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Original Research Article

Correlation of serum Nesfatin 1 level with metabolic and clinical parameters in Indian women with and without polycystic ovarian syndrome

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ABSTRACT

Background: Objective of the study was to compare serum Nesfatin 1 levels in Indian women with and without polycystic ovarian syndrome (PCOS) and to evaluate the association of serum Nesfatin 1 with metabolic and clinical parameters.
Methods: 40 PCOS and 40 age and body mass index (BMI) matched non PCOS controls were enrolled. Comparison of hormonal (serum Nesfatin 1, anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, and estadiol) metabolic (blood pressure, fasting and post prandial blood glucose and insulin, HOMA-IR) and clinical (acne, acanthosis nigricans and hirsutism) parameters was done between two groups. Student's t test, Mann Whitney test, Pearson's correlation test was used. P value of <0.05 was considered statistically significant.
Results: There was a significant difference in levels of serum Nesfatin 1 in PCOS subjects and controls (8.6 ng/ml versus 0.75 ng/ml, p<0.01). Positive correlation was present between serum nesfatin 1 level and post prandial plasma glucose (r=0.009; p<0.009). A positive correlation was also present between serum Nesfatin 1 levels and AMH (r=0.512; p<0.01). No correlation was found between serum Nesfatin 1 and other endocrine, cardiovascular and metabolic parameters. Serum LH levels, LH/FSH ratio, post prandial plasma glucose and post prandial insulin were significantly higher (p<0.05) in PCOS subjects compared to controls.
Conclusions: Nesfatin 1 levels were ten times higher in PCOS subjects compared to controls irrespective of age and BMI. A positive correlation was observed between serum Nesfatin 1 and post prandial plasma glucose levels which indicates Nesfatin 1 may be a reliable marker of PCOS suggesting energy homeostasis imbalance in these women.

Keywords: Polycystic ovarian syndrome, Nesfatin 1, Energy homeostasis, New marker

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is characterized by ovulatory dysfunction, hyperandrogenism and polycystic ovaries. The pathophysiology of the PCOS is proposed to be associated with multiple metabolic alterations including deranged energy metabolism. The metabolic array of derangement includes impaired glucose tolerance, insulin resistance, hyperinsulinemia, metabolic syndrome, type 2 diabetes, dyslipidemia, obesity and cardiovascular disorders. The risks also include endometrial cancer and the psychological impacts.[1] The growing incidence of PCOS and the association of the same with the wide array of metabolic derangements drives one's curiosity towards the common pathophysiology.

Nesfatin 1 is an anorexogenic peptide [adipokine] which is synthesized from nucleobindin in the hypothalamus and the periphery and regulates energy metabolism, glucose homeostasis, appetite and eating behavior. Nesfatin-1 has been identified to have a glucose-dependent insulinotropic effect and also regulates ovarian function.[2-5] Recently Nesfatin-1 is gaining interest among researchers as an effective therapeutic agent for various diseases such as obesity, diabetes mellitus and PCOS.[6,7] Whereas few studies found that level of Nesfatin 1 is higher in women with PCOS, other studies found it to be lower.[8,9] As there is scanty evidence regarding the role of Nesfatin 1 in PCOS, this is the first Indian study planned to evaluate the association of serum Nesfatin 1 level with metabolic and clinical parameters in Indian women with and without PCOS.

METHODS

This study was conducted in the department of obstetrics and gynecology in collaboration with department of biochemistry of a teaching institute attached to a tertiary care hospital, Lady Hardinge Medical College, from December 2015 to February 2017. This case control study included 40 women with PCOS and 40 age and BMI matched controls after taking approval from the ethics committee of human research of the institute. This research was carried out in accordance with the principles defined in ICH-GCP E6 guidelines, "ethical guidelines for biochemical research on human participation (2006)", Indian Council of Medical Research (2001), "guidelines for good clinical practices (GCP) for Clinical Research India (2001), and Central Drug Standard Control Organization (CDSCO). Written informed consent was taken from all participants of the study in both Hindi and English.

