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A New Immune-Biochemical Study To Identify Indicators Of The Response Of Infertile Polycystic Ovary Syndrome Patients To Metformin Treatment

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Abstract

Background: *Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women throughout their lifespan. The prevalence of PCOS varies depending on the population studied, but it is estimated to affect between 10% and 13% or ~140 million women globally. PCOS in adults is diagnosed according to the International Evidence-based Guideline Criteria based on two of either ovulatory dysfunction, clinical or biochemical hyperandrogenism, and/or polycystic ovary morphology (PCOM) on ultrasound or elevated anti-Müllerian hormone levels. Infertility is a serious global health problem affecting an estimated 50 to 70 million couples worldwide. Infertility is a condition in which pregnancy does not occur even after regular unprotected sex for more than one year ، for women under 35 years of age, a couple's failure to conceive after 12 months of regular sexual activity without using contraception, and after 6 months for women 35 years of age or older. Infertility results in disability expressed through impaired function, social exclusion, and psychological trauma; thus, it is ranked by the World Health Organization as the fifth highest serious disability worldwide.*

Population of the study: *This study included samples of 90 women of reproductive age (20-40 years). They were distributed into three groups. The first: included 30 patients undergoing treatment for infertility due to PCOS, the second group included 30 patients who were diagnosed with PCOS through the simultaneous appearance of 3-4 symptoms that are essential for diagnosis. The third group (the control group) included 30 women who were completely free of symptoms of PCOS and all of whom had children without any medical intervention and did not suffer from any disease, based on clinical examinations and ultrasound examinations.*

Kits and technique: *Sandwich-ELISA technique was applied to determine the level of integrin, CLEC10A and interleukin-42 in the serum samples of the study individuals.*

Results: *Respectable statistical differences in integrin levels were observed when comparing the group of PCOS patients treated with infertility drugs (p=0.000), as well as the untreated PCOS patients (p=0.010) with the healthy control group. The results showed a significant elevation in CLEC10A levels comparison to the untreated PCOS patients group (p=0.000).The results show a significant statistical differences when comparing interleukin-42 levels in the group of patients with PCOS patients treated with infertility drugs compared to the group of patients with Untreated PCOS patients group (p=0.000) or healthy women in the control group (p=0.002).The results indicated the significant positive correlations when studying the relationship between Integrin and CLEC10A in the group PCOS patients treated with Metformin (r=0.841 at p=0.000) and in the group of untreated PCOS patients (r=0.854 at p=0.000), as well as healthy women (r=0.834 at p=0.000). According to the results, there is a strong negative correlation between Integrin and Interleukin-42 in PCOS patients treated with Metformin (r=0.871 at p=0.000), while; the significant positive correlations were observed when studying the relationship between Integrin and Interleukin-42 in the group of untreated PCOS patients (r=0.879 at p=0.000) and the group of healthy females (r=0.516 at p=0.040). The statistical analysis indicates a strong negative correlation between CLEC10A and interleukin-42 in PCOS patients treated with Metformin (r=0.859 at p=0.000), while changing in the opposite direction the results indicated to positive correlations between CLEC10A and interleukin-42 in the group untreated PCOS patients (r=0.619 at p=0.009), as well as, in the group of healthy females (r= 0.902 at p=0.000). The study showed the highest sensitivity (97%) and specificity (93%) for interleukin-42 were*

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recorded in the Metformin-treated group. The study revealed that integrin exhibited the highest individual sensitivity (96%) and specificity (80%) in the group of untreated PCOS patients. ***Conclusions:*** *CLEC10A and interleukin-42 are promising tools for differentiating PCOS patients treated with Metformin and untreated PCOS patients.*

Key Words *PCOS, Integrin, CLEC10A and Interleukin-42.*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex common endocrine disorder affecting around 5–20% of reproductive aged women, depending on the diagnostic criteria used and population studied [1]. PCOS is characterized by hyperandrogenism, oligoovulation and/or polycystic ovaries, is a very common endocrine and metabolic disorder with an increased risk of reproductive abnormalities, often accompanied by insulin resistance (IR) [2].PCOS is a heterogeneous condition that can cause a variety of reproductive, metabolic, and psychological effects, and its severity may vary between individuals depending on factors such as age, diet, race, genetics, medications, contraceptive use, obesity, body mass index, and geographic region [3]. PCOS is recognized as the leading cause of anovulatory infertility, accounting for approximately 70% of infertility cases related to ovulation disorders [3]. Hyperandrogenism in women with PCOS can be detected clinically by evaluation of hirsutism, acne, or androgenic alopecia, or by biochemical testing of circulating androgen concentrations [4].Worldwide, the prevalence of PCOS ranges from 4% to 21% [5]. The prevalence of PCOS varies widely worldwide. A recent meta-analysis examining the prevalence of PCOS in women of reproductive age across different ethnic groups worldwide indicated that women in the Middle East had one of the highest prevalence rates, at 16.0% [6]. A comprehensive analysis of the Middle East and North Africa (MENA) region revealed an overall prevalence of clinical infertility of 7.2% [6]. Considering all studies, the prevalence of PCOS was 4.98% according to the NIH 1990, 8.80% according to the Rotterdam 2003, 4.74% according to the AE-PCOS 2006, and 1.69% according to other criteria/self-report, with an overall PCOS prevalence of 4.57%. All studies using the NIH 1990, Rotterdam 2003, and AE-PCOS 2006 were of high quality, and those using other criteria/self-report were of low quality, so subgroup analysis based on quality resulted in the same prevalence rates as above [7]. The main cause of infertility in Iraqi Arab women is PCOS, which accounts for about 46% of all infertility cases. The prevalence of PCOS appears to be increasing due to changes in lifestyle, nutrition, and obesity. This confirms the findings in other studies where prevalence studies were classified by geographic location. It should also be noted that even within the same ethnic group, i.e. Middle Eastern women [8]. There is a lack of comprehensive research on the prevalence of PCOS in Iraq. The recent study conducted in Erbil, the only local investigation available, focused on infertile women seeking assistance at an in vitro fertilization (IVF) center, using the Rotterdam 2003 criteria for diagnosis, and the results revealed a prevalence of PCOS of 33% among the participants [9]. C-type lectin domain family, member 10A (CLEC10A) is a member of the C-type lectin receptor family and is also called C-type lectin in macrophages [10]. which are non-enzymatically linked to carbohydrate structures [11]. Human CLEC10A is expressed by multiple cell types, namely monocyte-derived dendritic cells (moDCs) [12]. CLEC10A is expressed at several critical stages of human development with highest expression in oocytes. The pluripotent eight-cell stage of embryogenesis expresses CLEC10A at levels approximately 70-fold higher than later embryonic stem cells [11].CLEC10A is an endogenous receptor that has been proposed as a target for cancer immunotherapy. In general, C-type lectin receptors require calcium to bind the sugar ligandto achieve the correct structure of the binding site and to coordinate with the sugar hydroxyl groups. CLEC10A is expressed on epidermal dendritic cells, immature peripheral dendritic cells, alternatively activated M2a macrophages, and other tissues [13].

