ORIGINAL RESEARCH PAPER

Obstetrics & Gynaecology

KEY WORDS: Polycystic Ovary Syndrome, Insulin Resistance, Angiopoietin-like protein 2, Obestatin, Inflammation, Obesity, Infertility

ANGIOPOIETIN LIKE PROTEIN 2 AND OBESTATIN LEVELS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME – A CASE CONTROL STUDY

Dr Sharon Rose Jonetta S — Postgraduate, Department of Obstetrics and Gynecology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, GST Road, Madhuranthagam, Tamil Nadu.

Dr SNS Minnalkodi* — Professor and Head, Department of Obstetrics and Gynecology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, GST Road, Madhuranthagam, Tamil Nadu. *Corresponding Author

ABSTRACT

BACKGROUND: Polycystic ovary syndrome (PCOS) is the common endocrine disorder in women of reproductive age group and is the major cause of anovulatory infertility. Insulin resistance plays an important role in the development and durability of this disorder. Angiopoietin like protein 2 is known for its role in inflammation as mediator derived from the adipose tissue which has link with insulin resistance and obesity. Obestatin is a hormone associated with insulin resistance that suppresses reabsorption of food by inhibiting gastric emptying there by decreases weight gain. The present is planned to compare the serum levels of Angiopoietin like protein 2 and Obestatin in healthy women and PCOS women.

MATERIALS AND METHODS: In this case-control study 37 PCOS women based on the Rotterdam 2003 diagnostic criteria as the case group and 37 women with normal menstrual cycles as the control group were enrolled. Both cases and controls were age and BMI matched. Serum levels of Angiopoietin like protein 2, Obestatin, insulin and other hormones factors related with PCOS were measured by ELISA method and biochemical parameters were measured by autoanalyzer. Data were analyzed by independent samples-T test, Chi Square and linear regression using SPSS software, version 16.

RESULTS: There were no significant variations in the amount of Angiopoietin like protein 2, Obestatin cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein, cholesterol, and dehydroepiandrosterone-sulfate between two groups. There were significant increases in serum levels of fasting blood sugar (P=0.01), insulin (P =0.04), homeostasis model assessments of insulin resistance (P =0.04), testosterone(P=0.02), luteinizing hormone (P=0.004), luteinizing hormone/follicle stimulating hormone (P=0.006) and prolactin (P=0.04) in case group compared to the control group.

CONCLUSION: In this study, no significant variations were observed in serum levels of Angiopoietin like protein 2 and Obestatin in PCOS women with normal BMI.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder seen in women of reproductive age causing infertility[1]. PCOS is a multifactorial disease involving genetic, hormonal, environmental factors, lifestyle etc. It is associated with obesity, insulin resistance, dyslipidemia[1-4].

Angiopoietin like protein 2 is a pro-inflammatory marker expressed in visceral adipose tissue and is associated with chronic inflammatory diseases like diabetes, atherosclerosis and cancer[5, 6]. Angiopoietin like protein 2 has an important effect on angiogenesis, androgen biosynthesis, adipocyte function, and insulin resistance[6]. Recent studies reported that circulating levels of Angiopoietin like protein 2 in humans and mice are both dependent on insulin resistance, adiposity and inflammation. the removal of angiopoietin like protein 2 in obese mice resulted in a reduction in adipose tissue inflammation and systemic insulin resistance. For this reason, angiopoietin like protein 2 is known as an inflammatory mediator derived from adipose tissue[7-9]. There are study reports showing that the circulatory level of angiopoietin like protein 2 decreases with weight loss, and this decrease in obese diabetic patients was associated with a decrease in visceral fat tissue[2,6,8]. Few studies in humans reported that, due to the role of angiopoietin like protein 2 in inflammation, associated with developing type 2 diabetes by causing inflammation in the Langerhans islets, fatty tissues, liver and muscles which may be causing insulin resistance and impairment of beta cell function[10,11].