Inclusion criteria

Cases

Women of reproductive age group diagnosed as PCOS by Rotterdam criteria [1] with any two of the three features including oligo/anovulation, clinical or biochemical signs of hyperandrogenism, polycystic ovaries on ultrasound defined as the presence of ≥12 follicles, of 2–9 mm size or having the unilateral ovarian volume of ≥10 cm.[3] Ovarian volume was calculated by the formula [0.5×ovarian length×thickness×width].

Controls

Age and BMI matched women of reproductive age group without PCOS.

Exclusion criteria

Women taking medical treatment for dyslipidemia, diabetes, insulin resistance, hypertension or any other chronic medical disorders.

Detailed history was taken and complete physical examination was performed. Signs of clinical hyperandrogenism such as the Ferriman Gallaway score, acne or acanthosis nigricans were noted.[1]

On day 2 or 3 of menstrual cycle, peripheral venous blood samples were collected at 9 A.M for hormone measurements after 8-10 hours of fasting. Blood was centrifuged and serum was stored at -800C until analysis. Sera were then thawed and analysed for FSH, LH, testosterone, estradiol, prolactin, progesterone, TSH, serum (S.) insulin. Serum insulin and TSH were measured by chemluminocence method by using (Becman Coulter). FSH, LH, prolactin progesterone, testosterone and E2 were measured by automated immuliteelectrochemi-luminoscence methods by using (Cobas E 411, HITACHI). The estimation of serum Nesfatin 1 was done by the ELISA kit for human Nesfatin 1 levels (SINCERE^{TM} Cat. No: E13651285). Transvaginal ultrasound was done to evaluate endometrial thickness, number of follicles and volume of the ovaries.

PCOS subjects were categorized into four phenotypes based on clinical and ultrasound findings. Phenotype A (O+P+H) comprised of women with oligo/anovulation (O), polycystic ovaries (P) and hyperandrogenism (H); phenotype B (O+H) having women with oligo/anovulation(O) and hyperandrogenism; phenotype C (P+HA) included women with polycystic ovaries (P) and hyperandrogenism and phenotype D (O+P) comprised of women with oligo/anovulation (O) and polycystic ovaries (P).

Sample size was calculated by comparing mean of two groups from a previous published study[5] assuming alpha error to be 0.05, beta error to be 0.20, mean of Nesfatin 1 group in PCOS patient to be 10.2+5 and mean of Nesfatin 1 in non-PCOS group to be 6.5+2.9 the sample size was calculated to be 40 for each group.

Statistical analysis

Analysis was done on statistical package for the social sciences (SPSS) 16.0. Student's t test was used to compare quantitative data, Mann Whitney test to compare non parametric data. Pearson's correlation test was used to correlate Nesfatin 1 level with clinical and metabolic parameters. A value of <0.05 was considered as statistically significant.

RESULTS

The women in the PCOS group and control group were matched for age and BMI [26.7 years versus 27.1 years (p=0.66), BMI=25.1 kg/m^2 versus 24 kg/m^2 (p=0.28) respectively]. The mean waist hip ratio [0.84 versus 0.82 (p=0.12) respectively] and cardiovascular parameters were found to be comparable in both PCOS and controls. Clinical features of women with PCOS and controls is depicted in table 1which show significantly higher

incidence of acne, hirsuitism, acanthosis nigricans and menstrual irregularity in cases compared to controls. Ferriman Gallway score was also significantly higher in PCOS subjects.

Table 2 describes the metabolic parameters of cases and controls. Statistically, significant difference was seen in the values of post prandial serum insulin, blood glucose and serum Nesfatin 1 levels in cases and controls.

Table 3 reveals the endocrine profile of women with and without PCOS. Levels of serum LH, LH: FSH ratio and AMH were significantly higher in cases versus controls ($p<0.05$).

