivity to Specificity 83.333-96.667%; and Accuracy: 90.000% in identifying infertility.

4. Discussion

By inhibiting the activin-receptor connection, Inhibin-B prevents activin from inducing FSH secretion. This process occurs when the pituitary gonadotropes work to restrict the production and release of FSH but not LH. This modulatory effect of Inhibin-B is defined as negative feedback. The current study, as presented in Table 2, found that elevated levels of Inhibin-B in patients can serve as good indicators, but not the best fertility indicators. This finding is by that of Jankowska *et al.* (2022). The current study found elevated levels of anti-TPO in patients. The pathophysiological

Table 7. Receiver Operative Characteristic Curve (ROC) for Inhibin-B, Anti-TPO parameters

Metrics		Inhibin-B (pg/ml)	Anti-TPO (ng/ml)
Std. Error		0.024	0.01
Asymptotic Sig.		0.009	0.002
Asymptotic 95%	Lower bound	0.907	0.970
Confidence interval	Upper Bound	1.000	1.000
Cutoff point		77.347	9.306
Area under curve (AUC)		95.42%	98.86%
Sensitivity		91.67%	96.68%
Specificity		98.33%	96.67%
Accuracy		95.00%	97.00%
Positive predictive value		98.21%	96.67%
Negative predictive value		92.19%	96.67%

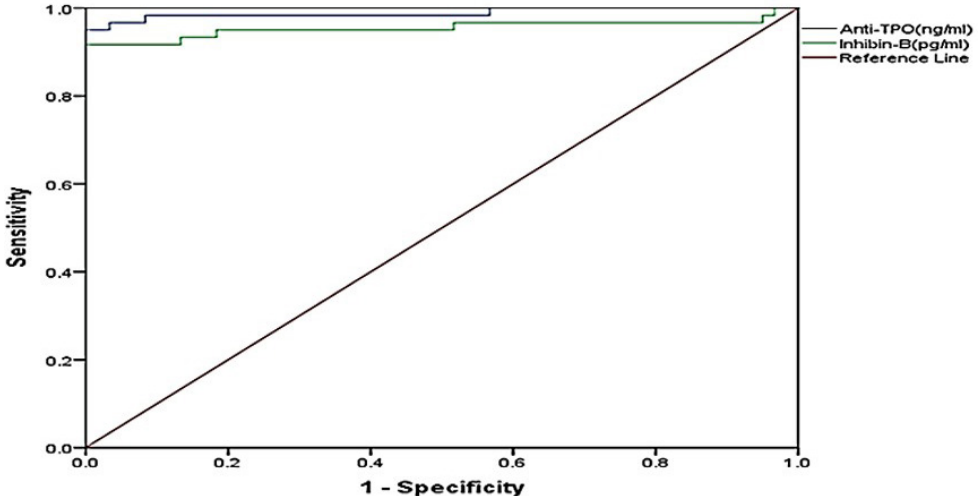

Figure 2. ROC Curve Demonstrating the Sensitivity and Specificity Values for Inhibin-B, Anti-TPO

Table 8. Receiver operative characteristic curve (ROC) for hormonal parameters

Metrics		Prolactin (ng/ml)	Testosterone (pg/mL)	Estrogen (pg/mL)	LH (mlU/mL)
Std. Error		0.019	0.025	0.052	0.024
Asymptotic Sig.		0.004	0.002	0.003	0.001
Asymptotic 95%	Lower bound	0.907	0.900	0.668	0.891
Confidence interval	Upper Bound	0.982	0.997	0.871	0.985
Cutoff point		19.373	7.254	56.945	11.325
Area under curve (AUC)		94.472%	94.875%	76.917%	93.778%
Sensitivity		93.333%	88.333%	76.667%	83.333%
Specificity		85.000%	98.333%	93.333%	96.667%
Accuracy		89.167%	93.330%	85.000%	90.000%
Positive predictive value		86.154%	98.150%	92.000%	96.150%
Negative predictive value		92.727%	89.390%	80.000%	85.290%

mechanism of anti-TPO is explained during the maturation period. Anti-TPO may generate a cytotoxic environment that damages the maturing oocyte when it crosses through the blood follicular barrier. Thus, it is proven that the rise in these antibodies can lead to impaired fertility and miscarriage, and sometimes lead to premature birth, which is in accordance with (Silva *et al.* 2022). Studies have shown that Inhibin B levels decline with age due to the aging of the ovaries. Inhibin-B levels have been found to increase due to the elevated number of small growing follicles that are characteristic of PCOS. Due to this, excess Inhibin-B production inhibits the overabundance of FSH levels, contributing to the hormonal irregularities in PCOS (Fazil *et al.* 2023). Patients with POF under 40 years showed a decrease in Inhibin-B levels. Inhibin-B is

