Original Article

# Serum Claudin-5 Level As A Predictor Of Changes In The Microvascular Endothelium In Young Women With Polycystic Ovary Syndrome

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## Abstract

Background: Polycystic ovary syndrome (PCOS) is a complex syndrome involving both endocrine and metabolic disorders. Additionally, the most common reason for infertility in women of reproductive age, with a variety of clinical symptoms. Claudin-5 (CLDN5) is the most enriched tight junction protein and is critical to controlling endothelial cell polarity and pericellular permeability.

Aim of the study: In the current study, the serum CLDN5 level and its affecting factor were examined in patients with recently diagnosed polycystic ovarian symptoms (PCOS).
Materials and methods: An age-matched case-control study included 90 recently diagnosed PCOS women as patients group using the Rotterdam criteria and 90 normally infertile women as the healthy control group. ELISA was determined CLDN-5, fasting insulin (FINS) and other hormone levels. The homeostasis model assessment of insulin resistance (HOMA-IR) assessed insulin resistance. The outcomes were examined using suitable statistical analysis.

Results: According to the study's findings, PCOS women had significantly higher of BMI, LH, LH/FSH ratio, TT, FAI, FSG, FINS, HOMA-IR, TC, TG, and LDL-C levels than healthy fertile women, PCOS patients' have a significant lower of SHBG and HDL-C levels. Serum level of CLDN5 was a highly significant increase in PCOS women compared with healthy women (17.18±3.30 vs10.01±4.21), respectively. Furthermore, the result showed a significant positive correlation between CLND5 level with age, LH, FINS, and HOMA-IR in the PCOS patients group.

Conclusion: Serum CLND5 levels were increased in PCOS patients than in healthy control women and strongly correlated with elevated HOM-IR levels, Serum CLDN5 may serve as helpful indications for the management of alterations in the microvascular endothelium in PCOS patients.

**Keywords:** Polycystic ovary syndrome, LH, HSH, Cluadin5, HOMA-IR

## INTRODUCTION

The most prevalent endocrine illness is polycystic ovarian syndrome (PCOS), which is associated with major metabolic system disorders like obesity, hyperinsulinemia, elevated lipid levels, and reproductive system problems like hyperandrogenism, anovulation, and infertility (Li *et al.*, 2019; Alhassan *et al.*, 2022), affecting women with a prevalence of 15–20% amongst reproductive age women according to the Rotterdam diagnostic criteria (Azziz *et al.*, 2004; Alhassan *et al.*, 2022).
Even if the clinical definition is as stated, PCOS affects women's lives in more ways than just these symptoms because it has long-lasting repercussions that last for the whole of a woman's lifetime. Finding the time of life take values for androgen levels is advised because PCOS patients' fasting blood glucose, fasting insulin, and androgen levels are all impacted and change with age (de Medeiros *et al.*, 2020), infertility is a prevalent symptom of PCOS, and in addition to ovarian reasons, this condition is also described by a reduction in endometrial receptivity caused by impacted endometrial receptivity indicators ( Teed *et al.*, 2019).
Furthermore, all phenotypes of PCOS are associated with metabolic disorders (Maffazioli *et al.*, 2020). In addition to these characteristics, chronic inflammation is a component of PCOS, and current research suggests that people with PCOS have higher levels of inflammatory markers like C-reactive protein (CRP), leukocytes/white blood cells (WBCs),

certain interleukins, and tumor necrosis factor- (TNF-) ( Maffazioli *et al.*, 2020). Diabetes mellitus and overweight are associated with ongoing reduced inflammatory response, which has been associated to endothelial dysfunction, atherosclerosis, and cardiovascular disease(McCracken, Monaghan and Sreenivasan, 2018).

Although the exact etiology of the persistent inflammation reaction associated with PCOS is unknown, it is thought that persons who have already begun the inflammatory reaction have a greater risk of developing insulin resistance, overweight, and heart problems (Özay and Özay, 2021),Follicular improvement is an organized series of occasions controlled by endocrine criticism frameworks of the nerve center pituitary-gonadotropic pivot and is calibrated via autocrine and paracrine correspondence signals inside follicular cells of the ovary including the development of the oocyte, expansion and separation of granulosa cells (GC) and theca cells ( Buccione, Schroeder and Eppig, 1990; Hsueh *et al.*, 2015).

Typical sticky structures that epithelial cells generate to facilitate communication, contact, and regulate paracellular permeability. To create cell-cell interactions and seal the paracellular space of the cell layer, adherence junctions (AJ), desmosomes, and TJ together make up the apical junctional complex. These cells are abundantly expressed in epithelial and endothelial cells (Paris and Bazzoni, 2008; Van Itallie and Anderson, 2014). Among all the three junctional complexes, only TJ control the paracellular permeability for ions, water and other molecules (Suzuki, 2013).

