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Research Article

The Effect of Leptin on the Regulation of Immune Responses in Women with Polycystic Ovary Syndrome

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Abstract

Background and Objective: Leptin is a hormone produced by fat cells in adipose tissue that plays a biological role in metabolism, immune system control and energy flow regulation. The study's objective was to investigate serum leptin levels in PCOS females and determine how they are related to immunological and hormonal parameters. **Materials and Methods:** Thirty PCOS women were chosen for the study and compared to thirty healthy women as control samples, with both case and normal samples ranging in age from 18-36 years. WBC count, absolute numbers of neutrophils, lymphocytes, monocytes, eosinophils, leptin, Prolactin and serum IgA levels were measured. **Results:** The results showed the mean WBC, lymphocytes and eosinophil absolute numbers in PCOS women were significantly different. However, there was no significant difference in the absolute numbers of neutrophils and monocytes. The findings also revealed a significant increase in BMI, IgA, leptin and prolactin levels in PCOS when compared to controls. The results showed strong positive correlation coefficients between BMI and IgA (1.000**), WBC with neutrophils, lymphocytes and monocytes (0.797**, 0.790**, 0.712**), respectively and finally leptin and prolactin (0.474**). The same test, on the other hand, revealed an inverse correlation coefficient between BMI, IgA and prolactin (-0.376*, -0.376*, respectively with $p \leq 0.05$). **Conclusion:** A rise in the levels of the hormones leptin and prolactin, which were positively associated with the body mass index in women with PCOS, was found in the current study. The findings revealed that the hormones leptin and prolactin have an effect on some immune parameters in women with PCOS.

Key words: PCOS, leptin, prolactin, BMI, WBC, differential WBC, IgA

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

PCOS is a well-known endocrinopathy that affects reproductive-age women and is one of the most frequent endocrine-reproductive metabolic diseases. Hyperandrogenemia, infertility, hirsutism, obesity and menstrual abnormalities afflict roughly 5-10% of women of reproductive age^{1,2}.

PCOS is a complicated genetic disorder characterised by a significant degree of heterogeneity, the exact cause is unknown. In addition, in PCOS, hormones and immune white defence cells (innate and specific) are thought to interact. Low-grade chronic inflammation increases the likelihood of autoimmune disorders. This might be linked to estrogen levels as autoimmune disorders strike women at a younger age than they do males³. Furthermore, contacts between the cells of the immune system as well as their products such as growth factors, steroids, peptides, hormones and intermediaries and the ovary are important in the regulation of ovarian function⁴. Inflammatory reactions can cause infertility by disrupting important physiological functions such as embryo implantation and ovulation^{5,6}. Obesity in childhood (before the age of 12) appears to increase the likelihood of female infertility later in life⁷.

Patients with PCOS may experience persistent immune system activation as a result of obesity, with elevated levels of androgen and estrogen, resulting in an increase in pro-inflammatory cells such as T-helper1 and T-helper17 and a decrease in anti-inflammatory cells such as regulatory T-cells as well as a dichotomous change in antigen-presenting cells. Autoantibodies are produced as a result of an immune microenvironment imbalance, which triggers autoimmune disorders. It's also conceivable that the body's immunological tolerance is damaged, resulting in a persistent inflammatory state that impairs follicular ovulation creation⁸. Obesity-induced alterations in tumour necrosis factor- α (TNF), interleukin (IL-6), leptin and resistin were discovered in a recent study^{9,10}. The lifestyle and eating habits of PCOS patients were also linked to blood lipids¹¹. The accumulation of fat, which contributes to the recruitment of immune defence cells¹² is one of the most important risk factors for PCOS.

This syndrome has been defined as a chronic inflammatory condition that affects not only adipose tissue but also other organs such as the ovaries, especially in obese people¹³. When compared to healthy controls, the number of peripheral white blood cells in PCOS patients is substantially higher. Furthermore, in PCOS patients, the number of lymphocytes, mononuclear cells and eosinophil's is

significantly increased¹⁴. Some data has shown a link between deregulated leptin expression and the development of obesity-related diseases like PCOS^{15,16}.

