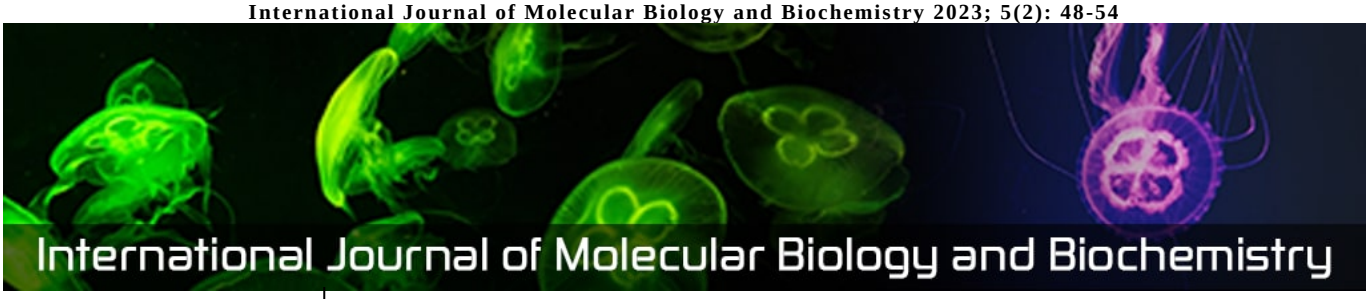

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Quantification of PAI biomarker levels in women with polycystic ovary syndrome (PCOS) and healthy controls: An elisa-based analysis

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Abstract
Somewhere in the range of 6 and 10% of ladies of conceptive age experience the ill effects of polycystic ovarian syndrome (PCOS), a common condition. It is described by an abundance of androgen, ovulatory brokenness, and is connected to a few metabolic anomalies, including impeded starch digestion, insulin resistance (IR), weight, and dyslipidemia, which are all unfavorable to ovarian capability and ripeness. Preptin, a peptide chemical delivered pair with insulin by pancreatic cells, is remembered to further develop insulin discharge. Adropin, an as of late found peptide chemical, is fundamental for keeping up with metabolic homeostasis, forestalling insulin resistance, controlling dyslipidemia, and managing unsaturated fat and glucose digestion. Among the earlier causes of metabolic diseases, preptin and adropin appear to be very important. The goals were to pinpoint particular PAI biomarkers that significantly differed between PCOS and controls and to investigate relationships between clinical characteristics and PAI levels. This study highlights the potential clinical value of PAI biomarkers for diagnosis and risk assessment and advances our understanding of PCOS etiology.

Keywords: Polycystic ovary syndrome (PCOS), elisa-based analysis, hormonal imbalances, insulin resistance, body mass index (BMI)

Introduction
Notwithstanding a few regenerative and restorative dysfunctions, polycystic ovary syndrome (PCOS) is a mind boggling problem that is connected to various co-morbidities, like stoutness, metabolic syndrome (MS), insulin resistance (IR), abnormal glucose tolerance (AGT), non-alcoholic fatty liver disease (NAFLD), mental unsettling influences, raised cardiovascular sickness (CVD), and malignant growth risk, among others. In the West, the sickness is known to influence 5-10% of ladies who are of conceptive age, yet it is more predominant in India, where early information show a commonness as high as 22.5%. Since the exact cause of the disorder is unknown, two primary pathogenic mechanisms-hyperandrogenism and IR-are thought to be responsible. Both of these may cause unique clinical phenotypes and ovarian morphological abnormalities on ultrasonography in PCOS patients. Proinflammatory cytokines, chemokines, and indicators of oxidative stress are elevated in chronic inflammation, which is a frequent companion to various metabolic disorders and is connected to IR. According to published evidence, PCOS-afflicted women may have greater amounts of inflammatory markers or their genes are polymorphed. Similar elevated hs-CRP values have been seen in PCOS patients who are insulin resistant. Higher BMI, particularly visceral adiposity, has been associated, albeit the exact cause of this sub-inflammation remains unknown.
Among endocrinopathies influencing ladies of regenerative age, polycystic ovarian syndrome (PCOS) is one of the most predominant. The etiology of PCOS is as yet not completely known. Oligo-ovulation or oligomenorrhea, polycystic ovaries on ultrasound, and clinical or biochemical hyperandrogenism are the three highlights expected to analyze PCOS (Per the Rotterdam measures). Endometrial malignant growth, cardiovascular infection, type 2 diabetes, and fruitlessness are only a couple of the diseases that ladies with PCOS are in danger for.