Integrins are heterodimeric transmembrane adhesion receptors, formed by the non-covalent linkage of one out of 18 α- and one out of 8 β-subunits [14]. The term "integrin" originates from its function as an integral membrane protein complex that links the extracellular matrix to the cytoskeleton [15]. When bound to extracellular matrix (ECM) proteins, they are able to transmit bidirectional signals across cell membranes,

thus mediating biological processes, such as cell adhesion, migration/invasion, proliferation, and survival [16, 17]. According to the different binding properties of integrins, integrins can be divided into four types: leukocyte adhesion integrins, RGD-binding integrins, collagen-binding integrins (GFOGER), and laminin-binding integrins[13]. Integrins play a key role in most mechanical signal transduction pathways, and integrin-mediated mechanotransduction is mostly responsible for tissue differentiation, development, and maintenance of homeostasis [18]. Integrin transmits this biomechanical signal to the cytoplasm in biochemical form and stimulates subsequent signaling pathways via focal adhesion (FA) formation. These subsequent signaling pathways then produce the final cellular response to the biomechanical signal [17]. Integrin-mediated cell adhesion is regulated at multiple levels, these levels include regulation of integrin expression, ligand availability, dimmer specificity and activity, and recruitment of various proteins within cells [16]. Integrins are metalloproteins whose functions are strictly dependent on free calcium and free magnesium, which are physiologically present in the serum at the level of millimoles [19]. Previous studies have shown that Ca^{2+} maintains vegetables in an inactive state by binding to a metal ion-binding site called ADMIDAS in the β1 subunit domain of vegetables (βI), and removal of extracellular Ca^{2+} rapidly induces the activation of vegetables within seconds[20]. Itgb1 has emerged as a key player in PCOS research due to its involvement in regulating cell morphology and signaling pathways [21]. Interleukins are a group of protein compounds that belong to a class of cytokines that are produced by many cells in the body, including immune cells. They are involved in many important cellular processes, including proliferation, maturation, migration, and adhesion, and are also involved in the activation and differentiation of immune system cells[22]. Interleukins exhibit three characteristic mechanisms of action on other cells: autocrine, paracrine, and endocrine [22].The classification of interleukins adopted in the literature was the division based on their biological effect in the inflammatory response. There are three groups distinguished: the first, the largest group are inflammatory cytokines, which include 22 molecules, such as IL-1, IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, IL-14 and IL-15. The second group includes anti-inflammatory molecules, which are 14 interleukins, such as: IL-7, IL-10, IL-30 and IL-37. The last group consists of interleukins with a dual function, which, in appropriate situations, can act as inflammatory and anti-inflammatory molecules—IL-2, IL-3, IL-11 or IL-12 [23]. Interleukins include more than 60 types of cytokines [24].

MATERIALS AND METHODS

The Population: Over a period of five months, from October 2024 to March 2025, 90 women participated in the current study. These participants were divided into three groups.

The first group included 30 samples from patients undergoing infertility treatment with metformin, collected from the Fertility Center - Al-Sadr Medical City in Najaf Governorate. The second group included 30 samples from patients with PCOS who had been diagnosed with infertility and had not received any treatment at all. These samples were collected from Al-Zahraa Teaching Hospital and Al-Furat Al-Awsat Hospital. The study included patients with primary or secondary infertility due to PCOS, aged 20–40 years.

The third group included 30 healthy women with normal testosterone levels, regular menstrual cycles, normal ovulation, healthy ovaries, natural childbearing without surgical intervention, no use of contraception, no history of PCOS, and within the study age group (20-40 years). Samples of Control group were collected from a hospital work environment, in addition to graduate students at the College of Education for Girls and their relatives.

Inclusion Criteria:.The current study included the participation of patients characterized by the following:

- **Symptoms of the Syndrome:** the patients must exhibit at least 3-4 symptoms of PCOS.

- **Physiological Puberty:** the participating patients must have normal puberty and not suffer from primary amenorrhea, before symptoms of the PCOS appear.
- **Marital Status:** all patients must be married for at least one year and suffer from primary or secondary infertility due to PCOS.
- **Age:** the age of participants (patients and controls) must be at least 20 years old, and no more than 4o years old.

Exclusion Criteria: a number of female were excluded, which included: Unmarried, pregnant women, females (patients or controls) who use contraceptives, menopause females, female patients who suffered from cancerous or chronic disease (diabetes, cardiovascular diseases and hypothyroidism), females with autoimmune diseases, smokers.