Leptin is an adipocyte-derived hormone that circulates in the plasma as a free or protein-bound adipokine and is encoded by the 'ob' gene¹⁷. Leptin suppresses hunger, increases energy expenditure and lowers hypothalamic synthesis of neuropeptide Y. Leptin, which acts at several levels of the hypothalamus-pituitary ovarian axis, may also have a role in reproductive function¹⁸. According to certain research, circulating leptin levels are favourably linked with body fat regardless of the presence of PCOS^{19,20}.

Breast feeding, luteal function, reproduction, appetite, fertility suppression, balance, osmotic protection and blood clotting are all regulated by prolactin (PRL), a single-chain polypeptide hormone produced by the pituitary gland. Prolactin is a powerful lipogenic and diabetogenic hormone that has an impact on energy balance and fuels metabolism²¹.

The study examine the leptin influenced several immunological and hormonal alterations in PCOS women and compare them to BMI- and age-matched healthy women. To understand more about how limiting chronic inflammation plays a role in the development of PCOS, explored if white blood cell count is connected to the immunologic, physiological and hormonal aspects of the patients.

MATERIALS AND METHODS

Study area: From 1 July, 2021 to 1 February, 2022, the research was conducted at the Department of Biology's Advanced Immunology Lab in Iraq.

Study subjects: Thirty women with PCOS were chosen for the study and compared to thirty healthy women as control samples, for both patients and the control group ages ranged from 18-36 years. Age, weight and height were obtained from the patient and control groups and any cases of acute or chronic disease were excluded.

Blood and serum collection: Each participant had 5 mL of venous blood taken from them. About 1 mL of blood was placed in an EDTA tube for the complete blood count. The leftover material was centrifuged for 5 min at 3000 rpm after coagulating for 5 min at 37°C in a gel tube. Separated serum was collected in Eppendorf tubes and kept at -20°C for subsequent analysis.

Complete Blood Count (CBC): A Mythic™ 18 (Switzerland) automated system was used to read 18 haematological parameters²².

Prolactin hormone: The level of prolactin hormone was quantified using a Korea Boditech Med, Inc. AFIAS-6 instrument, a fluorescence immunoassay method for the quantitative detection of PRL in human serum. Its automated testing approach enabled it to conduct several tests for six distinct samples at once. A capillary tip was used to collect a small volume (10 L) of serum for quantitative testing²³.

Determination of leptin concentration in serum by human

LEP ELISA kit: A sandwich enzyme-linked immune-sorbent assay technology was used to develop this product (MyBioSource, USA). In a 96-well plate, the capturer antibody was pre-coated. As detecting antibodies, biotin-conjugated antibodies were utilized. The standers, test samples and biotin-conjugated detection antibody were then added to the well, followed by a wash buffer. Unbound conjugates were washed away with wash buffer after HRP-Streptavidin was added. The HRP enzymatic reaction was observed using the TMB substrate. TMB was converted to a blue-coloured product by HRP, which became yellow when exposed to an acidic stop solution. The yellow density is linked to the amount of sample trapped in the plate. The concentration of leptin was determined using the O.D. absorbance at 450 nm in a microplate reader.

Determination of IgA concentration: The concentration of IgA antibodies was calculated using the BMI value and the following formula²⁴:

$$\text{IgA} = \beta_0 + \beta_1 \text{ BMI}$$

$$\beta_0 = -309.608, \beta_1 = 12.215$$

Statistical analysis: To compare the means statistically, an ANOVA with a probability of $p \leq 0.05$ was utilized. BMI, WBC, neutrophil, lymphocyte, monocyte, leptin, prolactin and IgA all had correlation coefficients. To determine the significance of the correlation coefficients, a basic linear regression coefficient analysis was conducted to analyse the connection between the variables. The statistical analyses were carried out using the SPSS 24 statistical analysis tool and the SPSS 24.0 software.

RESULTS AND DISCUSSION

The current study involved tracking the most important immunological indicators, the total and calculated absolute numbers of white blood cells, which play a significant role in resisting infectious agents and in autoimmune diseases. These

include neutrophils, which are the first immune line of defence against infection factors in the blood, mononuclear cells, which are responsible for phagocytic activity in tissues, lymphocytes, which represent the immune system's specialised immunological arm as well as eosinophil's, which cause allergy symptoms.