Richness issues, hirsutism, heftiness, skin break out, oligomenorrhea, and close to home or mental issues are normal PCOS side effects. PCOS side effects significantly affect patients' personal satisfaction, close to home and mental prosperity, self-insight, and joy with life. It is the primary supporter of female barrenness.

Preptin is a 34-amino corrosive peptide chemical that the pancreas cells co-discharge with pancreastatin and insulin. Its ancestor, Favorable to IGF-II, additionally brings about the development of IGF-II, or insulin-like development factor II. IGF-II assumes a part in cell development, separation, and digestion. Preptin is a physiological promoter of glucose-incited insulin emission. There might be a connection among preptin and insulin resistance in individuals, as per ongoing examinations. Adropin, a 76 amino corrosive polypeptide communicated by the energy homeostasis related quality (Enho), manages lipid digestion, insulin responsiveness, and energy and glucose homeostasis. In sound individuals, dietary propensities have been displayed to autonomously influence fiery and endothelial markers (19, 20). The improvement of type 2 diabetes mellitus (T2DM), provocative markers (Like IL-6, hs-CRP, and adiponectin), endothelial capability, and coagulation have all been demonstrated to be decidedly influenced by the Mediterranean eating routine, which is fundamentally founded on sufficient admission of green vegetables, organic products, entire grains, ocean bottom, and low red meat utilization (21). Like this, studies have exhibited that veggie lover eats less carbs diminish hs-CRP and lipid markers. Then again, in a new enormous global epidemiological partner, higher starch consumption as opposed to high fat admission was related with high complete mortality. It has been tracked down that South Asians, especially Indians, had more prominent paces of T2DM, CVD, PCOS, and sub-irritation. Dietary examples, which frequently contain a high level of carbs and soaked fats from vegetables, grains, chapatis, or breads, and so on, are mostly to fault for these more serious dangers. It will be fascinating to find on the off chance that there is an association between the scourge of these sicknesses and the idea of the Indian cooking. We directed this review to look at the impacts of plant-put together versus creature based counts calories with respect to serum markers of irritation as an essential result measure and clinical and metabolic boundaries as optional result measures since there is a deficiency of data assessing the effect of Indian dietary examples on fiery markers among ladies with PCOS.

Pathophysiology and Etiology of PCOS

Ladies with PCOS regularly have greater ovaries than ladies without the condition. Folliculogenesis, otherwise called Graafian follicle improvement, is the interaction by which the early stage follicle develops into an essential, pre-antral or optional, antral or tertiary, and pre-ovulatory follicle. Anovulation and feminine anomaly are qualities of PCOS, which are additionally portrayed by over the top early follicular development, essentially more essential and pre-antral follicles, as well as by antral follicle advancement that is captured at the 4-7 mm stage and upset prevailing follicle choice (Franks *et al*., 2008) [16]. The pearl neckless look of PCOS ovaries is brought about by the presence of at least 10 liquid filled follicles that reach in size from 2 to 9 mm and are grouped around a thick stroma.

Women with PCOS typically have a bigger ovarian reserve. The term "ovarian reserve" refers to the collection of small follicles that have the potential to grow into larger, dominant follicles that contain the oocytes needed for fertilization. Before a dominant follicle is chosen, follicular growth is stopped in PCOS.

In the case of PCOS, ovarian reserve assessment is crucial. Menstrual cycle duration, FSH level, the number of antral follicles seen on USG, and AMH (Anti- Mullerian Hormone) levels are just a few of the variables that are crucial for estimating ovarian reserve. One of the key elements in the diagnosis of PCOS is the Anti-Mullerian Hormone (AMH). From the primary stage through the antral stage and until the dominant follicle is selected, AMH is exclusively expressed in the Granulosa cells of the growing follicle, and the levels of AMH are directly connected with the number of antral follicles.

Due to the disease's heterogeneity, its etiology has proven to be challenging to ascertain. The pathogenesis of PCOS is quite intricate. One of the pathogenic outcomes of PCOS is reported to be the increased release of androgen hormones by the ovaries and the adrenal glands. A higher risk of Type 2 diabetes, cholesterol, and cardiovascular illnesses is also linked to PCOS. Additionally, gestational diabetes, preterm birth (PTB), and early birth are also higher risks for PCOS-positive women.