Assessment of Lectins Concentration: sandwich enzyme linked immune sorbent assay (Sandwich-ELISA) method was applied to determine the level of Integrin, CLEC10A and Interlukin-42in the serum samples of the study individuals.

The Statistical Analysis of the Data: The outcomes of the present study were analyzed through the statistical package for the social sciences (SPSS) version 26 software application statistical analysis system and excel (statistical package). The variables were illustrated by mean ± S.D, minimum, maximum, frequencies, and percentages. Graphics are presented using pie and bar charts. Inferential data analysis includes analysis of variance (ANOVA) test was applied to assess differences between the levels of the studied parameters. Pearson's correlation was applied to determine the relation among the parameters of the present study. The probability of deflection than controls are considered statistically significant if p-value is below 0.05. Receiver operating characteristic (ROC) curve was applied to present the sensitivity of the evaluated parameters. Combined sensitivity and specificity percentages were calculated according to biomedical statistical.

RESULTS AND DISCUSSION

- **Evaluation of Integrin in The Sera Samples of The Study Groups:** The ANOVA test was unsuccessful to demonstrate statistically significant variations **(p=0.064)** in the integrin level when comparing the groups of PCOS patient together. In contrast, respectable statistical differences were noticed when the comparisons between the group of patients with PCOS treated with infertility drugs **(p=0.000),** as well as patients with Untreated PCOS **(p=0.010)** to the healthy control group were done, as illustrated in **Table 1**.

Table 1: Integrin Levels in the Serum Samples of the Studied Groups

Subjects (n)	Integrin(µg/mL) Mean ± SD	Minimum-Maximum	p-value
G1 PCOS Patients (30)	34.116±4.546	28.107-47.996	0.064 For G1 vs G2 0.000 For G1 vs C 0.010 For G2 vs C
G2 PCOS Patients (30)	32.017±3.493	24.313-38.043	
Controls (30)	29.061±4.845	12.726-35.229	

G1: Group of PCOS patients treated with infertility drugs, G2: Untreated PCOS patients group and Controls: A group of healthy individuals. The mean difference is significant at the 0.05 level.

The focus observation indicated that the highest concentration of integrin (47.996 µg/mL) was recorded in the G1while the lowest concentration of integrin (12.726 µg/mL) was noted in the sample of Controls. Integrins are heterogeneous cell surface adhesion molecules found in all nucleated cells. The 18 alpha subunits and 8 beta subunits form 24 distinct heterodimers, each with distinct functional and

histological properties. These subunits integrate processes in the intracellular space with those in the extracellular environment [25].

The term "integrin" arises from its function as an integral membrane protein complex that links the extracellular matrix and the cytoskeleton[18]. Integrins are divided into four types according to the different binding properties of the integrins: leukocyte adhesion integrins, RGD-binding integrins, collagen-binding integrins (GFOGER), and laminin-binding integrins [18]. As a key regulator of cell adhesion, migration, and cytoskeletal organization, integrin beta 1 (Itgb1) is essential for maintaining tissue morphogenesis and cellular homeostasis in neuroglia systems [26, 27]. Its involvement in neuroglia interactions suggests a potential role for it in regulating hypothalamic tanycytes and GnRH cells. In addition, focal adhesion kinase (FAK) is involved in integrin-mediated signal transduction, activating transforming growth factor receptor beta 1 (TGF-βR1) and its downstream effector Smad2 [25,28]. These properties and physiological functions make Itgb1 a pivotal candidate for exploring the molecular mechanisms underlying abnormal neural homeostasis in PCOS [28]. A study showed that endometrial ITGB3 mRNA expression levels were lower in PCOS patients before LOD compared to healthy controls. A significant increase in ITGB3 mRNA expression was detected in endometrial biopsies taken three months after LOD. The study found a 3.8-fold increase in ITGB3 mRNA in the endometrium after LOD. Subtle changes in integrin expression were observed after LOD. Progesterone resistance may be one possible mechanism behind the increased expression of ITGB3 after LOD. Dysregulation of progesterone receptor expression in PCOS may lead to decreased expression of progesterone-responsive ITGB3 mRNA. was concluded that subfertility in PCOS is not only due to impaired folliculogenesis but also to impaired expression of endometrial integrins and fibroblast-activating factor [29]. Integrin inhibitors suggest as potential therapies [30, 31]. It is noteworthy that metformin reduced levels of testosterone, androstenedione, and the free androgen index (FAI), but did not decrease the HOMA-IR index. This suggests that hyperandrogenism and/or hyperinsulinemia may induce cell-specific molecular changes. These findings highlight the role of metformin in targeting integrin signaling and dysregulated pathways to improve endometrial health in women with PCOS [30].

The current study demonstrated that integrin expression levels were higher during infertility treatment in patients with PCOScompared to untreated patients and healthy controls. Progesterone resistance may underlie the increased integrin levels observed during infertility treatment in PCOS patients.

- **Evaluation of C-Type Lectin Domain Family 10, Member A Levels in the Study Groups:** C-type lectin domain family 10member A (CLEC10A) levels were appraised in the sera samples of the PCOS patients treated with infertility drugs, The results showed a significant elevation in CLEC10A levels comparison to the untreated PCOS patients group **(p=0.000),** On the other hand, The results of the current study did not show statistical differences in CAEC10A concentration when comparing PCOS patients treated with infertility drugs with healthy women **(p=0.497).** CLEC10A levels were evaluated in serum samples of Untreated PCOS patients group and healthy women. The results showed a significant elevation in CLEC10A levels comparison to the healthy women **(p=0.000)**; as shown in **Table 2.**

Table 2: Levels of C-Type Lectin Domain Family 10, member A in the Serum Samples of the Studied Groups

Subjects (n)	CLEC10A (ng/mL) Mean ± SD	Minimum-Maximum	p-value
G1 PCOS Patients (30)	398.377±23.086	361.614-462.857	0.000 For G1 vs G2 0.497 For G1 vs C 0.000 For G2 vs C
G2 PCOS Patients (30)	340.659±45.136	207.352-398.636	

Controls (30)	**389.723±68.244**	**171.928-514.392**	

G1: Group of PCOS patients treated with infertility drugs, G2: Untreated PCOS patients group and Controls: A group of healthy individuals. The mean difference is significant at the 0.05 level.