The overall WBC count as well as the absolute number of lymphocytes and eosinophil's, were significantly increased by 6723.33 ± 1536.3 , 5150.03 ± 792.5 , 336.16 ± 77.06 , respectively when comparing PCOS patients to the control group, 5946.66 ± 1235.6 , 1607.60 ± 349.4 , 297.66 ± 61.52 (0.035^* , 0.001^* , 0.304^*) with $p \leq 0.05$. However, there was no significant difference in the number of neutrophils and monocytes as shown in Table 1.

In PCOS patients, the study found a significant increase in BMI and the concentrations of both leptin and prolactin hormones (31.23 ± 4.780 , 162.1098 ± 198.68 , 20.617 ± 10.870) respectively, when comparing PCOS patients to the control group, (24.25 ± 3.192 , 49.3375 ± 22.140 , 13.344 ± 2.245) respectively as well as a significant decline in the IgA immunoglobulin (71.9701 ± 58.39) compared with control (137.3800 ± 29.19), (0.000^* , 0.003^* , 0.001^* and 0.000^*) respectively with $p \leq 0.05$ as shown in Table 2.

Correlation coefficients were calculated to identify the link between leptin concentration and each immunological parameter as well as BMI and prolactin hormone. The results showed a strong positive correlation coefficient between BMI and IgA (1.000^{**}), WBC with neutrophils, lymphocytes and monocytes (0.797^{**} , 0.790^{**} and 0.712^{**} , respectively) and finally between leptin and prolactin (0.474^{**}), with $p \leq 0.01$. On the other hand, the same test observed an inverse correlation coefficient between BMI, IgA and prolactin (-0.376^* and -0.376^* , respectively) with $p \leq 0.05$ as shown in Table 3.

PCOS is one of the most common reproductive disorders, with a varied clinical character that involves, in addition to hormonal problems, several additional symptoms. Several articles discuss WBC's function in the pathophysiology of PCOS, In comparison to normal, healthy women, WBC levels were consistently shown to be higher in women with PCOS. The key question is whether this leucocytosis is an innate feature of the disease or a result of its metabolic features²⁵⁻²⁷. The present study's findings corresponded with the findings of many other studies, which indicated that an increase in weight is one of the most important factors causing an increase in the number of white blood cells in women with PCOS^{28,29}. Leptin influences immune system development, reproduction, anti-apoptotic activity, maturation, ovulation and activation³⁰. Neutrophils, monocytes and lymphocytes have all been shown to have leptin receptors. Leptin has been

Table 1: Comparison of the total WBC count and the absolute numbers of neutrophils, lymphocytes, monocytes and eosinophils between PCOS patients and controls

Cell (mm ⁻³)	Samples	Numbers	Mean±SD (cell mm ⁻³)	Extreme value (cell mm ⁻³)	p-value
WBC count	PCOS	30	6723.33±1536.3	10400-4000	0.035*
	Control	30	5946.66±1235.6	8800-4500	
Neutrophils	PCOS	30	3798.43±914.0	5115-2187	0.096
	Control	30	3289.73±1371.9	6116-2505	
Lymphocytes	PCOS	30	5150.03±792.5	4241-1387	0.001*
	Control	30	1607.60±349.4	3596-1235	
Monocytes	PCOS	30	438.70±146.2	842-193	0.289
	Control	30	364.93±348.10	418-155	
Eosinophil's	PCOS	30	336.16±77.06	520-200	0.304*
	Control	30	297.66±61.52	440-225	

*p≤0.05

Table 2: Comparison between BMI, IgA, Leptin and Prolactin in PCOS patients and control

	Samples	Numbers	Mean±SD	Extreme value	p-value
BMI	PCOS	30	31.23±4.780	1094-2769	0.000*
	Control	30	24.25±3.192	2100-6291	
IgA (mg dL ⁻¹)	PCOS	30	71.9701±58.39	205.445-9.725	0.000*
	Control	30	137.3800±29.19	422.2-95.1	
Leptin (pg mL ⁻¹)	PCOS	30	162.1098±198.68	270.948-43.24	0.003*
	Control	30	49.3375±22.140	95.933-31.25	
Prolactin (ng mL ⁻¹)	PCOS	30	20.617±10.870	54.19-10.53	0.001*
	Control	30	13.344±2.245	19.17-10.8	

