Original Research

Study of serum VASPIN level and its effect on insulin sensitivity in polycystic ovary syndrome patients, in southern Odisha

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is a reproductive disorder of growing concern in women of child bearing age group. The presence of metabolic and endocrine disorder along with the reproductive derangement necessitates a multi-omics approach to understand its pathophysiology. The adipocytokines have been quite instrumental in this aspect of learning about the metabolic defects in PCOS. VASPIN a visceral adipose tissue derived adipokine is a novel molecule which has been associated with metabolic syndrome, is being researched in PCOS.

Objective : We aim at studying the serum VASPIN levels in PCOS patients and correlate them with BMI, plasma insulin, HOMA-IR in these patients.

Material and Methods: 90 participants were included in the study with 60 PCOS patients (30 women with BMI≥ 25 and 30 women with BMI<25) and 30 age matched healthy controls. Anthropometric measurements were done. Fasting blood glucose, plasma insulin, Homeostatic model assessment for insulin resistance (HOMA-IR), serum vaspin levels were measured. All data were analysed using IBM SPSS version 20 and presented as mean and standard deviation(±SD), ANOVA and Pearson's correlation was done.

Result : There was no significant difference between the age in the three groups. The fasting blood glucose, plasma insulin, HOMA -IR were significantly higher in both lean and obese PCOS groups compared to the controls. The mean of serum VASPIN level in obese PCOS (4.06 ± 2.39) and lean PCOS (1.47 ± 0.28)was higher than control (1.06 ± 0.35) which was statistically significant. ($p<0.001$). The Serum VASPIN level positively correlated with BMI, plasma insulin, HOMA –IR in both obese and lean PCOS.

Conclusion : The increase in serum VASPIN levels and its positive correlation with BMI, HOMA-IR and plasma insulin in PCOS patients could contribute to the diabetogenic ,atherogenic and steroidogenic risk in PCOS. Therefore VASPIN could be a good diagnostic and predictive marker to prevent the complication in PCOS.

Key word: Polycystic ovary syndrome (PCOS), VASPIN , HOMA-IR, BMI

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a polygenic multifactorial disease which is featured by elevated levels of androgens, ovulatory dysfunction, and morphological abnormalities.[1] It affects an estimated **8–13%** of reproductive-aged women globally (**5.8-10%** in India).[2]

The presence of irregular menses and polycystic ovaries was the core of Stein and Leventhal's original study (1935). Since then, innumerable of other

symptoms have also been associated with PCOS such as acne, male pattern balding (alopecia), hirsutism (excessive hair growth), infertility, obesity (truncal adiposity), skin tags and high androgen hormone levels.[3] It is one of the main cause of anovulatory infertility in women and is the most common endocrinopathy affecting reproductive-aged women.[4]

The multifactorial aetiology of PCOS is underpinned by a complex genetic architecture that has only recently been taken into account. Many genes have been associated with PCOS, which affect fertility either directly or indirectly.[5] It was previously considered as disease of adult women but recent evidence suggests that PCOS is a lifelong disease manifestation occurring since prenatal age.[6]

Notably, the increase in incidence of obesity in the recent decades was accompanied by an elevated prevalence of PCOS.[7]This brings into light the role of adipocyte as an endocrine organ taking pivotal roles in metabolic functions. Adipokines or adipocytokines are proteins or peptides with hormone like properties, released from adipose tissue.[8]Adipokines affect metabolic and endocrine signalling in women with PCOS, are known to affect the regulation of the hypothalamic–pituitary–gonadal axis or to locally alter ovarian steroidogenesis.[9]

VASPIN or the Visceral Adipose Tissue Derived Serine Protease Inhibitor, one of the more recently discovered adipokine, found both in the visceral and in subcutaneous adipose tissue. It is a member of the SERPIN A12 family, and an adipocytokine isolated from the OLETF (Otsuka Long-Evans Tokushima Fatty) rats. It is coded by the gene SERPINA12 located on the long arm of chromosome 14 (14q32.13). Although the exact mechanism of action of VASPIN in glucose metabolism and insulin sensitization is not known, it has been postulated to be inhibiting a protease kallikrein 7(a protease degrading insulin),[11] which plays a role directly or indirectly in lowering blood glucose. It plays an important role by inhibiting serine phosphorylase among the insulin receptor-1(irs-1) and 2(irs-2).[10]Visceral adipose tissue-derived serine protease inhibitor (vaspin) is another adipokine,which protects from inflammation, liver steatosis, atherosclerosis and insulin resistance. However, studies show, a positive association between the vaspin concentration in the blood serum and the indicators of obesity, T2DM, polycystic ovary syndrome (PCOS) and coronary artery disease (CAD).[12]

Several researchers suggest that this adipokine increases the insulin sensitivity of adipose tissue in the condition of obesity. The increase of VASPIN expression acts like a compensatory mechanism and being a reaction to growing obesity and insulin resistance.[13]

VASPIN being a novel adipokine, there has been only a few research on it in India . With most researches taking metabolic syndrome and diabetes mellitus into consideration, we aim at studying VASPIN level and its relation with insulin resistance in patients of PCOS in our region and this will be the first study taking PCOS into account.