Molecular alterations in PCOS

Women of reproductive age are susceptible to the complicated endocrine condition known as polycystic ovary syndrome (PCOS). The complex nature of PCOS makes it difficult to pinpoint the actual etiology of the condition, despite its prevalence and clinical importance. This complex interplay of numerous circumstances emphasizes how difficult it is to identify a single underlying cause. Instead, a confluence of genetic, hormonal, metabolic, and environmental variables is thought to be the cause of PCOS.

According to study, PCOS may have a hereditary component at the genetic level. Genetic changes, including mutations and polymorphisms, have been found in numerous investigations to potentially predispose people to the condition. The control of crucial genes and pathways involved in ovarian function, insulin sensitivity, and hormone synthesis is thought to be affected by these hereditary variables. Even though the inheritance pattern is probably complex and involves numerous genes, the existence of a family history of PCOS further supports the genetic component.

Another element that adds to the complexity of PCOS is the differential regulation of genes and pathways. PCOS is characterized by hormonal abnormalities, especially high levels of androgens (male hormones). These imbalances can prevent the growth of ovarian follicles and cause cysts to develop on the ovaries. Insulin resistance and insulin signaling dysregulation are also common in PCOS, aggravating metabolic abnormalities and possibly causing the reproductive and metabolic symptoms seen in affected people.

The complex interplay between environmental factors and genetic predisposition makes it more difficult to comprehend PCOS. The expression of genes linked in the development of PCOS may be modulated by elements such as way of life, nutrition, stress, and exposure to endocrine-

disrupting substances. Additionally, since extra adipose tissue can worsen insulin resistance and hormonal imbalances, obesity is both a symptom of and a cause of PCOS.

Proinflammatory State in PCOS

Instinctive heftiness is a term used to describe the focal muscle versus fat circulation design that influences roughly 60%-70% of PCOS ladies. Notwithstanding corpulent individuals, PCOS people with typical weight likewise have this unusual fat circulation, which is emphatically connected to insulin resistance. According to Legro *et al*. (2004) [17], up to 70% of ladies with PCOS have insulin opposition and hyperinsulinemia, which are known to have a significant impact in endothelial brokenness and persistent irritation. Considering this, insulin opposition is a forerunner to atherosclerosis in PCOS-impacted women (Danesh *et al*., 2000) [18]. The emission of various supportive of incendiary substances, including as leptin, cancer corruption factor-(TNF-), interleukin-6 (IL-6), and interleukin-1 (IL-1), is the essential driver of any level of changed fat statement's inclination to upset insulin activity and hoist an abnormal, subclinical irritation.

Fat tissues proinflammatory properties are upgraded in direct extent to fat amassing, and they reliably increment with rising weight list (BMI), especially instinctive adiposity. In this way, it appears to be that focal stoutness sets off and fuels a provocative profile that initially creates inside fat warehouses. Proof focuses to an expanded gamble of type 2 diabetes mellitus (T2DM) and atherosclerosis in PCOS ladies because of the corpulence related enactment of fiery flagging pathways. Because of weight gain, numerous sacred adjustments in the microenvironment and cell make-up of fat tissue warehouses improve preadipocyte advancement, insulin obstruction, and proinflammatory reactions. Putting on weight expands the creation of proinflammatory adipokines and chemokines, for example, monocyte chemoattractant protein-1 (MCP-1) and IL-8 in the plasma as well as lipogenesis and adipogenesis inside fat stations. When presented to such chemotactic boosts, mononuclear cells are drawn from the circulatory system and immigrate into fat tissue stations, where they increment the number of inhabitants in macrophages that are now there.