*p≤0.05

Table 3: Correlation coefficient test between BMI and leptin, prolactin and immunological parameters in PCOS patients

	BMI	WBC	Neutrophils	Lymphocytes	Monocytes	Leptin	Prolactin	IgA
BMI								
Pearson correlation	1	-0.007	0.010	-0.050	0.132	-0.121	-0.376*	1.000**
Sig. (2-tailed)		0.971	0.957	0.795	0.485	0.524	0.041	0.000
N	30	30	30	30	30	30	30	30
WBC								
Pearson correlation	-0.007	1	0.797**	0.790**	0.712**	0.114	-0.066	-0.007
Sig. (2-tailed)	0.971		0.000	0.000	0.000	0.550	0.731	0.971
N	30	30	30	30	30	30	30	30
Neutrophils								
Pearson correlation	0.010	0.797**	1	0.265	0.271	0.183	-0.117	0.010
Sig. (2-tailed)	0.957	0.000		0.158	0.148	0.332	0.538	0.957
N	30	30	30	30	30	30	30	30
Lymphocytes								
Pearson correlation	-0.050	0.790**	0.265	1	0.815**	-0.027	0.016	-0.050
Sig. (2-tailed)	0.795	0.000	0.158		0.000	0.886	0.933	0.795
N	30	30	30	30	30	30	30	30
Monocytes								
Pearson correlation	0.132	0.712**	0.271	0.815**	1	0.134	-0.009	0.132
Sig. (2-tailed)	0.485	0.000	0.148	0.000		0.480	0.961	0.485
N	30	30	30	30	30	30	30	30
Leptin								
Pearson correlation	-0.121	0.114	0.183	-0.027	0.134	1	0.474**	-0.121
Sig. (2-tailed)	0.524	0.550	0.332	0.886	0.480		0.008	0.524
N	30	30	30	30	30	30	30	30
Prolactin								
Pearson correlation	-0.376*	-0.066	-0.117	0.016	-0.009	0.474**	1	-0.376*
Sig. (2-tailed)	0.041	0.731	0.538	0.933	0.961	0.008		0.041
N	30	30	30	30	30	30	30	30
IgA								
Pearson correlation	1.000**	-0.007	0.010	-0.050	0.132	-0.121	-0.376*	1
Sig. (2-tailed)	0.000	0.971	0.957	0.795	0.485	0.524	0.041	
N	30	30	30	30	30	30	30	30

*Correlation is significant at the 0.05 level (2-tailed) and **Correlation is significant at the 0.01 level (2-tailed)

shown in recent studies to increase *in vivo* cellular proliferation in Siberian hamsters and splenocyte proliferation in mice as also the percentage of CD⁴⁺ lymphocytes in endometriosis patient's peritoneal fluid. Even in the elimination of monocytes, the effects of leptin on T-cells may be observed. When activated, human leptin has a direct influence on circulating T-cells³¹. The current study found that an increase in lymphocytes and eosinophil's is correlated with an increase in leptin levels. Furthermore, leptin may stimulate eosinophil chemokinesis and result in the production of IL-1 and IL-6 as well as the inflammatory mediator IL-8³².

The ovary is a multi-compartmental organ with constantly changing tissue that is primarily regulated by the hypothalamus and pituitary gland. Leptin has been identified as a possible regulator and peripheral indicator of a variety of reproductive processes, including the ovary's propensity to be gametogenic and steroidogenic. There is thought to be a connection between nutrition and reproduction³³.

Women's reproductive capability is harmed by significant changes in either direction in nutritional status and energy availability. When the nutritional status is restored, these adaptive alterations can be reversed^{34,35}. Leptin regulates energy balance and as a result, has a variety of effects on the reproductive system³⁶. During the menstrual cycle, leptin concentrations fluctuate inside the ovary³⁷. Estrogens, progesterone, androgens and insulin are all proven to have a strong relationship with serum leptin.