C-type lectin domain family 10 member A (CLEC10A), also known as macrophage galactose-type C-type lectin, is a part of the C-type lectin receptor (CLR) family[32]. The only known receptor on APCs to show preferential binding for N-acetylgalactosamine (GalNAc) (KD> 100 μM for monovalent GalNAc). Though rarely found on healthy human cells, GalNAc is abundant on the surface of numerous pathogens and tumor cells [33].

The studies suggest that CLEC10A plays a dual role; it can either suppress or activate the immune system depending on the antigen structure and the composition of the bound lectin [33,34]. CLEC10A is expressed at several critical stages of human development, and its expression is highest in oocytes. The eight-cell stage of pluripotent embryogenesis expresses CLEC10A at levels approximately 70-fold higher than in later embryonic stem cells [13].

Although many interactions mediated by lectin receptors have been described as potentially relevant to fertilization and pregnancy, galectins have received considerable attention. They have been shown to be involved in embryo implantation, placental formation, angiogenesis, and the development of maternal immune tolerance. However, there are no reports on the role of CLEC10A-another galactose-specific lectin-in interactions related to reproduction [35]. Several studies have found an association between CLEC10A expression and positive or negative disease outcomes [13].

There are no previous studies linking CLEC10A with metformin in the context of PCOS or infertility, and our study is the first. Our current study showed that CLEC10A expression levels were higher during infertility treatment in patients with PCOScompared to untreated patients, and the results were similar to those of healthy controls, suggesting that metformin has an effective disease-suppressing effect. It can also be hypothesized that metformin may improve the endometrial environment by reducing inflammation and enhancing the immune response. CLEC10A also contributes to the regulation of immune responses in the endometrium, so there may be an interaction between metformin and CLEC10A regulation.

• Assessment of Interleukin-42 in the Serum Samples of the Studied Groups

Interleukin-42 levels were measured in the sera samples of the study groups. The results show a significant statistical differences when comparing interleukin-42 levels in the group of patients with PCOS patients treated with infertility drugs compared to the group of patients with Untreated PCOS patients group (p=0.000) or healthy women in the control group (p=0.002). On the other hand, the results of the current study did not show statistical differences in interleukin-42 concentration when comparing Untreated PCOS patients with healthy women (p=0.142); as shown in **Table 3.**

Table 3: Levels of Interleukin-42 in the Serum Samples of the Studied Groups

Subjects (n)	Interleukin-42 (pg/mL) Mean ± SD	Minimum-Maximum	p-value
G1 PCOS Patients (30)	**9.159±0.702**	**8.01-10.62**	**0.000For G1 vs G2**
G2 PCOS Patients (30)	**10.265±0.918**	**8.65-12.41**	**0.002 For G1 vs C**
Controls (30)	**9.908±1.125**	**8.17-12.50**	**0.142 For G2 vs C**

G1: Group of PCOS patients treated with infertility drugs, G2: Untreated PCOS patients group and Controls: A group of healthy individuals. The mean difference is significant at the 0.05 level.

The current study of patients treated with metformin appears to significantly affect IL-42 levels. Chronic inflammation in women with PCOS may improve with metformin treatment compared to untreated patients. Metformin improves insulin sensitivity and blood glucose control, which also reduces androgen levels and improves ovulation. In this sense, low IL-42 levels in patients undergoing treatment are a useful biochemical marker for predicting the outcome of metformin treatment in PCOS.

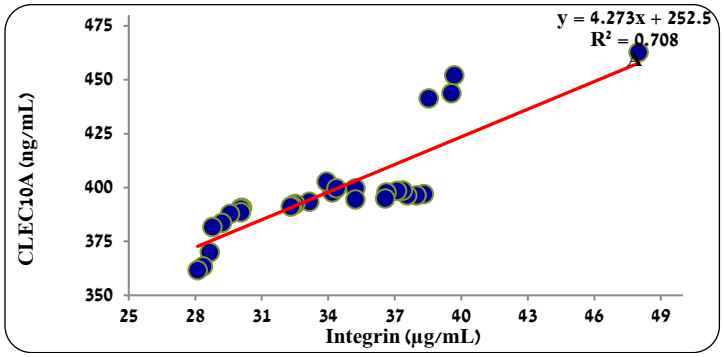
Insulin resistance and associated hyperinsulinemia lead to hyperandrogenism through decreased hepatic production of sex hormone-binding globulin (SHBG) and dysfunction of the hypothalamic-pituitary-ovarian (HPO) and hypothalamic-pituitary-adrenal (HPA) axis. This occurs through effects on the brain, pituitary gland, ovaries, and adrenal glands (associated with increased secretion of GnRH, LH, and androgens, and adrenocorticotropic hormone (ACTH), respectively) [36].

These negative effects of insulin resistance contribute to the decreased fertility associated with PCOS. Elevated androgen levels may also exacerbate insulin resistance by inhibiting insulin recruitment of the GLUT4 glucose transporter, impairing insulin's ability to stimulate glucose uptake [37]. Metformin reduces insulin resistance in women with polycystic ovary syndrome [38], reduction of hyperinsulinaemia during metformin treatment [37].