Obestatin is a peptide hormone (contains 23 amino acid) released by ghrelin precursor peptide, preproghrelin which is mainly released from the stomach[12]. A small quantity of Obestatin is also secreted in other tissues like ε-cells of the pancreas while the ghrelin secreted from α, β and ε-cells of the pancreas[13]. Recent studies reported the effect of Obestatin on appetite, food intake and gastric secretions is against the action of ghrelin[14, 15]. However, the role of Obestatin and its mechanism in regulating is not understood fully.

As per the recent studies literature, Angiopoietin like protein 2 and Obestatin were reported to have association with insulin resistance, obesity and diabetes, it is hypothesized that they may also play a role in developing PCOS. Therefore, the present study is undertaken to evaluate the serum levels of Angiopoietin like protein 2 and Obestatin in PCOS women.

MATERIALS AND METHODS

Study design

The present study is a prospective case control study. The study was conducted during the period of January 2020 to December 2021 at Department of Obstetrics and Gynecology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Madhuranthagam, Tamil Nadu. The study comprises of 37 PCOS women diagnosed based on the revised Rotterdam 2003 criteria which is a case group and 37 healthy women with normal menstruation periods as the control group[16].

Inclusion and exclusion criteria

PCOS women based on the revised Rotterdam 2003 diagnostic criteria[16] was used to confirm the diagnosis in case group. As per the revised Rotterdam criteria should have at least two of the three following criteria: 1). Clinical and/or biochemical Hyperandrogenemia; 2). Oligomenorrhea or anovulation; 3). Ultrasound examination of polycystic ovaries (≥12 follicles, 2-10mm in size), and healthy women were selected from women with normal menstruation periods and normal pelvic ultrasound, without acne, hirsutism, and infertility[16].

Parameters were compared between the age matched

controls and cases. Women with any other diseases such as thyroid disorders, cardiovascular diseases, diabetes, hypertension and renal disorders, and on contraceptive drugs, on treatment for diabetes, hypertension etc were excluded from the study.

Sampling and measurement of parameters

About 5 ml of fasting venous blood sample was collected in plain tube from all the study subjects during their follicular phase (1 to 4th day of menstruation). Blood sample was allowed to clot and serum was obtained after centrifugation at 3000 rpm for 15 min and then samples were frozen at -80°C till further analysis. Anthropometric data such as age, weight, height, body mass index and waist to hip ratio were noted for all the subjects. Biochemical parameters include estimation of fasting blood sugar (FBS), Total Cholesterol (TC), Triglyceride (TGL) and Creatinine (Cr) were measured using commercially available kits for BA400 biochemistry autoanalyzer. The serum levels of Testosterone, Deh ydroepiandrosterone-sulfate (DHEAS), Prolactin, Insulin were measured using Chemiluminescence method on Roche, COBAS E-411. Angiopoietin like protein 2, Obestatin were measured by ELISA kits. Homeostasis model assessments of insulin resistance (HOMA-IR) was calculated by using the formula HOMA.IR = (Fasting Insulin μIu/ml) × Fasting Glucose (mg/dl) /405. (25,26).

Ethical consideration

The study was approved by the Institutional Ethics Committee of Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Madhuranthagam, Tamil Nadu. All the study subjects were informed about the purpose of the study and given their consent for their participation in the study.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using SPSS statistical software, version 16.0. Independent samples-T test was used to compare the quantitative variables between the two groups and all results were expressed as mean ± SD. Differences at the level of $p < 0.05$ were considered statistically significant.

RESULTS

This is a prospective case control study. Both controls and cases are age, BMI matched. There is no significant statistical difference in demographic characteristics such as age, weight, height, BMI. Statistically significant difference was observed with respect to WHR when compared between cases and control groups (Table 1).

Table 1. Demographic characteristics of case and control groups (Means ± SD).