However, their role in the regulation of circulating leptin levels is uncertain. Even though leptin is prevalent in reproductive tissues, little is known about how it interacts with reproductive hormones^{38,39}. Because PCOS patients' leptin

levels were greater than the control group's and there was no correlation coefficient between leptin concentration and BMI, The current study's major finding is that the PCOS population had higher leptin levels regardless of BMI. Because adipocytes create the bulk of leptin, PCOS patients with a higher BMI have a higher prevalence of the condition.

To measure the increase in BMI in connection with the concentration of IgA, a simple linear regression coefficient statistical test is shown in Fig. 1. The estimations of IgA (Y-axis) depending BMI value (X-axis), It appears that there is a direct relationship between BMI and IgA concentration as whenever the IgA value increases by one unit, the BMI value will rise by (0.082) with a significant level of 0.000* as shown in Table 4.

The presence of foreign antigens in obese people could be explained by the presence of foreign antigens in fat tissue or blood vessels, which are required for free immunoglobulin intervention to control the inflammatory state in fat tissue. The findings of Al-Sufyani and Mahassni⁴⁰, who found a significant rise in the number of neutrophil cells responsible for phagocytosis, which increases when the body is in an inflammatory state, in obese patients, backed up this theory.

To measure the increase in BMI in connection with the concentration of prolactin, a simple linear regression coefficient statistical test is shown in Fig. 2. The estimations of BMI value (X-axis) depending prolactin concentration (Y-axis), It appears that there is an inverse relationship between BMI and prolactin concentration as whenever the prolactin value increases by one unit, the BMI value will decrease by (-0.855) with a significant level of 0.041* as shown in Table 5.

Prolactin is produced and released by both adipose and breast tissue in response to specific inflammation or

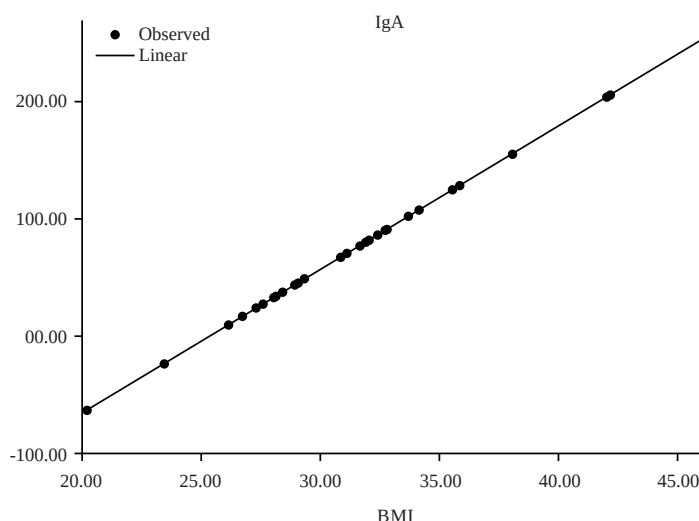


Fig. 1: A simple linear regression of the relationship between BMI and IgA levels

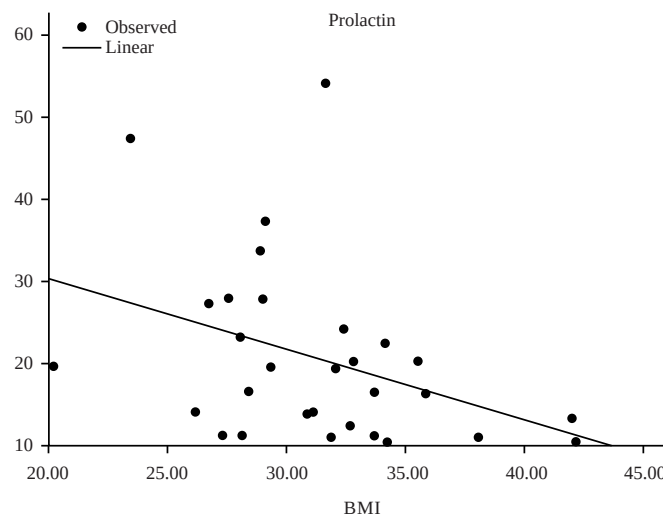


Fig. 2: A simple linear regression based on the relationship between BMI and prolactin levels