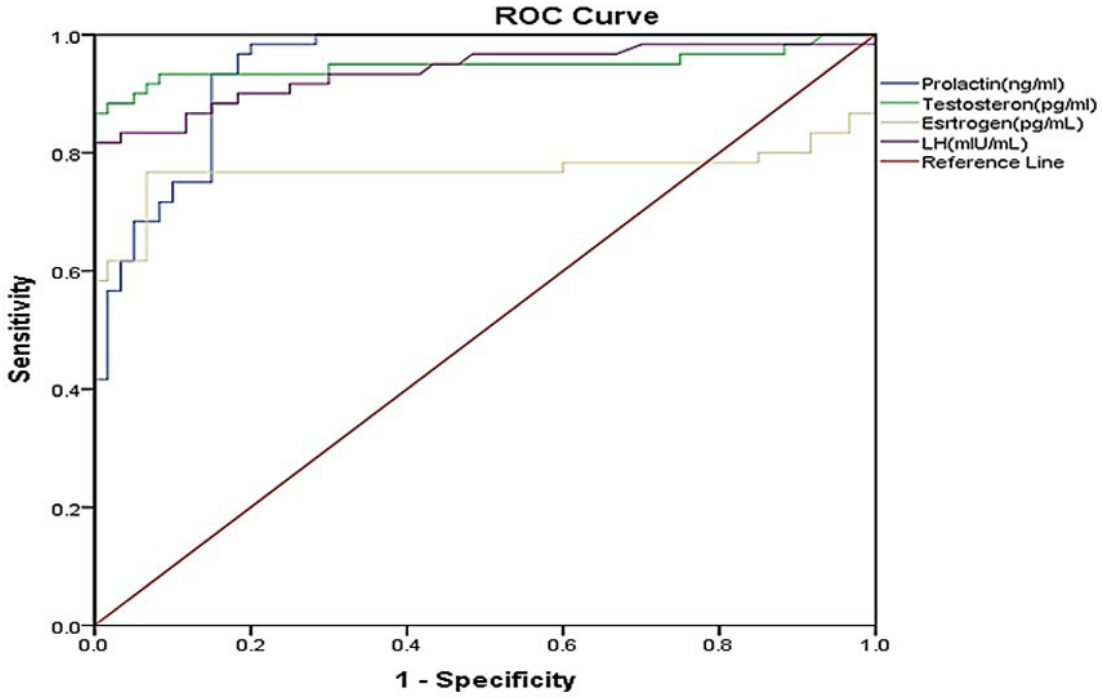

Figure 8. ROC Curve Demonstrated the Sensitivity and Specificity Values for hormones

considered one of the predictors of resuming ovarian function in POF patients. Recently, studies have demonstrated a marked ongoing decrease in Inhibin-B as POF progresses. When the level of inhibin-B decreases, the excretion of the hormone FSH increases, indicating a hormonal imbalance that suggests a failure of ovarian function (Zhu *et al.* 2021). The high Anti-TPO level in both PCOS and POF has a negative impact on pregnancy and fertility as it reduces ovum quality and fertilization potential by creating a toxic environment that affects ovum maturation (Bucci *et al.* 2022; Akdulum *et al.* 2022). Inhibin-B levels are elevated in secondary infertility more than in primary infertility, and when compared to their controls, the elevation is very significant, according to Anso (2023). This is due to an increase in FSH levels, resulting in a greater need to release larger amounts of Inhibin-B to discourage. As for Anti-TPO levels, they were higher in primary and secondary infertility patients compared to their controls. Due to a defect in vital processes, the body releases larger amounts of anti-TPO as an immune response to these abnormal processes (Safarian *et al.* 2023). What explains the increased levels of Inhibin-B is that obesity leads to polycystic ovaries. PCOS ovaries contain many follicles capable of producing large amounts of Inhibin-B. Therefore, the ovaries of PCOS may perfectly release more Inhibin-B than normal ovaries, and that agrees with Ibrahim *et al.* (2022) and Al-Ezairjawi *et al.* (2020) and Al-Fatlawi (2022). The levels of Anti-TPO are elevated in women with obesity, as obesity causes polycystic ovaries, which will lead to higher levels of Anti-TPO. This increases as a result of the high response of autoimmune thyroid in patients with PCOS. So, the importance of screening during the investigation of PCOS in infertile females should be not only in thyroid hormone levels but also in levels of anti-thyroid peroxidase antibodies (Sharma *et al.* 2022).