By preventing the passage of the membrane components between both the apical and basolateral regions, TJ also aid in maintaining the cell polarity (Cereijido *et al.*, 2008). The blood mind boundary, made out of endothelial cells, astrocytes and pericytes, assumes a focal part in keeping up with the vascular homeostasis of the focal sensory system (Ballabh, Braun and Nedergaard, 2004), Major cardiovascular conditions like high blood pressure are more prevalent in the elderly population (Hajjar, Keown and Frost, 2005), and stroke (Mikulis, 2005). All participate in the malfunctioning of the blood–brain barrier,

The permeability of the blood-brain barrier is influenced by a number of circumstances, including elevated levels of inflammatory cytokines (Banks, Kastin and Broadwell, 1995) and free radicals (Greenwood, 1991).

Claudins are molecules that tight junctions (TJs) (Schlingmann, Molina and Koval, 2015) There are 27 claudins that have been identified, and changes in the permeability of cell layers to electrolytes and other solutes are caused by their development (Lappi-Blanco *et al.*, 2013). Human bronchi and bronchioles produce claudins 1, 3, 4, 5, 7, 8, and 18 Claudin-5 (CLDN5), an integral membrane protein, is primarily produced by the vascular endothelium, such as in the pulmonary microcirculation, and is a vital part of endothelial TJs that regulate pericellular permeability. Vascular endothelial cells demonstrate a significant amount of expression and in vascular tumors( Zhu *et al.*, 2006).

## MATERIALS AND METHODS

A case-control study involved 90 newly PCOS women in age range (18-38 years), were conducted at AL-Zahra teaching hospital for obstetrics and gynecology and the fertility center in AL-Sader medical city in Najaf- Iraq, during the period from December 2021 to April 2022, To compare with 90 non-PCOS women apparently healthy with age matched PCOS women volunteers as control group, Using Rotterdam ESHRE/ASRM 2003 criteria, PCOS was diagnosed ( Rotterdam, 2004). The study was approved by the Ethics Committee of Faculty of Science, University of Kufa and the Najaf health directorate of the administration of obstetrics and Gynecology hospital.

Each participant signed informed consent. Patients with any type of chronic illness, including type 1 and type 2 diabetes, hypertension, dyslipidemia, cardiovascular diseases, thyroid disorders, Cushing's syndrome, androgen-secreting tumors, enzyme deficiencies (particularly 21-hydroxylase), diminished ovarian reserve, and smokers were excluded in this study. Anthropometric measurements of body mass index (BMI) was calculated as the ratio of weight (Kg) to height squared ($m^2$), with unit $kg/m^2$. Five ml tests of venous blood were collected in cycle day 2 at time 8.00 and 9.00 a.m., after fasting (12 hrs), and the serum was separated and store at - 20°C until they were for examination. Fasting investigation of Serum glucose (FSG), and lipid profile (total cholesterol (TC), triglyceride (TG), high-density lipoprotein -cholesterol (HDL-C), low-density lipoprotein- cholesterol (LDL-C) levels were estimated by colorimetric methods using the commercially available test kits. The level of serum free testosterone was determined by ELISA kits (Monobind, USA).

Fasting Insulin (FINS), Sex hormone binding globulin (SHBG), Claudin-5(CLDN5) were determined by ELISA kits(ELabscience/USA), luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (TT) were assessed by using the immune-fluorescence method (Minividas, Biomerieux, France). Insulin resistance was calculated by using the homeostatic model assessment (HOMA-IR), which was computed using a standard calculation as follows: HOMA-IR is defined as fasting insulin (IU/L) fasting glucose (mmol/L) /22.5, with > 2.5 being the cutoff value (Weir and Jan, 2019).

In addition, the free androgens index (FAI) was calculated using the standard formula FAI = total testosterone (TT)/SHBG × 100.

## 2.3. STATISTICAL ANALYSIS

For statistical analysis, SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA) was utilized, The mean and SD of each result were recorded, Using an unpaired student t-test, the statistical significance of the study's groups was determined, Two independent samples were compared using t- tests. For the comparison of parameters among PCOS-affected women, Pearson’s correlation analysis was used to examine the association between variables, Statistical significance was defined as a P-value 0.05.