Table 4: Analysis of simple linear regression equation depending on BMI value

Dependent variable: BMI							
Model summary						Parameter estimates	
Equation	R square	F	df1	df2	Significant	Constant	b1
Linear	1.000	7564289405.118	1	28	0.000	25.347	0.082
Independent variable is IgA							

Table 5: Analysis of simple linear regression equation depending on prolactin concentration

Dependent variable: Prolactin							
Model summary						Parameter estimates	
Equation	R square	F	df1	df2	Significant	Constant	b1
Linear	0.141	4.610	1	28	0.041	47.325	-0.855
Independent variable is BMI							

hyperglycemia^{41,42} and also adipose tissue macrophages⁴³. It stimulates adipose tissue development and prevents lipid breakdown in adipose tissue⁴⁴. The research found that the amount of prolactin generated by subcutaneous fat cells obtained from obese people is lower than the prolactin generated by the same type of cells in non-obese individuals^{42,45}. Although weight appears to restrict the synthesis of prolactin, the mechanism of this reduction is unclear, however, one study found that prolactin concentration is unrelated to BMI⁴⁶. The deficiency in lipid metabolism in PCOS patients may be one of the most significant causes. As the hormone prolactin was directly connected to lipid metabolism, a low physiological level of PRL signals a very serious lipid metabolism issue.

Prolactin has a range of effects on energy output, one of which is functioning as a key regulator of food consumption.

One of PRL's orexigenic effects is the development of central leptin resistance and a reduction in cellular responses to leptin receptor activation within the paraventricular hypothalamic nucleus⁴⁷.

One of leptin's most significant tasks is connecting dietary status and immunological response. The immune response is regulated by leptin in both normal and pathological conditions.

Leptin affects the immune system in a variety of ways, including proliferation, replication, anti-apoptosis, maturity and stimulation.

Leptin controls both innate and adaptive immune responses and has the power to impact all types of cells in the immune system. Macrophages (monocytes), macrophages (neutrophils) and lymphocytes all have leptin receptors belonging to the group I cytokine receptor family. In the

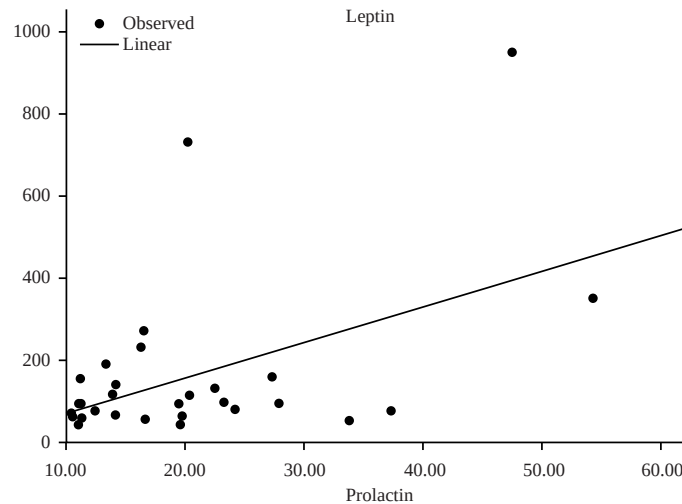


Fig. 3: A simple linear regression based on the relationship between leptin and prolactin levels

Table 6: Analysis of simple linear regression equation depending on prolactin concentration with respect to leptin levels

Dependent variable: Prolactin							
Model summary						Parameter estimates	
Equation	R square	F	df1	df2	Significant	Constant	b1
Linear	0.224	8.094	1	28	0.008	16.417	0.026
Independent variable is Leptin							

Table 7: Analysis of simple linear regression equation depending on IgA concentration