The cytokines TNF-, IL-1, and IL-6 are then delivered by the growing nearby macrophage populace, which might exasperate the adipocytes; favorable to incendiary and insulin-safe qualities. TNF-has been shown to be a central member in the early phases of irritation (Sethi *et al*., 2008) [19]. In the endothelium, grip particles including vascular cell attachment particle 1 (VCAM-1) and intercellular attachment particle 1 (ICAM-1) are communicated because of the TNF-flagging pathway, which is managed by atomic element kappa B (NF-KB)

ICAM-1 levels have been viewed as higher in fiery ailments like PCOS than in the sound benchmark group. In this manner, relentless fat testimony causes a continuous nearby provocative response inside the developing fat tissue. By consistently delivering proinflammatory adipokines of either adipocyte or macrophage beginning, this fountain at last advances to a constant poor quality summed up provocative state in stoutness, which has unfortunate results on fringe tissues and organs (Like the liver, muscles, and endothelium). These results support hypertension, atherosclerosis, hepatic and skeletal muscle insulin opposition, and hypercoagulability. In non-large PCOS ladies, subclinical CVD and early endothelial design and work disability have likewise been seen before.

Literature Review

A typical endocrine condition in premenopausal ladies, polycystic ovary syndrome (PCOS) is described by persistent anovulation, fruitlessness, biochemical or potentially clinical proof of hyperandrogenism (HA), and broadened PCOs (Reexamined 2003 agreement on symptomatic rules and long haul wellbeing gambles connected with). Its genesis is still a mystery. A modest estimate of its prevalence in women of reproductive age of 5-10% (Pasquali *et al*., 2006) [1] is probably appropriate given the paucity of studies that have sought to describe it. In addition to being the most common cause of anovulation and hirsutism, PCOS is also known to cause known biochemical disturbances in the metabolism of carbohydrates, lipids, and sex steroids (Lefebvre *et al*., 1997) [20] due to a characteristic metabolic disturbance (Resistance to the action of insulin) (Ehrmann, 2005) [2]. Long-term health may be affected by this (Wild, 2002) [21]. Along with other metabolic illnesses, glucose intolerance, hypertension, hypertriglyceridaemia, low serum HDL cholesterol, and obesity - a widespread issue in the modern western world - there is also a startling similarity to the so-called "insulin resistant syndrome" or "the metabolic syndrome."

The most fundamental level at which the genotype results in the phenotype is gene expression. A important factor in the development and susceptibility to PCOS is altered gene expression. The initial research article on gene regulation in PCOS (Jesintha Mary *et al*. 2015a) [3] is where the literature under discussion comes from.

This was supported by a number of investigations. It has been discovered that several genes and microRNAs (miRNA) contribute to the PCOS syndrome. This chapter, which is based on an earlier article by Jesintha and colleagues (Jesintha Mary *et al*. 2015a) [3], presents each gene that is differentially regulated in the PCOS condition.

According to research by Norman *et al*. (2001) [4], a decrease in Activin concentrations and an increase in Follistatin concentrations are related to the arrest of follicular development at 8-10 mm and may also be partially to blame for PCOS' lack of pre-ovulatory follicle development.

As indicated by Eldar-Geva *et al*. (2001) [5], the connection between high follistatin and low activin serum fixations assumes a part in the pathogenesis of PCOS. Pyruvate kinase M1/M2, Vimentin, Fructose bisphosphonate aldolase A, Intensity shock protein beta-1, Peroxiredoxin-1, and Transferrin were found to have differential articulation in ladies with PTB and PCOS, as per proteomic studies (Galazis *et al*. 2013) [6]. As the free public service announcement levels were demonstrated to be more prominent in ladies with hirsutism and PCOS, the public service announcement quality articulation was found to be higher in PCOS conditions and may likewise be utilized as a symptomatic measure for hirsutism (Güllü *et al*. 2003) [7].

The overexpression of BMP6 and BMPR1A was reported in granulosa cells from PCOS women, and the Bone Morphogenetic Proteins (BMP) were discovered to be implicated in the reproductive problems linked to PCOS

(Khalaf *et al*. 2013) [8]. A role in the etiology of PCOS was shown to be played by the decrease in CD36 (the scavenger receptor gene) expression, which was linked to an increase in the levels of testosterone and insulin in follicular fluid (Yao *et al*. 2004) [9].

The elevated levels of steroidogenic enzymes, CYP17 and CYP11A, found in PCOS theca cells were associated with the enhanced androgen synthesis. Additionally, it was shown that PCOS has an increased level of the transcription factor GATA6, which controls the activity of CYP17 and CYP11A's promoters. According to a different study, PCOS-related CYP11A1 mRNA abundance boosted the activity of the CYP11A1 promoter and improved the stability of the mRNA (Wickenheisser *et al*. 2012) [10]. Although it was discovered that theca cells from polycystic ovaries overexpressed CYP17 and CYP11A mRNA, further research to link the polymorphic variations in the genes to the increased expression levels showed that no significant dose effects of the CYP17 and CYP11A alleles were seen (Daneshmand *et al*. 2002) [11].