## RESULTS

In Table 1 summarizes the demographic and biochemical characteristics of the participating women and their statistical significance as Mean ± SD. There is nonsignificant difference in age between PCOS and healthy women. Also, revealed a significant increase in BMI, WHR, LH, LH/FSH ratio, free testosterone, total testosterone, FIA, FSG, FSH, insulin, HOMA-IR, TC, TG, and LDL-c levels in patients compared with fertile healthy women. In addition, significantly decreased levels of SHBG, HOMA-%S, and HDL-c in PCOS patients compared with healthy fertile women. Serum levels of CLDN-5 in women with PCOS group had increased significantly than the healthy women as shown in **Figure 1**. As revealed in **Table 2** the current results were shown a significant positive correlation between CLND5 with age, LH, FINS, and HOMA IR (r=0.320, p=0.045), (r=0.372, p=0.026), (r=0.392, p=0.021), and (r=0.331, p=0.041) respectively. In addition, serum CLND5 level has negative correlation with HDL-C, and SHBG levels in PCOS women group.

**Table 1:** The demographic and biochemical parameters in women with PCOS compared with healthy women.

| parameters | PCOS patients' group Means ± S. D | Healthy control group Means ± S. D | P - Value |
|---|---|---|---|
| **No.** | 90 | 90 | - |
| **Age (Years)** | 29.35± 7.67 | 28.11± 8.04 | 0.185 |
| **BMI (kg/m²)** | 29.66 ± 1.19 | 23.62 ± 1.04 | 0.001 |
| **WHR** | 0.98± 0.06 | 0.77± 0.04 | 0.011 |
| **LH ( IU/L )** | 10.98±2.70 | 5.86±1.12 | 0.009 |
| **FSH ( IU/L )** | 5.16±2.01 | 6.29±2.18 | 0.054 |
| **LH/FSH** | 2.30±0.87 | 0.721±0.22 | 0.001 |
| **TT ( nmol/L )** | 2.32±0.84 | 1.42±0.64 | 0.025 |
| **FT ( pmol/L )** | 12.29±1.96 | 2.98±2.57 | 0.0001 |
| **SHBG ( nmol/L )** | 33.86±9.27 | 72.4±14.04 | 0.001 |
| **FAI** | 9.87±2.25 | 4.41±1.2 | 0.029 |
| **FSG (mg/dL)** | 103.61±9.74 | 85.32±7.34 | 0.048 |
| **Insulin (mIU/L)** | 14.39±6.75 | 9.24±3.75 | 0.0001 |
| **HOMA-IR** | 3.97±1.15 | 1.89±0.62 | 0.007 |
| **HOMA % S** | 68.30±21.52 | 88.7±17.82 | 0.03 |
| **TC (mmol/L)** | 4.88±1.72 | 3.41±1.01 | 0.015 |
| **TG (mmol/L)** | 1.78±0.43 | 0.81±0.07 | 0.022 |
| **HDL-C (mmol/L)** | 1.21±0.25 | 1.36±0.20 | 0.047 |
| **LDL-C (mmol/L)** | 3.28±0.99 | 1.92±0.82 | 0.018 |
| **VLDL-C (mmol/L)** | 0.362±0.07 | 0.165±0.01 | 0.01 |
| **CLDN-5 (ng/mL )** | 17.18±3.30 | 10.01±4.21 | 0.002 |

SD **:**Standard deviation, WHR**:** Waist-to-hip ratio , BMI: Body mass index, FSG: fasting serum glucose , HOMA%S: HOMA of insulin sensitivity , HOMA-IR: Homeostatic Model Assessment for Insulin Resistance , FAI: fasting insulin, LH: luteinizing hormone , FSH: follicle stimulating hormone, TT: total testosterone , FT: free testosterone, FAI:free androgen index, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein -cholesterol, LDL-C : low-density lipoprotein- cholesterol and CLDN-5: claudin-5

Abnormal gonadotropin production is one of the most distinctive hormonal characteristics and is linked to the persistence of the anovulatory condition in PCOS women(Song and Li, 2021) . Elevated serum LH concentrations and an elevated LH/FSH ratio are two characteristics of the aberrant gonadotropin production pattern in PCOS (Alhassan *et al.*, 2022), and this pattern is related to increases in both the amplitude and frequency of LH secretion secondary to increased pulse frequency of hypothalamic gonadotropin releasing hormone(Atoum, Alajlouni and Alzoughool, 2022) . PCOS is characterized by altered LH-FSH ratio, which is caused by accelerated heart frequency of gonadotrophin-releasing hormone (GnRH). Typically, the proportion is 1:1. When LH levels are higher than FSH, it results in PCOS, which produces too much ovarian androgen and has ovulatory failure (Abinaya *et al.*, 2019). Different LH-FSH ratio cut-offs were suggested, but the cut-off > 1.0 was proven to be the most effective in diagnosing PCOS when taking into account both sensitivity and specificity (Ramezani Tehrani *et al.*, 2021).