Dependent variable: IgA							
Model summary						Parameter estimates	
Equation	R square	F	df1	df2	Significant	Constant	b1
Linear	0.141	4.610	1	28	0.041	113.613	-2.020
Independent variable is Prolactin							

immune system, leptin activates pro-inflammatory cells, promotes T-cell responses and regulates the secretion of inflammatory cytokines such as TNF, interleukin IL-2 and IL-6. Synthesis of the leptin receptor is also increased by some proinflammatory signals. It has been shown that decreased leptin production is linked to an elevated risk of infection^{8,48}. Given the established link between leptin and obesity, it is reasonable to believe that leptin may play a role in PCOS. Inflammatory circumstances and metabolic failure cause certain adipose tissue to produce more prolactin, according to a study that found a link between impaired metabolism and low prolactin levels. In his investigation, Glinborg *et al.*⁴⁹ discovered that prolactin levels were decreased in PCOS patients. Zandi *et al.*⁵⁰ concluded that PCOS patients had greater prolactin levels than controls, although this was not statistically significant.

To measure the increase in the leptin concentration in connection with the concentration of prolactin, a simple linear

regression coefficient statistical test is shown in Fig. 3. The estimations of leptin concentration (Y-axis) depending prolactin concentration (X-axis), It appears that there is a direct relationship between prolactin and leptin concentration as whenever the leptin value increases by one unit, the prolactin value will rise by (0.026) with a significant level of 0.008* as shown in Table 6.

To measure the increase in the IgA concentration in connection with the concentration of prolactin, a simple linear regression coefficient statistical test is shown in Fig. 4. The estimations of prolactin concentration (X-axis) depending on IgA concentration (Y-axis), It appears that there is an Inverse relationship between prolactin and IgA concentration as whenever the IgA value increases by one unit, the prolactin value will decrease by (-2.020) with a significant level of 0.041* as shown in Table 7.

The function of IgA immunoglobulin within tissues includes resistance to infection influences and resistance to

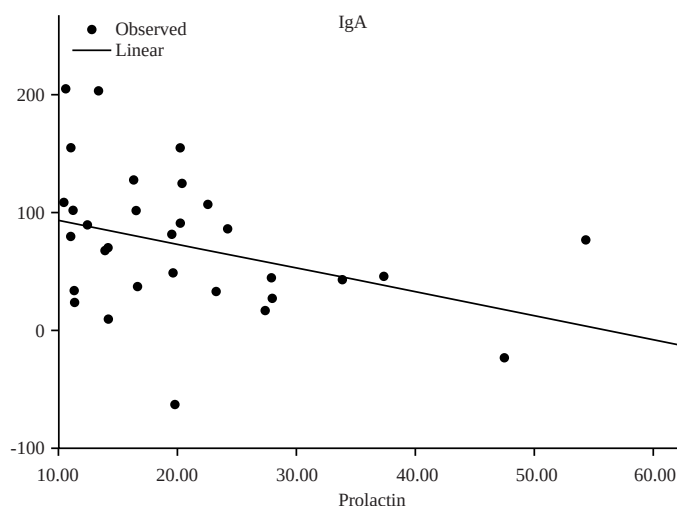


Fig. 4: A simple linear regression based on the relationship between IgA and prolactin levels

the emergence of foreign antigens in a specific tissue. The relationship between immune responses and leptin hormone activity in PCOS patients may not be apparent but after reviewing the results of the simple linear regression, it was discovered that IgA concentration increased in a direct relationship with BMI as shown in Fig. 1. As shown in Fig. 2, BMI is inversely correlated with the hormone prolactin. Prolactin has an inverse relationship with IgA. As a result, a decrease in IgA concentration is correlated with an increase in the hormone leptin. An increase in leptin levels may impact the humoral immune response and immunoglobulin, which is responsible for resistance to infection factors, particularly in the sebaceous and secretory tissues.

CONCLUSION

A rise in the levels of the hormones leptin and prolactin, which were positively associated with the BMI in women with PCOS, was found in the current study. The findings revealed that these hormones have an effect on some immune parameters in women with PCOS. The relationship between leptin and reproductive hormones is still unclear and more research is needed.

SIGNIFICANCE STATEMENT

In addition to an increase in body mass index, women with PCOS experience immune changes, at both the humoral and cellular immune levels as well as hormonal changes. The current study will aid in reaching a better understanding of the relationship between hormonal and immune variables in

PCOS patients as well as in the search for the hormonal or immune causes of the syndrome's onset.

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