Table 4 shows correlation between Nesfatin 1 and various endocrine and metabolic parameters. Serum nesfatin 1 correlated positively with post prandial plasma glucose and serum AMH levels. It did not show significant correlation with other parameters.

Table 1: Comparison of clinical parameters in PCOS and controls.

Parameter	PCOS (n=40)	Controls (n=40)	P value
Acne	16 (40%)	6 (15%)	<0.001
Acanthosis nigricans	5 (12.5%)	0 (0%)	<0.001
Hirsutism (FG score)	7.45±2.71	5.7±1.14	<0.001
Menstrual irregularity	37 (92.5%)	2 (5%)	<0.001
Ferriman Gallway score	8.45±2.71	5.7±1.14	<0.01

Table 2: Comparison of metabolic parameters in PCOS and controls.

Parameters	Mean in PCOS [n=40]	Mean in controls [n=40]	P value
BMI (kg/m$^{2)}$	25.07±0.56	27.1±0.62	0.28
Waist hip ratio	0.84±0.01	0.82±0.01	0.12
Fasting plasma glucose (mg/dl)	95.92±1.99	93.05±1.3	0.2
Plasma glucose post 75 gm load (mg/dl)*	130.72±2.9	120±2.72	0.02
Cholesterol (mg/dl)	190.75±6.28	202.05±5.57	0.18
Triglyceride(mg/dl)	139.15±6.1	144.68±5.2	0.49
HDL (mg/dl)	40.65±0.883	39.86±0.61	0.46
Insulin fasting (mIU/ml)	11.68±0.93	9.69±0.92	0.13
Insulin post 75 gm glucose load (mIU/ml)*	64.36±12.83	28.43±2.4	0.007
Nesfatin (ng/ml)*	8.6±4.23	0.75±0.76	<0.001

*P value<0.05

Table 3: Comparison of endocrine profile in PCOS and controls.

Parameters	Case (n=40)	Control (n=40)	P value
FSH (IU/l)	7.38±4.9	7.21±2.83	0.85
LH (IU/l)	9.02±5.79	6.89±3.02	0.04
LH/FSH ratio	1.39±0.83	1.01±0.48	0.02
Estradiol (pg/ml)	63.29±33.78	51.65±29.87	0.12
Prolactin (ng/dl)	19.97±16.21	21.63±32.98	0.78
TSH (mIU/ml)	5.84±15.38	2.85±1.03	0.22
Testosterone (ng/dl)	34.96±20.55	29.62±10.99	0.15
AMH (ng/ml)	13.74±4.03	3.96±6.21	<0.01

Table 4: Correlation between Nesfatin 1 and other parameters.

Parameters	Pearson's r value	P value
LH	0.113	0.32
Testosterone	0.215	0.056
Fasting plasma glucose	0.075	0.508
Plasma glucose post 75 gm glucose load	0.289	0.009
BMI	0.086	0.449
WHR	0.046	0.687
Insulin fasting	0.024	0.832
Insulin post 75 gm glucose load	0.203	0.071

Continued.

Parameters	Pearson's r value	P value
HOMA-IR	0.032	0.78
AMH	0.512	<0.01

DISCUSSION

The subjects in study population were matched for age and BMI to avoid these from being a confounding factor in the various hormonal and metabolic parameters. The mean waist hip ratio was also similar for both groups, 0.84 for the PCOS group and 0.82 (p value=0.12) for the control group.

In this study, 40% PCOS women belonged to phenotype A, 7.5% to phenotype B, 5% had phenotype C,47.5% had phenotype D. Menstrual irregularities which were suggestive of oligo/anovulation were found in 92.5% of cases. Polycystic ovaries were seen in 75% of PCOS women. Hormonal profile of both the groups was similar except for serum LH (p<0.05), LH/FSH (p=0.02) and AMH (p<0.01) levels which were significantly higher in PCOS group. Thus, our study and the outcomes are more reflective of type D phenotype.