MATERIAL AND METHOD

Study population – A total of 90 participants were included in the study among which 60 participants were diagnosed PCOS cases(according to the revised 2003 Rotterdam European Society for Human Reproduction (ESHRE)/American Society of Reproductive Medicine (ASRM) PCOS Consensus Workshop Group diagnostic criteria, namely, *1*) oligo- and/or anovulation, *2*) clinical and/or biochemical signs of hyperandrogenism, and *3*) polycystic ovaries)The participants were resourced from the department of obstetrics and gynaecology as well as from the department of endocrinology. Thirty (30) age matched healthy control females were chosen from amongst attendant of patients, MBBS students, nursing students and from general populations.All my study participants were females between the age of 17 - 35 years and residing in the southern districts of Odisha

Exclusion criteria for the study included age <16years or >35years ,Women with known cases of type 2 DM, thyroid disease (TSH), hyperprolactinemia (prolactin),Non-Classical Congenital Adrenal Hyperplasia (NCCAH), Cushing's syndrome/disease, hypogonadotropic hypogonadism or androgen producing tumours which exhibit similar manifestations (clinical/biochemical/morphological) as that of PCOS, Women using OCPs or insulin sensitizing drugs at least 3 months prior to study period.

Ethics- Institutional ethics committee clearance(IEC 1040) was obtained for the study and informed written consent were obtained from the participants prior to their participation in the study

All participants underwent anthropometric measurements like weight, height, waist circumference and BMI was calculated

Sample collection– Taking aseptic measures, fasting blood samples was collected on 2nd -3rd day of the menstrual cycle for the control group where as in the PCOS cases it was collected independent to the menstrual cycle owing to their cycle irregularity.Estimation of the biochemical parameters like, Fasting blood glucose(FBG), plasma insulin, serum VASPIN. Homeostasis model assessment of insulin resistance (HOMA -IR) was calculated using the formula (fasting glucose(mg/dl)×fasting insulin(μIU/L)/405)

Fasting blood glucose was estimated by Toshiba 120FR automated biochemical analyser. Serum Prolactin and serum TSH(thyroid stimulating hormone) were assayed using the commercially available kits of Roche Cobas e411

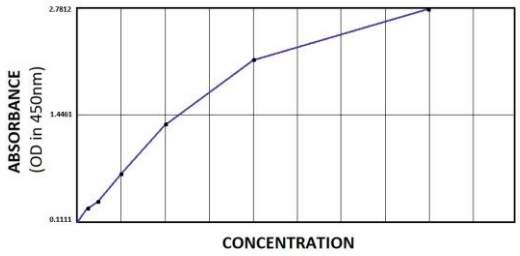
electrochemiluminescence assay, to rule out hyperprolactinaemia or hypothyroidism.

Estimation of Serum VASPIN

Estimation of Serum VASPIN and plasma Insulin using ELISA.VASPIN was assayed by Abbkine human VASPIN ELISA Kit that employs a two-site sandwich ELISA, with a detection range from 0.5ng/ml - 8 ng/ml and the plasma insulin was assayed using Raybio human insulin ELISA kit (detection range 4µIU/ml-300µIU/ml).

Absorbance of VASPIN standard solution with standard curve plot

Standards	Concentration (ng/ml)	Absorbance OD
Std 1	0	0.1111
Std 2	0.5	0.2603
Std 3	1.0	0.3401
Std 4	2.0	0.6497
Std 5	4.0	1.3558
Std 6	8.0	2.0818
Std 7	16.0	2.7894



STASTISTICS

The statistical analysis was done using IBM SPSS version 20, where in two-way ANOVA test was done to compare the mean and standard deviation of various parameters between the groups and the Pearson's correlation was used to establish correlation between VASPIN levels and other parameters.