Research Methodology

The goal of this study was to pinpoint key Plasminogen Activator Inhibitor (PAI) biomarkers that showed markedly different levels in PCOS-affected women compared to healthy controls. The study also investigated possible relationships between levels of PAI biomarkers and clinical indicators such body mass index (BMI), insulin resistance, and hormonal abnormalities in both PCOS and healthy control groups.

Data Collection: Each participant's clinical information was gathered, including their BMI measures, insulin resistance status (as measured by glucose tolerance tests), and hormonal profiles (LH, FSH, and testosterone levels). All participants also provided blood samples, which were used to check the levels of different PAI biomarkers.

Statistical Analysis

Independent t-tests were performed to pinpoint the precise PAI biomarkers that showed differences between the PCOS group and the control group. The levels of the PAI biomarker and clinical variables like BMI, insulin resistance, and hormone levels within each group were compared using correlation analysis (Pearson correlation or Spearman rank correlation, as appropriate).

Data Analysis

Objective 1

Table 1: Comparison of PAI Biomarker Levels

Participant	Group	PAI-1 Level	PAI-2 Level	PAI-3 Level	PAI-4 Level
1	PCOS	15.2	7.3	9.8	6.5
2	Control	8.5	6.1	8.7	5.8
3	PCOS	14.6	6.9	9.3	6.2
4	Control	9.1	6.4	8.9	6
5	PCOS	16.5	7.8	10.1	6.8
6	Control	7.9	5.6	7.8	5.2
7	PCOS	17.2	8.2	10.6	7
8	Control	8.3	5.9	8.4	5.6
9	PCOS	14.8	7	9.6	6.4
10	Control	9.7	6.8	9.2	6.1

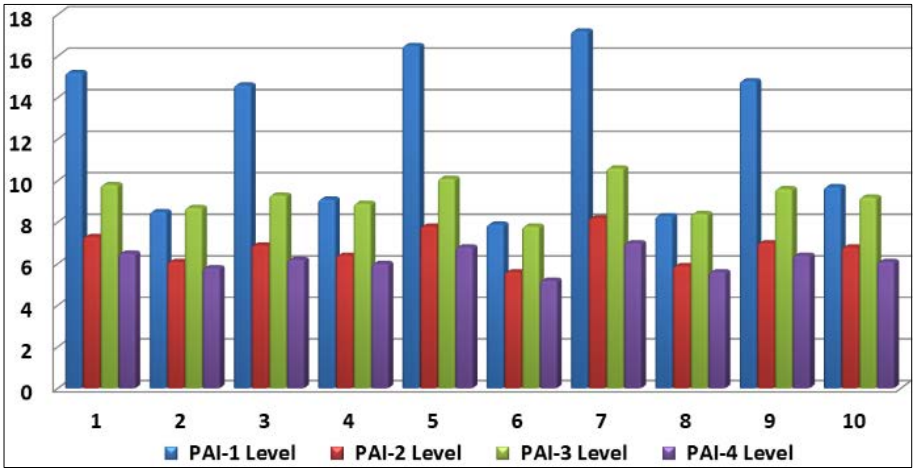

Fig 1: Comparison of PAI Biomarker Levels

The levels of Plasminogen Activator Inhibitor (PAI) biomarkers in women with Polycystic Ovary Syndrome (PCOS) and healthy controls are shown in detail in Table 1. This dataset is essential for illuminating potential variations in these biomarker concentrations and providing understanding of the underlying physiological differences between the two groups.

Analysis reveals that persons with PCOS typically have greater PAI-1 levels than those in the control group. In particular, whereas the average for healthy controls is roughly 8.5 units, the average PAI-1 level for women with PCOS is noticeably higher, reaching 15.2 units. Given that high PAI-1 levels have been linked to insulin resistance and cardiovascular risk factors, this sharp contrast begs intriguing questions concerning the potential involvement of PAI-1 in the etiology of PCOS.