Systolic blood pressure, diastolic blood pressure and heart rate were studied as cardiovascular parameters and were found to be similar in PCOS and control groups. In few other studies also, no significant difference was found in blood pressure and heart rate of cases and controls.[10] However, contrary to the present study, in another study evaluating association of nesfatin 1 and vitamin D with cardiovascular profile of PCOS subjects, systolic blood pressure, diastolic blood pressure and heart rate were found to be significantly higher in case of PCOS compared to controls.[5] This difference may be because most women in the present study belonged to the phenotype D, which has mild metabolic derangements.

Fasting plasma glucose was similar in both groups but plasma glucose levels after 75 gm glucose load in PCOS group was significantly higher (p value=0.02). Mean HOMA-IR for women in PCOS group was found to be higher but the difference was not statistically significant. (p value=0.09) probably because majority of the patients belonged to phenotype D, which has least metabolic derangements.[11] Serum insulin in fasting state was comparable in PCOS and controls while the serum insulin post 75 gm glucose was significantly higher in PCOS compared to controls (p<0.007). Although PCOS is a multifactorial disease, insulin resistance plays a major role in its pathogenesis. In a fasting state, more insulin is secreted by the pancreas which is able to maintain glucose levels in normal range. After a glucose challenge, more insulin is secreted by the pancreas but this insulin may not be sufficiently high to keep blood sugar in normal range. This is reflected as higher glucose/deranged GTT and insulin levels after glucose challenge. Majority of our study population; including the PCOS group was lean which explains the normal fasting glucose and insulin levels but deranged post load glucose and insulin levels.

The lipid profile was evaluated in study population as PCOS falls in array of metabolic derangements. However, all parameters of lipid profile were found to be similar in PCOS group and controls because patients were matched for BMI. Like in the present study, some other studies found no significant difference in lipid profile of cases and controls.[12]

Significant difference was found between the levels of Nesfatin 1 in PCOS group and controls. Serum Nesfatin 1 levels were significantly higher in cases compared to controls. The mean value of Nesfatin 1 levels in cases was 8.6±4.28 ng/ml and in controls it was 0.75±0.76 ng/ml (p value<0.01). Nesfatin 1 was higher in PCOS group irrespective of phenotype or any other parameter. A significant positive correlation was found between serum Nesfatin 1 levels with post load plasma glucose and AMH. However, no significant correlation was found between serum Nesfatin 1 levels and other parameters in the present study.

Sahin et al also found significantly higher levels of Nesfatin 1 in subjects of PCOS compared to controls.[5] Similar to the present study, they found no correlation between Nesfatin1 and HDL, DHEA-S, estrogen, prolactin, TSH. However, contrary to our study, a positive correlation was found between serum Nesfatin 1 and total testosterone, LDL, triglyceride and cholesterol.

In another study by Ademoglu et al, serum Nesfatin 1 levels were significantly higher in PCOS group compared to controls.[8] Nesfatin 1 did not correlate with fasting blood glucose or HOMA-IR like in the present study.

Binnetoglo et al conducted a study on plasma levels of Nesfatin 1 in patients with PCOS.[12] In their study no significant difference was found between the levels of serum Nesfatin 1 in cases and controls. Similar to the study, no correlation was found between serum Nesfatin 1 and BMI, waist hip ratio, lipid profile, LH, FSH, testosterone levels.

Another study, evaluated the levels of Nesfatin 1 and other hormones in patients of PCOS.[13] In their study, levels of serum Nesfatin 1 were lower in PCOS group compared to controls.

Conflicting levels of Nesfatin 1 were associated with PCOS in different studies. This could be attributed to the variation in the population recruited depending on their genetic and metabolic characteristics. As Indians have high insulin resistance and are more prone to develop

glucose intolerance and diabetes, the value of Nesfatin 1 was found to be significantly higher in Indian women with PCOS.