RESULTS

In this study the PCOS cases were divided into two groups basing on BMI i.e Lean PCOS group with BMI < 25kg/m^2 and Obese PCOS group with BMI ≥ 25kg/m^2 and thirty (30) aged matched healthy control were taken. On comparing the BMI in the three groups, the mean and standard deviation BMI in obese PCOS group was higher than the healthy controls (table 1) which was statistically significant. The mean serum VASPIN, HOMA -IR, plasma insulin and fasting blood glucose (table 2) was also compared in the three groups and the levels were found to be higher in the obese PCOS group which was statistically significant. Serum VASPIN levels were found to positively correlate significantly with the BMI, insulin and HOMA-IR, in PCOS group. (table 3) Serum VASPIN levels were found to positively correlate significantly with insulin and HOMA-IR in both lean and obese PCOS groups (table 4)

Table 1: Anthropometric parameters

PARAMETERS	CONTROL N=30 BMI≤25	LEAN PCOS N=30 BMI<25	OBESE PCOS N=30 BMI≥25	'P' VALUE (anova)
Age(Years)	24.75 ± 5.19	23.23± 2.75	23.27 ± 3.02	0.334
Height(Cm)	159.5 ± 6.7	155.0 ± 6.7	159.9 ± 6.7	**0.010**
Weight(Kg)	55.5 ± 8.3	54.8 ± 6.1	76.4 ± 13.5	**<0.001**
Waist ircumference (Cm)	74±3.0	72.24±3.1	84.3±5.06	**<0.001**
BMI(KG/M^2)	21.47 ± 2.64	22.53 ±1.91	29.24 ± 4.77	**<0.001**

. p value < 0.05 is significant

Table 2: Biochemical parameters and HOMA-IR

PARAMETERS	CONTROL N=30 BMI≤25	LEAN PCOS N=30 BMI<25	OBESE PCOS N=30 BMI≥25	'P' VALUE (anova)
FPS (mg/dl)	85.46 ± 8.56	102.5 ± 9.94	106.3 ± 15.3	0.000
INSULIN(µIU/ml)	4.59 ± 2.31	8.36 ± 3.33	16.68 ± 6.05	**0.000**
HOMA-IR	1.03 ± 0.61	2.13 ± 0.86	4.55 ± 1.53	**0.000**
VASPIN (ng/ml)	1.06 ± 0.35	1.47 ± 0.28	4.06 ± 2.39	**0.001**

. p value < 0.05 is significant

Table 3: Correlation of serum VASPIN levels with BMI, plasma insulin and HOMA-IR in PCOS cases

PARAMETRES	SERUM VASPIN	
	'r' value	**'p'value**
BMI (kg/m^2)	0.71	<0.001
INSULIN(μIU/ml)	0.64	<0.001
HOMA-IR	0.69	<0.001

. p value < 0.05 is significant

Table 4: Correlation of serum VASPIN levels , plasma insulin and HOMA-IR in PCOS cases

PARAMETERS	Serum VASPIN			
	Lean PCOS		**Obese PCOS**	
	'r' value	**'p' value**	**'r' value**	**'p' value**
INSULIN(μIU/ml)	0.48	**0.007**	0.57	**0.001**
HOMA-IR	0.45	**<0.001**	0.42	**0.021**

. p value < 0.05 is significant

DISCUSSION

PCOS is a heterogeneous metabolic, endocrine and reproductive disorder. Metabolic syndrome playing a pivotal role in the disease spectrum of PCOS, the adipokines have a instrumental role in the pathogenesis of insulin resistance, diabetes and cardiovascular risk that accompanies PCOS.

The mean age of all the participants in this study was 23.5 years. The mean value of weight and BMI was also higher in the obese PCOS group in comparison to the lean PCOS and control group, it was statistically significant.

Different BMI thresholds have been determined to define obesity in various ethnic groups. BMI cut-offs have been lowered to 25 and 23 kg/m2 to reflect the risk in South Asians and East Asians respectively (Chen *et al.* 2010)[14]. Sendur et al 2021, conducted a review on effect of ethnicity on various prospects of PCOS and opined that, because of the increased central adiposity of Asian and South Asian subjects the lowered thresholds of BMI may still not be proper for these ethnic populations[15]. Therefore, while evaluating the increased adiposity in women with PCOS of various ethnic origins, using population-specific anthropometric measures such as WHR may provide a better assessment of metabolic risk.

Hiya Islam et al in 2022, in a review article suggested that the implementation of adult thresholds in polycystic ovary morphology detection by ultrasonography (including use of transvaginal ultrasonography) and biochemical parameter estimation might lead to over-diagnosis of PCOM in adolescents.[16]

In our study we observed a higher mean value of fasting plasma glucose in the obese and the lean PCOS group in comparison to the control. It was within a range for impaired glucose tolerance (105-120mg/dl) for the obese PCOS group. Women with PCOS and impaired glucose metabolism had higher incidences of glucose intolerance and type II diabetes mellitus (Wei et al, 2009).[17]

Doddapa et al (2018) found higher level of fasting plasma glucose in obese women with PCOS, 105 +15.0 mg/dl, non-obese women with PCOS had 96+10.2 mg/dl compared to controls who had 92+7.8 mg/dl.[18] These values were statistically significant (p<0.001). Our study corroborated with the finding in this study where the mean value of FPS in obese PCOS and lean PCOS group was higher than that of the control group