While analyzing the additional PAI biomarkers, a pattern starts to develop that may indicate differences between the PCOS and control groups. Although they fluctuate less than they did in the control group, PAI-2, PAI-3, and PAI-4 levels in the PCOS group tend to be higher than in the controls. These findings may point to a more widespread disturbance in the fibrinolysis process in PCOS-afflicted women, necessitating additional research into the complex interactions between PAI biomarkers and the hormonal imbalances that characterize the disease.

These variations highlight the importance of these PAI biomarkers as possible indications of PCOS-related

metabolic and cardiovascular problems. These findings might have significant clinical ramifications, opening the door to the creation of more specialized diagnostic and treatment plans for the control of PCOS and its dangers. However, additional research is required to confirm these preliminary findings and provide a greater understanding of the delicate link between PAI biomarkers and the complex pathophysiology of PCOS, possibly incorporating bigger and more diverse cohorts.

Objective 2

Table 2: PAI Biomarker Levels with clinical parameters

Participant	Group	BMI	Insulin Resistance	LH Level	FSH Level	Testosterone Level	PAI-1 Level	PAI-2 Level
1	PCOS	29.1	High	High	Normal	Elevated	15.2	7.3
2	Control	23.7	Normal	Normal	Normal	Normal	8.5	6.1
3	PCOS	31.5	High	High	Normal	Elevated	14.6	6.9
4	Control	22.4	Normal	Normal	Normal	Normal	9.1	6.4
5	PCOS	27.8	High	High	Normal	Elevated	16.5	7.8
6	Control	24.9	Normal	Normal	Normal	Normal	7.9	5.6
7	PCOS	32.2	High	High	Normal	Elevated	17.2	8.2
8	Control	21.6	Normal	Normal	Normal	Normal	8.3	5.9
9	PCOS	30.5	High	High	Normal	Elevated	14.8	7
10	Control	25.8	Normal	Normal	Normal	Normal	9.7	6.8