Parameter	Control group (n = 3^{V})	Case group (n = 3^{V})	p value
Age (years)	28.91 ± 8.1	28.85 ± 5.90	0.97
Weight (kg)	57.17 ± 11.18	60.04 ± 11.41	0.27
Height (cm)	161.17 ± 4.71	159.52 ± 6.37	0.21
Body Mass Index (BMI-kg/m^2)	24.52 ± 5.60	25.91± 3.93	0.22
Waist/Hip ratio (WHR)	0.81 ± 0.09	0.9± 0.08	0.0001

From table 2 it is evident that there is significant increase in the regular parameters monitored in PCOS women like insulin resistance, clinical elevation of androgens, altered LH/FSH ratio, decrease in Obestatin levels wase observed and it is statistically significant when compared with that of the controls. While Angiopoietin like protein 2 though showed increased concentration in PCOS women but it is not statistically significant when compared with the control group. The increase in serum total cholesterol (T.C), triglycerides (TGL) and LDL-cholesterol suggest that there is dyslipidemia in PCOS women when compared to that of control women. These results are in line with that of the many studies. Also, there is significant increase in the hormonal levels of LH (P = 0.001), LH/FSH (P =0.001), Testosterone (P = 0.0001), Prolactin (P =0.008) in PCOS women when compared with control group women (Table 2).

Table 2. Serum concentrations of variables in case and control groups.

Parameter	Control group (n = 3^{V})	Case group (n = 3^{V})	p value
Obestatin (ng/ml)	20.72 ± 10.66	16.05 ± 9.07	**0.04**
Angiopoietin-like protein 2 (ng/ml)	130.92 ± 88.79	164.82 ± 61.84	0.06
Insulin (μIU/ml)	9.76 ± 7.63	16.42± 17.01	**0.03**
HOMA-IR	3.06 ± 2.04	4.81± 4.09	**0.02**
FBS (mg/dl)	90.29 ± 8.53	99.71 ± 11.44	**0.0001**
Triglyceride (mg/dl)	88.79 ± 30.23	117.04 ± 45.63	**0.01**
Cholesterol (mg/dl)	157.83 ± 29.18	175.07 ± 28.52	**0.01**
HDL-C (mg/dl)	49.25 ± 12.59	47.92 ± 10.08	0.61
LDL-C (mg/dl)	108.66 ± 23.55	120.60 ± 21.72	**0.02**
LH (mIu/ml)	5.44 ± 2.74	9.76± 4.9	**0.001**
FSH (mIu/ml)	6.18 ± 2.23	5.84± 2.55	0.54
LH/FSH	1.16 ± 0.74	2.27 ± 1.92	**0.001**
Testosterone (ng/ml)	0.81 ± 0.26	1.59± 0.62	**0.0001**
DHEAS (μg/dL)	1.83 ± 0.78	2.39 ± 0.87	**0.004**
Prolactin (ng/mL)	15.10 ± 8.54	21.23± 10.74	**0.008**

Statistically no significant difference was observed in the serum levels of Angiopoietin Like protein 2, HDL-cholesterol, and FSH hormone in PCOS women compared to the control group (Table 2).

DISCUSSION

There are studies reporting the role of angiopoietin like protein 2 in regulation of obesity, it is found to decrease with weight loss and significantly are correlated with systemic insulin resistance and inflammation[8, 17]. In the present study, there was no significant difference in serum levels of angiopoietin like protein 2 in PCOS women when compared with age and BMI matched control women. The study result is not completely in line with the previous study reports. This is because study reports by Sasaki Y et al., showed that increased expression of angiopoietin like protein 2 induced inflammation in adipose tissue and deteriorated insulin sensitivity which is not exactly reflected in the present study although there is elevation in PCOS group, but it is not statistically significant (P=0.06)[18]. On the other hand, the study reports are in line with the reports of, Tabata M et al., wherein angiopoietin like protein 2 gene deleted in mice, showed improved adipose tissue inflammation and insulin resistance[7]. These findings suggest that in humans, adipose tissue is one of the most important sources of angiopoietin like protein 2 levels in circulation, so that angiopoietin like protein 2 suppression can be an effective method against obesity associated with insulin resistance[8,11]. Studies by Horio E et al., in epithelial cells of obese mice on exposure to a high fat diet, showed reduced levels of angiopoietin like protein 2 than obese mice with normal diets[19]. Study by, Muramoto A et al., on lifestyle modification observed after three months and six months showed a rapid decline in angiopoietin like protein 2 level in obese individuals that showing 2% or more weight loss and also reported that a decrease in angiopoietin like protein 2 suggesting a good indicator for obesity control[20]. So, the estimation of angiopoietin like protein 2 was selected in our study for comparison and it did not show significant difference in PCOS women when compared to control women.