In this study the fasting plasma insulin and insulin resistance (HOMA-IR) was observed to be higher in the obese and lean PCOS group in comparison to the control group, it was statistically significant too. Akbarzadeh et al in 2012 reported a higher value of serum insulin and HOMA-IR in PCOS patient in comparison to healthy control. Insulin resistance is present in both obese and nonobese women with PCOS compared to normal women with the matched age and weight.[19]

Dodappa et al in 2020 opined that serum insulin, and IR were increased in both obese and nonobese women with PCOS compared to their BMI adjusted controls with *p* value of <0.001, the values were within reference range in nonobese women. In PCOS patients, it was observed that although the receptors affinities of insulin were similar to normal females, but there was a ddecreased insulin binding recorded at pancreatic *β*-cell and adipose tissues resulting in low glucose uptake and insulin insensitivity in PCOS females compared to normal females.[18]

This might be due the reduced numbers of GLUT4 in subcutaneous adipose tissues in PCOS patients, which leads to insulin insensitivity.[17]

PCOS females who are obese and overweight are at greater risk of the disturbances in glucose metabolism and they therefore are required to check their glucose regularly with proper metabolic profiling. (P.S Shepherd et al 1999).[20]

In this study we have observed a higher mean value of serum VASPIN in obese and lean PCOS, Women with PCOS had significantly higher serum VASPIN levels

than the healthy controls (3.52±1.38 vs. 0.36±0.19 ng/ml, p<0.0001) as was recorded by Cakal et al , 2011 [21]

A number of studies found higher VASPIN levels in obese[22,23](Cho et al. 2010; Derosa et al. 2013) and T2D patients[24,25] (Zhang et al. 2011; Teshigawara et al. 2012) Obese patients diagnosed with polycystic ovary syndrome (PCOS) were at higher risk for impaired glucose tolerance, insulin resistance, dyslipidaemia and T2D. Serum VASPIN levels were significantly higher in PCOS patients compared to healthy subjects (2.02 ng/ml versus 0.28 ng/ml; p= 0.048) (Koiou et al. 2011).[26]In the most recent study by Kozlowski et al 2022, amongst Polish women, it was found that VASPIN concentrations above the median was considered independent favourable prognostic factors for endometrial cancer.[27]

Wang et al in 2022 found that VASPIN level was positively correlated with BMI, WHR, TG, TC, LDL-C, HOMA-IR, ($P < 0.05$).[28]

Our finding which has significant positive correlation between serum VASPIN and BMI in PCOS (p <0.001) which was in accordance with study findings of Cakal et al (2011).[21]In another study by tan et al higher circulating as well as omental tissue concentration of VASPIN was found in PCOS women. They also found a significant positive correlation between these levels with BMI and waist to hip ratio.[29]

Our study deduced a positive correlation of serum VASPIN level with fasting plasma Insulin and HOMA-IR in each group where in for Lean (r = 0.48, p = 0.007 and r = 0.45, P =< 0.001) for Obese (r = 0.57, P = 0.001 and r = 0·42 p = 0.021)however **Dogan et al, 2020**, found no significant increase in serum VASPIN level in PCOS group despite an increase in BMI which was statistically significant.[30]

It has been postulated that the increase in VASPIN levels might be representing a compensatory response against obesity and Insulin resistance (IR). Therefore, VASPIN expression might represent a defence mechanism against Insulin Resistance. It may down regulate the expression of genes associated with IR; this action is more prominent in the abdominal fat. The increase in serum VASPIN levels in classical PCOS phenotypes might be attributed to the more adverse metabolic profile of these patients, including greater total and abdominal obesity, IR and risk factors for cardiovascular diseases.

Hernández-Rodríguez et al in 2019 found that fetal VASPIN concentration was increased in response to elevated glucose, possibly from maternal circulation. As VASPIN improves insulin resistance an increase in concentration will have its effect of improved fetal insulin utilization. This appears to be a compensatory mechanism for reducing fetal glucose, possibly derived from maternal sources, in order to achieve an optimal intrauterine environment.[31]

CONCLUSION

PCOS is a significant disorder in the women of reproductive age group. The metabolic and endocrine derangement in this disorder is characterised by insulin resistance, glucose intolerance, obesity and dyslipidaemia. Owing to the increase in incidence of obesity being associated to this syndromic disorder, VASPIN as an adipokine may prove as one such marker in understanding the disease as well as help in development of specific therapeutic targets in lowering the risk factors for development diabetes mellitus, coronary artery disease and infertility in patients with PCOS.

Antiprotease inhibitor therapy, like VASPIN antagonist (candesartan, incretin based therapy), VASPIN receptor blockers (flutamide) may have a promising hope in PCOS cases.

This trial of novel therapy and use of serum VASPIN as a diagnostic and prognostic marker in PCOS might help clinicians to prevent PCOS and its complications.[32]

Conflict of interest

The author reports no conflict of interest pertaining to the study.

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