The reason for high LH and low or normal FSH levels in PCOS patients is that they interfere with the release of GnRH (LH and FSH), in a way that increases LH but decreases FSH in the peripheral circulation (Saadia *et al.* 2020). Nevertheless, it is the opposite of Pratama *et al.* (2024), who found that FSH levels increase in POF patients, which may be related to the presence of fewer follicles. The body produces less Inhibin-B, which is responsible for keeping FSH levels in the normal range. This means the pituitary gland continues to produce more FSH. So the body tries to work harder to grow and mature the remaining follicles. It agrees with Khudhair *et al.* (2024) but disagrees with Zafardoust *et al.* (2023). Prolactin levels are higher in PCOS patients due to the insulin resistance condition. Thus, reduced insulin levels could increase the likelihood of other causes of hyperprolactinemia (Naz *et al.* 2022). Prolactin levels are also elevated in

POF patients, and these high prolactin levels inhibit ovulation, which agrees with Yang & Yang (2023) but disagrees with Longobardi *et al.* (2024). The levels of testosterone in PCOS patients are very high due to the increase in androgen excretion. Since testosterone is considered an androgen family member, its rise in production is a result of either elevated insulin or high LH levels, which are typically observed in PCOS patients (Valdimarsdottir *et al.* 2021). In addition, there was no discernible variation in testosterone levels between the POF patients and the control groups.

The overproduction of estrogen by the ovaries is one of the leading causes of PCOS. So high levels of estrogen were recorded in PCOS patients. Additionally, impaired metabolism and disrupted hormonal feedback mechanisms contribute to higher estrogen levels, and androgens become increased in females with PCOS because of the high levels of LH. The increase in testosterone levels aligns with that of Luan *et al.* (2022). Estrogen levels decreased in POF patients due to the association of POF with hypogonadotropic (elevated FSH levels) and hypogonadism (Ghahremani-Nasab *et al.* 2020). High levels of LH were recorded in patients with polycystic ovaries. This increase in hyperandrogenic circumstances in PCOS may reduce hypothalamic sensitivity to negative feedback from estradiol and progesterone, leading to the pituitary producing more LH and less FSH (Wang *et al.* 2023). POF patients also have higher levels of LH hormone than PCOS patients because of the increase in the same negative feedback system brought on by progesterone and estradiol, which causes a decrease in FSH secretion and an increase in LH secretion.

Comparable results were documented in previous studies, showing the AUC, sensitivity, and specificity of Inhibin-B were 82.84%, 36.8%, and 86.6% (AL-Azawea & Mossa 2021), while Anti-TPO were 88.45%, 91.7%, and 87.1% (Rashad *et al.* 2024). Another previous study showed sensitivity and specificity for Inhibin-B were 95% and 86% (Al-Ezairjawi *et al.* 2020) and 95.7% and 88.5% (He *et al.* 2020). The patients and control individuals selected for this research, as well as variations in the kits and techniques employed, could account for the variations in sensitivity and specificity results. Results were documented in previous studies showed that the AUC, sensitivity, and specificity of prolactin were 83.2%, 77%, and 88% (Pedachenko *et al.* 2021), testosterone were 88.9%, 92.6%, and 85.4% (Wang *et al.* 2020), estrogen were 76.6%, 92.1%, and 55.8% (Deng *et al.* 2022), LH were 93.2%, 80%, and 93% (Weghofer *et al.* 2020). The patients and control individuals selected for this research, as well as variations in the kits and techniques employed, could account for the variations in sensitivity and specificity results.

We can conclude that the serum concentration of Inhibin-B and Anti-TPO was significantly increased in PCOS patient groups compared to healthy controls, and it was decreased in POF patients compared to healthy controls. This indicates the role of these biomarkers in the early identification and prediction of the type of female infertility and may facilitate supportive medical care for positive patient outcomes. A significant difference is evident in the Inhibin-B and Anti-TPO biomarkers between the patient group and the control group.

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