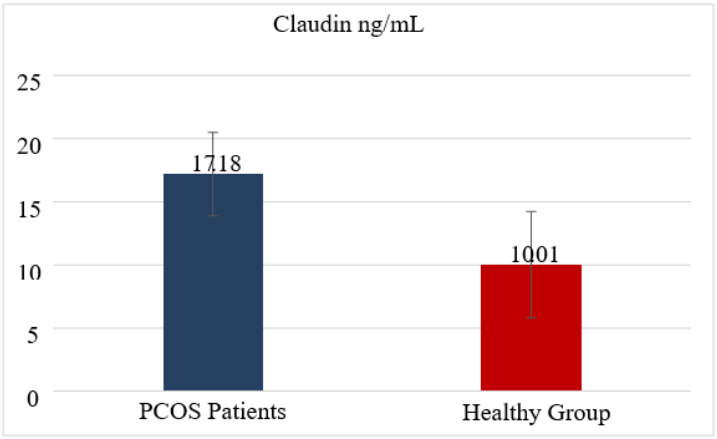


**Figure 1:** Comparisons Of Claudin-5 Levels Between Patients' Group With The Healthy Group

**Table 2:** Correlation analysis between serums claudin-5 level with other clinical parameters in PCOS patient's groups.

| Parameters | r | P-value |
|---|---|---|
| **Age (years)** | 0.320 | 0.045 |
| **BMI (kg/m$^2$ )** | 0.156 | 0.129 |
| **WHR** | 0.168 | 0.091 |
| **LH (IU/L)** | 0.372 | 0.026 |
| **FSH (IU/L)** | 0.163 | 0.09 |
| **LH/FSH** | 0.325 | 0.042 |
| **SHBG ( nmol/L )** | -0.304 | 0.042 |
| **TT ( nmol/L )** | 0.182 | 0.063 |
| **FT( Pmol/L )** | 0.140 | 0.138 |
| **FAI** | 0.132 | 0.142 |
| **FSG (mg/dL )** | 0.155 | 0.109 |
| **FINS ( mIU/L )** | 0.392 | 0.021 |
| **HOMA-IR** | 0.331 | 0.041 |
| **HOMA%S** | 0.317 | 0.045 |
| **TC (mmol/L )** | 0.116 | 0.301 |
| **TG (mmol/L)** | 0.087 | 0.350 |
| **LDL-C (mmol/L )** | 0.103 | 0.304 |
| **HDL-C (mmol/L )** | -0.126 | 0.182 |
| **VLDL-C (mmol/L )** | 0.124 | 0.184 |

SD **:**Standard deviation, WHR**:** Waist-to-hip ratio, BMI: Body mass index, FSG: fasting serum glucose , HOMA%S: HOMA of insulin sensitivity , HOMA-IR: Homeostatic Model Assessment for Insulin Resistance , FAI: fasting insulin, LH: luteinizing hormone , FSH: follicle stimulating hormone, TT: total testosterone , FT: free testosterone, FAI:free androgen index, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein -cholesterol, LDL-C : low-density lipoprotein- cholesterol and CLDN-5: claudin-5 The TJ proteins, or CLDN, which are hormone-regulated, are the main regulators of these functions by transferring membrane Claudin-5 into the cytosol and destroying Claudin-5 that has been deposited in the cytosol via auto phagosomes, the endothelial barrier's functional integrity is maintained (Schlingmann, Molina and Koval, 2015).

In post-menopausal women's microvessels had substantially disturbed claudin-5 distribution, and a similar result was seen in human microvessels (Nwafor *et al.*, 2021) CLDN-5 level has a limited supply of human tissue. This is the only study that we are aware of that looked at the amount of claudin-5 PCOS patients had in comparison to fertile, healthy women (Schlingmann, Molina and Koval, 2015; Nwafor *et al.*, 2021).

The regulation of pericellular permeability and endothelial cellular polarity depends on the tight junction protein claudin-5 (CLDN5). Claudin-5 (CLDN5), an integral membrane protein, is primarily expressed by the vascular endothelium and is a vital part of endothelial TJs that regulate pericellular permeability. Although CLDN5 is only slightly produced in the epithelium, it is substantially produced in the endothelium (Muranyi *et al.*, 2022) .