The Relationship among the New Evaluated Parameters in the Study Individuals: The relationships among the new evaluated criteria (Integrin, CLEC10A and Interleukin-42) were tested for the members in the study groups**.**

The Correlation between Integrin andC-Type Lectin Domain Family 10 Member A in PCOS patients treated with infertility drugs and Untreated PCOS patients in addition to Healthy Control Group

The results indicated a significant positive correlation (r=0.841 at p=0.000) when studying the relationship between Integrin and CLEC10A in the group PCOS patients treated with infertility drugs, as shown in Figure 1 A. On the other hand, the results indicated a significant positive correlation (r=0.854 at p=0.000) when studying the relationship between Integrin and CLEC10A in the group Untreated PCOS patients), as well as healthy women (r=0.834 at p=0.000) as shown in Figures 1 B and C, respectively.



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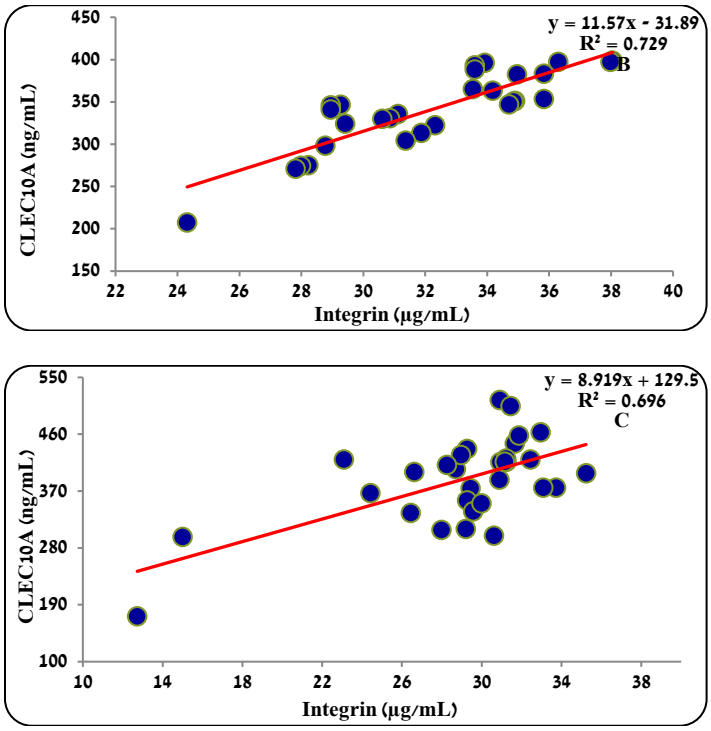
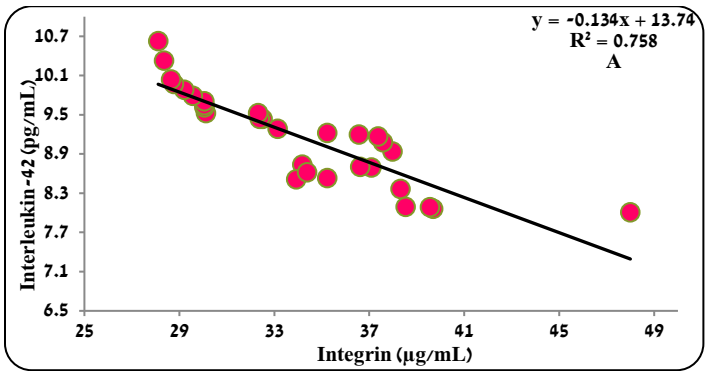


Figure 1: Relationship of Integrin to CLEC10A in: (A) G1 Patients Group (B) G2 Patients Group and (C) Healthy Individuals Group

The Correlation between Integrin and Interleukin-42 in PCOS patients treated with infertility drugs and Untreated PCOS patients in addition to Healthy Control Group Members

According to the data in Figure 2 A There is a strong negative correlation between Integrin and Interleukin-42, which is statistically significant in PCOS patients treated with infertility drugs (r=0.871 at p=0.000). This may indicate a potential regulatory relationship or direct interaction between the two proteins. The significant positive correlations were observed when studying the relationship between Integrin and Interleukin-42 in the group of Untreated PCOS patients (r=0.879 at p=0.000) and the group of healthy females (r=0.516 at p=0.040), as shown in Figures 2 B and 2 C, respectively.



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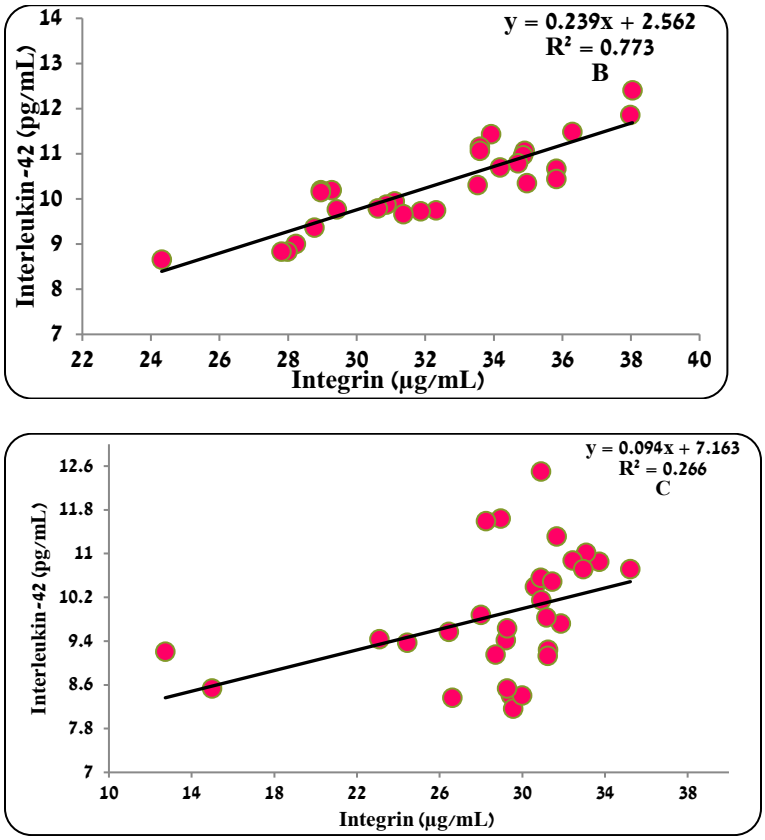