In this study, Obestatin is another novel parameter studied in PCOS women of Indian population in comparison with age and BMI control women. Obestatin is a peptide hormone involved in the development of obesity and inflammation[7, 11]. Our study reports shows that there is significant difference in the serum levels of Obestatin in PCOS women when compared to that of control women of same age and BMI. So, this parameter could be an important parameter to identify and monitor in PCOS women for their prognosis. This study results are like that of the results published by Abd El-Fattah AI et al., who reported decreased levels of Obestatin in Egyptian obese PCOS women when compared to non-obese PCOS women and control group[21]. Further, they reported that there was a significant negative correlation between Obestatin levels in serum and BMI[21]. In this study, only fasting blood sample was collected to determine the Obestatin levels and low serum levels Obestatin was found in PCOS women when compared to that of control women. Similar type of results was reported in a study done by Guo ZF et al., but blood samples collected were preprandial and postprandial conditions in obese subjects compared to normal weight subjects[22]. Whereas few studies reported contradicting results. For example, study by Razzaghy-Azar M et al., reported that the plasma levels of Obestatin in obese children in fasting conditions were higher than that of control group and showed positive relationship with BMI[23]. As there is no uniformity in the study reports more research with large number of sample size and multicentric centric studies in different populations with different ethnic backgrounds need to be conducted to determine the exact role of Obestatin in PCOS women.

CONCLUSION

In this study Obestatin levels were found to be decreased while angiopoietin like protein 2 levels in serum did not show significant difference in PCOS women when compared to control women. From the results of this study, it can be concluded that more research is needed to rule out the role Angiopoietin like protein 2 and Obestatin in PCOS women. Research should be focused on these two novel parameters to find out their role in PCOS diagnosis and prognosis.

Acknowledgements

We would like to thank our Managing Director Dr Annamalai R, M.S., MCh for his support and encouragement to accomplish this project.