Evidence supporting that PCOS is associated with chronic inflammatory state is emerging (Teede *et al.*, 2019). PCOS is associated with pro-inflammatory cytokines and chemokines elevation in plasma, such as interleukin-18 (IL-18) (Alhassan *et al.*, 2022). Leukocytes and plasma proteins are known to infiltrate blood vessel walls, although endothelial cells act as a gatekeeper to prevent this from happening. Additionally, endothelial cell-cell junctions can serve as either direct or indirect signaling structures (Adil, Narayanan and Somanath, 2021).

Claudins are transmembrane proteins, they can connect with one another in nearby cells through homo- or heterophilic trans-interactions. When TJ strands are formed, CLDN-5 interacts with nearby cells via face-to-face homophilic trans

interactions to block the paracellular gap (Yang *et al.*, 2021). Controlling the blood-brain barrier's and endothelial tissues' permeability qualities is a well-known function of the TJ protein CLDN-5 (Arvanitis, Ferraro and Jain, 2020).
When the brain ventricle expands due to hydrostatic pressure in zebrafish, CLDN-5 plays a crucial role in strengthening the neuroepithelial barrier (Yang *et al.*, 2021), However, its specific developmental functions within the vascular system are still unknown (Arvanitis, Ferraro and Jain, 2020).Claudins may affect cell attachment, which could alter endothelial or epithelial cell motility (Gupta and Ryan, 2010). While, CLDN-5 is limited to the arterial vascular system, including the dorsal aorta, Cldn5a has neuroepithelial and dorsal aorta expressions (Persidsky *et al.*, 2006). A minor inflammatory disease, PCOS. Therefore, inflammation may cause tissue damage, but late-stage inflammatory responses may be neuroprotective by promoting neurogenesis, angiogenesis, and neuroplasticity and promoting repair (Weir and Jan, 2019), Although the distribution of claudin-5 in tight junction (TJ) assemblies is unaffected by the passage of time, it is incredibly sensitive to persistent changes in the ovarian hormones' cyclicity (Cardoso, 2021). TJ proteins in follicular cells undergo hormonal regulation throughout follicular development, and they can serve as functional structures that receive and transmit signals from both inside and outside of cells to control the formation of cell-cell contacts and paracellular permeability as well as to modulate intracellular activities like cell growth and migration. The function of follicular cells and antrum expansion of ovarian follicles are regulated by TJ, which also regulates the paracellular permeability of cell layers and cell functions (Cong and Kong, 2020).
Vascular damage is a result of the impacts of inflammation (Hajjar, Keown and Frost, 2005), In this regard, endothelial cells investagte for a variety of chemicals implicated in various signaling cascades. Additionally, research is being done on the processes behind the pathophysiology of many diseases linked to endothelial dysfunction caused by cytokine pathways (Banks, Kastin and Broadwell, 1995;Zhu *et al.*, 2006), Numerous events, such as oxidative stress, metabolic irregularities, and cardiovascular problems, have been demonstrated to be associated with endothelial dysfunction (Amodio *et al.*, 2018), Therefore, one of the primary causes of endothelial dysfunction in PCOS patients may be alterations in inflammatory activity. Greater endothelial cell permeability causes increased vascular inflammation in illnesses (Tsukita, Tanaka and Tamura, 2019).
An increase in the paracellular leakage of plasma fluid and proteins is caused by the endothelial cells' increased permeability (Arvanitis, Ferraro and Jain, 2020). The integral membrane protein Claudin-5, which is involved in endothelial tight junctions, and the transcriptional and posttranslational regulation of this protein are involved in a variety of physiological and pathological events.
Claudins also regulate barrier function and pericellular permeability (Li *et al.*, 2019) .An earlier research performed on women with PCOS demonstrated a relationship between DBP and hyperandrogenemia even after age and BMI adjustments, as well as an interaction between hyperandrogenemia and hypertension (Naghshband and Malini, 2022) Since metabolic syndrome includes high blood pressure, PCOS sufferers are more likely to develop atherosclerotic heart disease. Regardless of insulin resistance, higher blood pressure may be linked to hyperandrogenemia due to increased vascular stiffness and endothelial dysfunction (Krentowska and Kowalska, 2022).

## CONCLUSION

In the current result concluded that the women with PCOS had higher serum levels of claudin-5, as sign of the tight junction proteins in follicular cells had changed during follicular development and were subject to hormonal regulation. Additionally, CLDN5 protein content was up-regulated in non-ovarian human endothelial cells may be related to tight junctions control paracellular permeability of cell layers as well as cellular activities and may be associated with changes in the microvascular endothelium and associated with inflammatory status. Further research should be done on the role of tight junctions in controlling antrum enlargement of ovarian follicles and function of follicular cells.


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## DECLARATION OF INTEREST

There is no conflict of interest, according to the authors.


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