Figure 2: Relationship of Integrin to Interleukin-42 in: (A) G1 Patients Group (B) G2 Patients Group and (C) Healthy Individuals

The Correlation between C-Type Lectin Domain Family 10, member A and Interleukin-42 in PCOS patients treated with infertility drugs and Untreated PCOS patients in addition to Healthy Control Group

The statistical analysis shown in the Figure 3 A indicates a moderate to strong negative correlation between CLEC10A and interleukin-42 in PCOS patients treated with infertility drugs (r=0.859 at p=0.000). This means that there is a clear tendency for levels of one variable to change in conjunction with levels of the other, while changing in the opposite direction.

The statistical analysis shown in Figure 3 B indicates a plausible positive correlation between CLEC10A and interleukin-42 in the group Untreated PCOS patients (r=0.619 at p=0.009). Although there is a positive trend, suggesting that an increase in one variable may be accompanied by an increase in the other, the strength of this relationship is not significant. This suggests that CLEC10A is not a strong predictor on its own for interleukin-42 levels, and that other factors are likely to be more influential. The statistical analysis indicates a strong positive correlation between CLEC10A and interleukin-42 in the group of healthy females (r= 0.902 at p=0.000). This means that as CLEC10A levels increase, interleukin-42 levels tend to increase as well, and that CLEC10A is a good predictor of interleukin-42 levels in this data set, as shown in Figure 3 C.

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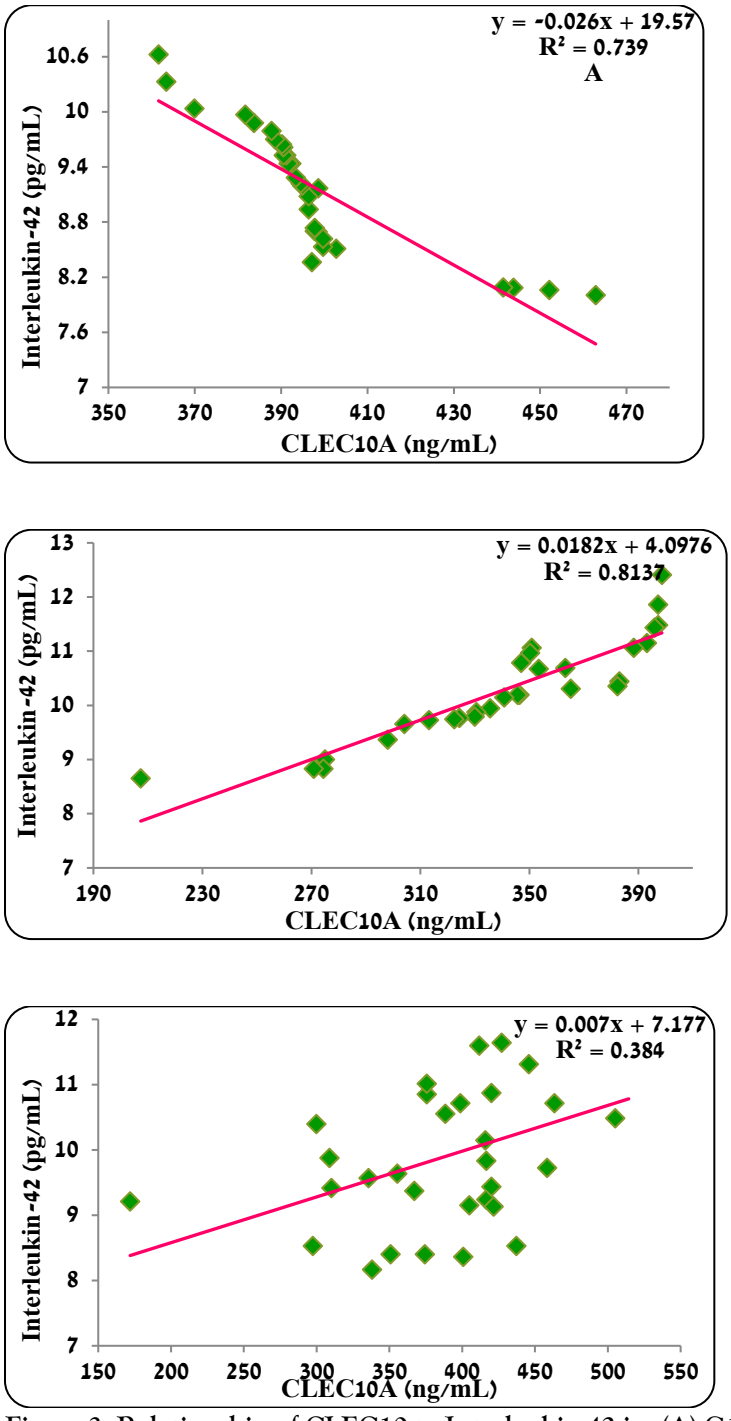

Figure 3: Relationship of CLEC10 to Interleukin-42 in: (A) G1 Patients Group (B) G2 Patients Group and (C) Healthy Individuals

Sensitivity and Specificity of the Evaluated Parameters

Sensitivity is known as the true positive rate or the probability of detection, it measures the proportion of positives that are correctly identified. Specificity is known as the true negative rate; it measures the proportion of negatives that are correctly identified. The calculation of sensitivity and specificity is used for assessing the

efficiency of the tested parameters to suggest them as diagnostic markers. The diagnostic efficiency of the included criteria in this work were evaluated by applying the receiver operating characteristic (ROC) as demonstrated in Figurers 4, 5, and 6 for Integrin, CLEC10A, and Interleukin-42; respectively.