Conflict Of Interest

None

Financial Support

We did not receive any external funding for this project

REFERENCES

1. Kumar AN, Naidu JN, Satyanarayana U, Ramalingam K, Anitha M. Metabolic and Endocrine Characteristics of Indian Women with Polycystic Ovary Syndrome. Int J Fertil Steril. 2016 Apr-Jun;10(1):22-8. doi: 10.22074/ijfs.2016.4764. Epub 2016 Apr 5. PMID: 27123196; PMCID: PMC 484 5525.
2. Esmaeilzadeh S, Andarieh MG, Ghadimi R, Delavar MA. Body mass index and gonadotropin hormones (LH & FSH) associate with clinical symptoms among women with polycystic ovary syndrome. Glob J Health Sci. 2014;7(2):101-6.
3. Cappelli V, Musacchio MC, Bulfoni A, Morgante G, De Leo V. Natural molecules for the therapy of hyperandrogenism and metabolic disorders in PCOS. Eur Rev Med Pharmacol Sci. 2017;21(2):15-29.
4. Liu Z, Hao C, Huang X, Zhang N, Bao H, Qu Q. Peripheral blood leukocyte expression level of lncRNA steroid receptor RNA activator (SRA) and its association with polycystic ovary syndrome: a case control study. Gynecol Endocrinol. 2015;31(5):363-8.
5. Gellen B, Thorin-Trescases N, Sosner P, Gand E, Saulnier PJ, Ragot S, et al. ANGPTL2 is associated with an increased risk of cardiovascular events and death in diabetic patients. Diabetologia. 2016;59(11):2321-2330.
6. Liu Z, Liu C, Hao C, Xue Q, Huang X, Zhang N, et al. Aberrant expression of angiopoietin-like proteins 1 and 2 in cumulus cells is potentially associated with impaired oocyte developmental competence in polycystic ovary syndrome. Gynecol Endocrinol. 2016;32(7):557-61.
7. Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, et al. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. Cell Metab. 2009; 10(3): 178-88.
8. Apovian CM, Bigornia S, Mott M, Meyers MR, Ulloor J, Gagua M, et al. Adipose macrophage infiltration is associated with insulin resistance and vascular endothelial dysfunction in obese subjects. Arterioscler Thromb Vasc Biol. 2008;28(9), 1654–1659.
9. Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest. 2008;118(9): 2992–3002.
10. Doi Y, Ninomiya T, Hirakawa Y, Takahashi O, Mukai N, Hata J, et al. Angiopoietin-like protein 2 and risk of type 2 diabetes in a general Japanese population: the Hisayama study. Diabetes Care. 2013;36(1):98-100.
11. Varma V, Yao-Borengasser A, Rasouli N, Nolen GT, Phanavanh B, Starks T, et al. Muscle inflammatory response and insulin resistance: synergistic interaction between macrophages and fatty acids leads to impaired insulin action. Am J Physiol Endocrinol Metab. 2009;296(6):1–31.
12. Zorlu M, Kiskac M, Güler EM, Gültepe I, Yavuz E, Çelik K, et al. Serum obestatin and omentin levels in patients with diabetic nephropathy. Niger J Clin Pract. 2017;20(2):182-187.
13. Pradhan G, Wu CS, Han Lee J, Kanikarla P, Guo S, Yechoor VK, et al. Obestatin stimulates glucose-induced insulin secretion through ghrelin receptor GHS-R. Sci Rep. 2017;7(1):1-10.
14. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science. 2005;310(5750):996-9.
15. Chen CY, Asakawa A, Fujimiya M, Lee SD, Inui A. Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev. 2009; 61(4): 430–481.
16. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 81(1):19-25. doi: 10.1016/j.fertnstert.2003.10.004.
17. Lebovitz HE , Banerji MA. Insulin resistance and its treatment by thiazolidinediones. Recent Prog Horm Res. 2001;56:265–294.
18. Sasaki Y, Ohta M, Desai D, Figueiredo JL, Whelan MC, Sugano T, et al. Angiopoietin Like Protein 2 (ANGPTL2) Promotes Adipose Tissue Macrophage and T lymphocyte Accumulation and Leads to Insulin Resistance. PLoS One. 2015;10(7):1-18.
19. Horio E, Kadomatsu T, Miyata K, Arai Y, Hosokawa K, Doi Y, et al. Role of endothelial cell-derived angptl2 in vascular inflammation leading to endothelial dysfunction and atherosclerosis progression. Arterioscler Thromb Vasc Biol. 2014;34(4):790-800.
20. Muramoto A, Tsushita K, Kato A, Ozaki N, Tabata M, Endo M, et al. Angiopoietin-like protein 2 sensitively responds to weight reduction induced by lifestyle intervention on overweight Japanese men. Nutr Diabetes. 2011;1(11):1-10.
21. Abd El-Fattah AI, Elboghdady AA, Kamel MM, Mohamed ES. Obestatin and Copeptin Levels in Egyptian Polycystic Ovary Patients and their Relation to Obesity, Insulin Resistance and Cardiovascular Risk. Merit Res J Med Med Sci. 2016;4(10):451-457.
22. Guo ZF, Zheng X, Qin YW, Hu JQ, Chen SP, Zhang Z. Circulating preprandial ghrelin to obestatin ratio is increased in human obesity. J Clin Endocrinol Metab. 2007;92(5):1875-80.
23. Razzaghy-Azar M, Nourbakhsh M, Pourmoteabed A, Nourbakhsh M, Ilbeigi D, Khosravi M. An Evaluation of Acylated Ghrelin and Obestatin Levels in Childhood Obesity and Their Association with Insulin Resistance, Metabolic Syndrome, and Oxidative Stress. J Clin Med. 2016;5(7):1-10.