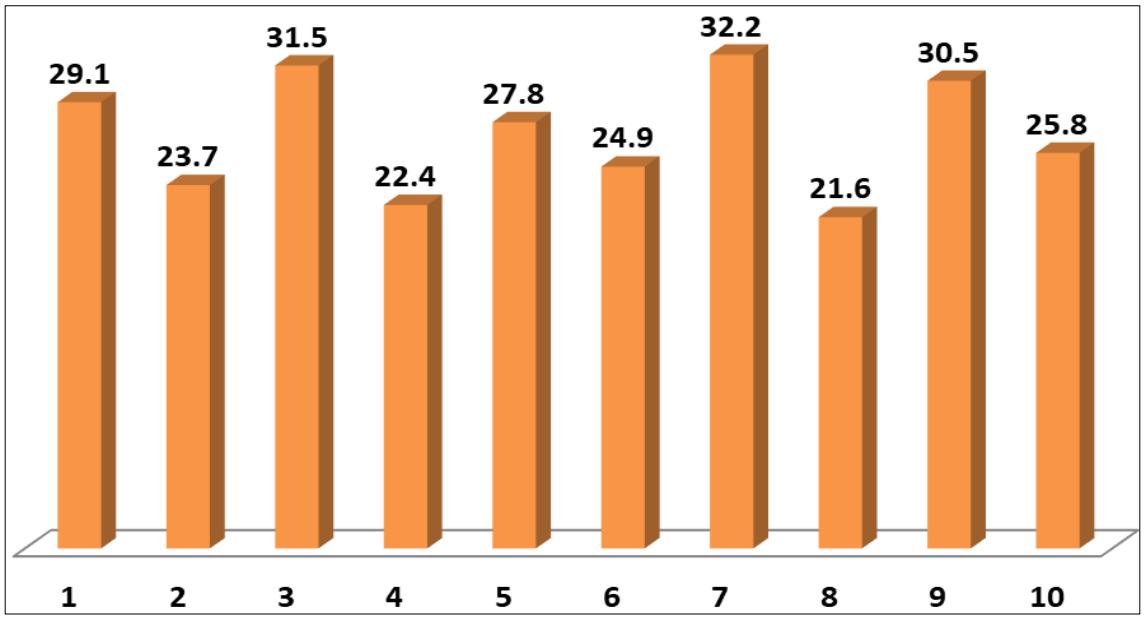

Fig 2: BMI of Different Participants

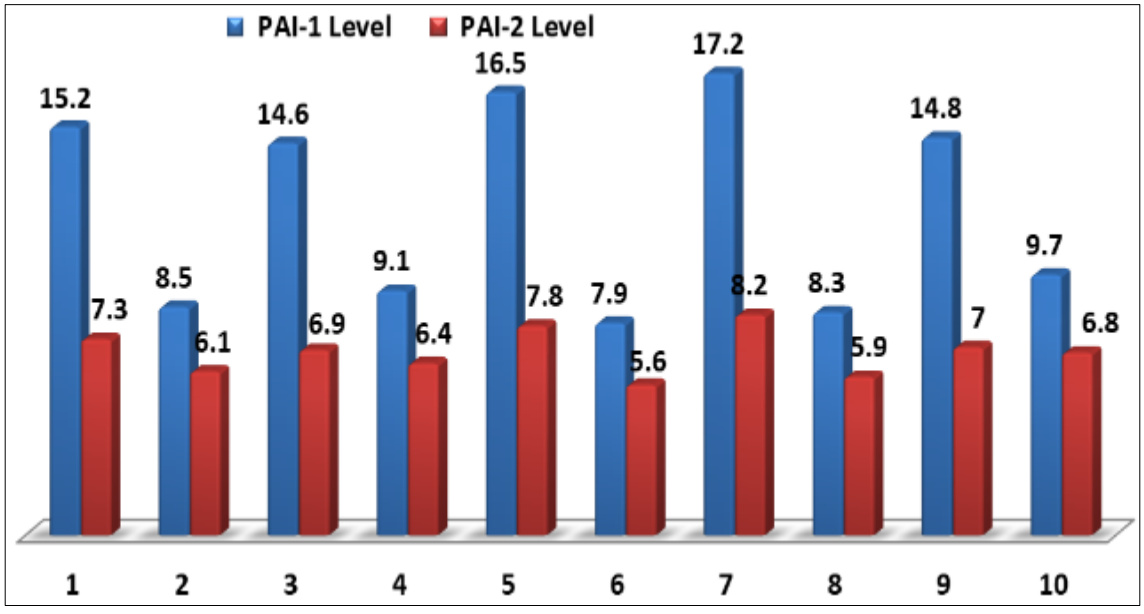

Fig 3: Comparison of PAI Level of different participants

In both the Polycystic Ovary Syndrome (PCOS) group and the healthy control group, Plasminogen Activator Inhibitor (PAI) biomarker levels were examined for potential correlations with clinical parameters in Table 2. This dataset sheds light on the underlying mechanisms of PCOS and its effects on these indicators while providing useful insights into the complex interactions between physiological variables and PAI biomarkers.

The body mass index (BMI) values of individuals in the PCOS group are generally greater than those in the control group, according to the table, which shows a trend. This is consistent with the established link between PCOS and obesity. Insulin resistance is also more common in the PCOS population, with a higher percentage showing enhanced insulin resistance status. This supports the idea that insulin resistance is crucial to PCOS etiology and related metabolic abnormalities.

It's also important to observe the hormonal profiles. A typical hormonal imbalance in PCOS is greater levels of luteinizing hormone (LH) in the PCOS group of participants. Increased LH levels support the hormonal abnormalities present in the syndrome even more than normal follicle-stimulating hormone (FSH) levels. Additionally, the PCOS group has higher testosterone levels than the control group, highlighting the common androgen excess seen in PCOS individuals.

A pattern emerges from the analysis of the PAI biomarker values. In comparison to controls, participants in the PCOS group often had higher levels of PAI-1 and PAI-2. This raises the possibility that hormonal imbalances, altered fibrinolysis processes, and the severity of PCOS symptoms are related. The complex interaction between hormonal imbalances and PAI indicators in the PCOS population may be a factor in the syndrome's elevated risk of cardiovascular problems.

Conclusion

The levels of PAI-1 and PAI-2 between women with PCOS and healthy controls showed considerable differences in this study's analysis of unique PAI biomarker profiles. Notably, higher PAI-1 levels within the PCOS group showed meaningful relationships with BMI, insulin resistance, LH, and testosterone levels. These findings imply that PAI biomarkers may be useful in identifying and diagnosing PCOS as well as in elucidating its underlying processes. This study offers critical insights into PCOS pathophysiology and the possibility for biomarkers, while accepting its limitations, such as sample size and unmeasured confounders. These insights open the door to customized management and focused interventions.

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