Table 4 shows the area under the curve and cut-off values for the criteria evaluated in the current study. The study demonstrates the efficacy (sensitivity) of the criteria evaluated in the current study in distinguishing between PCOS patients treated with Metformin and those not treated. The study showed the highest sensitivity (97%) and specificity (93%) for interleukin-42 were recorded in the Metformin-treated group.

Table 4: Receiver Operating Characteristic Analysis of Integrin, CLEC10A, and Interleukin-42 as Prognostic Markers for Polycystic Ovary Syndrome Patients Treated with Infertility Drugs

Criteria	AUC	SE	p-value	Cutoff value	Sensitivity%	Specificity%	CI (95%)
Integrin	0.767	0.062	0.000	28.652	93	77	0.645-0.889
CLEC10A	0.678	0.056	0.006	422.511	80	73	0.569-0.788
Interleukin-42	0.237	0.049	0.000	10.594	97	93	0.140-0.335

AUC: Area Under Curve, SE: Standard Error

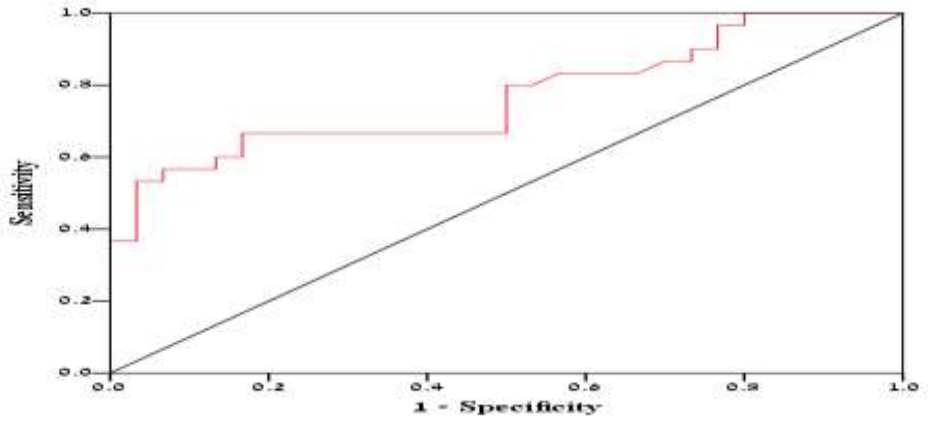

Figure 4: Receiver Operating Characteristic Curve of Integrin in G1 Polycystic Ovary Syndrome Patients

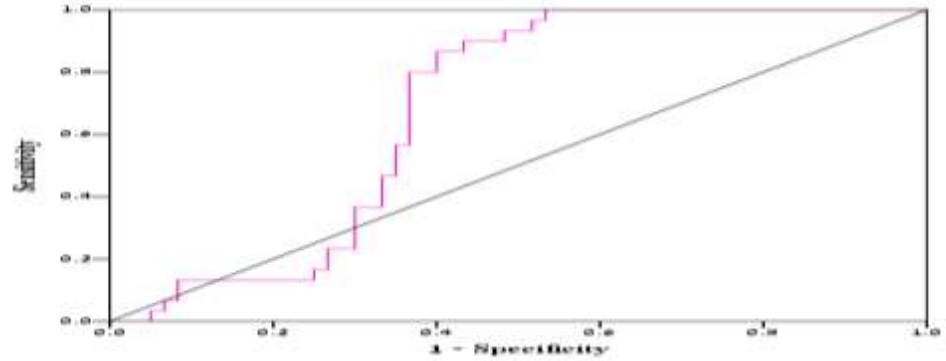

Figure 5: Receiver Operating Characteristic Curve of CLEC10A in G1 Polycystic Ovary Syndrome Patients

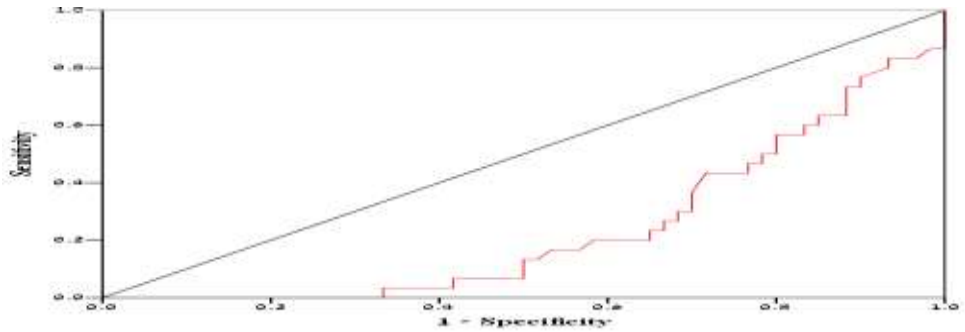

Figure 6: Receiver Operating Characteristic Curve of Interleukin-42 in G1 Polycystic Ovary Syndrome Patients

The combined sensitivity of the criteria Integrin, CLEC10A, and Interleukin-42 were examined in the current study, as summarized in **Table 5**. The outcomes of the study illustrate the highest sensitivity (100%) in the combination of every two evaluated parameters together.

Table 5: The Combined Sensitivity of the Evaluated Parameters

Parameters	Integrin	CLEC10A	Interleukin-42
Integrin	-	100	100
CLEC10A		-	100
Interleukin-42			-

Table 6 presents the area under the curve and cut-off values for the criteria assessed in this study. This research demonstrates the effectiveness (sensitivity) of these criteria in differentiating between untreated and treated PCOS patients. The study revealed that integrin exhibited the highest individual sensitivity (96%) and specificity (80%) in the group of untreated PCOS patients.

Table 6: Receiver Operating Characteristic Analysis of Integrin, CLEC10A, and Interleukin-42 as Diagnostic Markers for Polycystic Ovary Syndrome Patients

Criteria	AUC	SE	p-value	Cutoff value	Sensitivity%	Specificity%	CI (95%)
Integrin	0.671	0.071	0.023	27.629	96	80	0.532-0.810
CLEC10A	0.228	0.064	0.000	355.461	47	50	0.103-0.353
Interleukin-42	0.602	0.074	0.174	10.003	43	47	0.457-0.748

AUC: Area Under Curve, SE: Standard Error

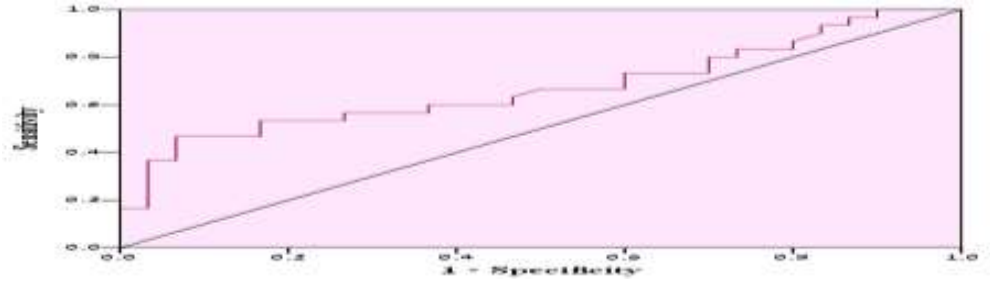

Figure 7: Receiver Operating Characteristic Curve of Integrin in G2 Polycystic Ovary Syndrome Patients

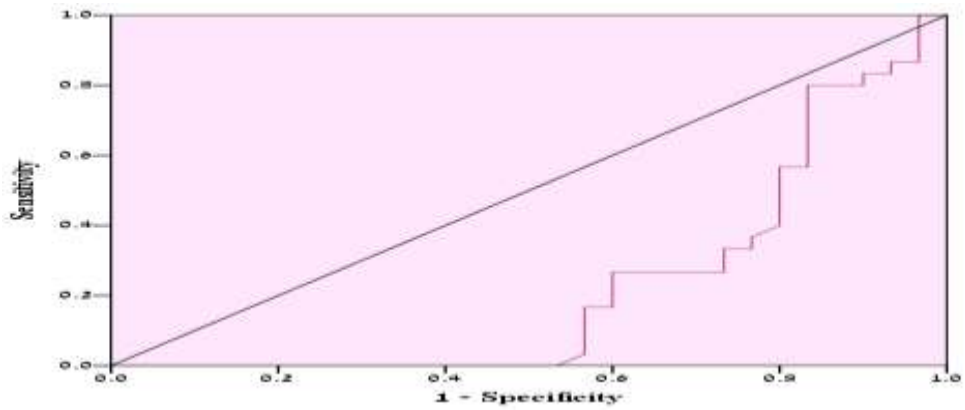

Figure 8: Receiver Operating Characteristic Curve of CLEC10A in G2 Polycystic Ovary Syndrome Patients

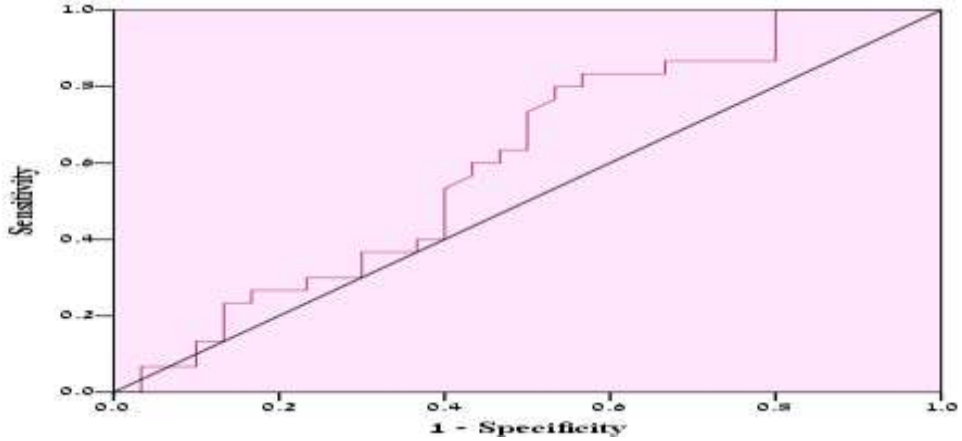

Figure 9: Receiver Operating Characteristic Curve of Interleukin-42 in G2 Polycystic Ovary Syndrome Patients

When the combined sensitivity was calculated, the combination of Integrin and CLEC10A yielded a value of 100%, as seen at their intersection, as shown in **Table 7.**

Table 7: The Combined Sensitivity of the Evaluated Parameters

Parameters	Integrin	CLEC10A	Interleukin-42
Integrin	-	100	100
CLEC10A		-	73
Interleukin-42			-

CONCLUSIONS

CLEC10A and interleukin-42 are promising tools for differentiating PCOS patients treated with infertility drugs and Untreated PCOS patients.One of the most important conclusions reached by the study there is a strong negative correlation between integrin and interleukin-42, as well as CLEC10A and interleukin-42, which is statistically significant in PCOS patients treated with infertility drugs.

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