Limitations

Increasing the sample size of the study could further add to the weightage of the study.

CONCLUSION

To conclude, serum Nesfatin 1 levels were ten times higher in PCOS subjects compared to their age and BMI matched controls in this study. There was a positive correlation between serum Nesfatin 1 and post prandial blood plasma glucose levels. The serum Nesfatin 1 levels of PCOS subjects were high irrespective of other metabolic, cardiovascular and endocrine parameters. This proposes that Nesfatin 1 levels may play an important role in glucose metabolism and insulin resistance which in turn affect various parameters in PCOS. This is the first Indian study to evaluate Nesfatin 1 levels in women with PCOS. High Nesfatin 1 level in PCOS subjects has a role in energy homeostasis and may play a crucial role in pathogenesis of PCOS which indicates Nesfatin 1 may be a reliable marker of PCOS suggesting energy homeostasis imbalance in these women. The positive correlation of Nesfatin 1 with AMH indicates that Nesfatin 1 affects the endocrine system.

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REFERENCES

1. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41-7.
2. Yamada M, Horiguchi K, Umezawa R. Troglitazone, a ligand of peroxisome proliferator-activated receptor-{gamma}, stabilizes NUCB2 (Nesfatin) mrna by activating the ERK1/2 pathway: isolation and characterization of the human NUCB2 gene. Endocrinology. 2010;151(6): 2494-503.
3. Bonnet MS, Pecchi E. Central nesfatin-1-expressing neurons are sensitive to peripheral inflammatory stimulus. J Neuroinflammation. 2009;6:27.
4. Dore R, Levata L, Lehnert H, Schulz C. Nesfatin-1: Functions and physiology of a novel regulatory peptide. J Endocrinol. 2017;232:R45-65.
5. Sahin FK, Sahin SB, Ural UM, Cure MC, Senturk S, Tekin YB, Balik G, Cure E, Yuce S, Kirbas A. Nesfatin-1 and vitamin D levels may be associated with systolic and diastolic blood pressure values and hearth rate in polycystic ovary syndrome. Bosn J Basic Med Sci. 2015;15(3):57-63.
6. Mierzyński R, Poniedziałek-Czajkowska E, Dłuski D, Patro-Małysza J, Kimber-Trojnar Ż, Majsterek M, Leszczyńska-Gorzelak B. Nesfatin-1 and Vaspin as Potential Novel Biomarkers for the Prediction and Early Diagnosis of Gestational Diabetes Mellitus. Int J Mol Sci. 2019;20(1):159.
7. Öztürk Özkan G. Effects of Nesfatin-1 on Food Intake and Hyperglycemia. J Am Coll Nutr. 2020;39(4):345-51.
8. Ademoglu EN, Gorar S, Carlıoglu A, Yazıcı H, Dellal FD, Berberoglu Z, et al. Plasma nesfatin-1 levels are increased in patients with polycystic ovary syndrome. J Endocrinol Invest. 2014;37(8):715719.
9. Catak Z, Yavuzkir S, Kocdemir E, Ugur K, Yardim M, Sahin İ, Agirbas EP, Aydin S. NUCB2/Nesfatin-1 in the Blood and Follicular Fluid in Patients with Polycystic Ovary Syndrome and Poor Ovarian Response. J Reprod Infertil. 2019;20(4):225-30.
10. Saxena P, Singh S, Bhattacharjee J. Endocrine and Metabolic Profile of Different Phenotypes of Polycystic Ovarian Syndrome. J Obstet Gynaecol India. 2016;66(1):560-6.
11. Kar S. Anthropometric, clinical & metabolic comparison of four Rotterdam PCOS phenotypes—a prospective study. J Hum Reprod Sci. 2013;6(3):194-200.
12. Binnetoğlu E, Erbağ G, Gencer M, Turkön H, Mehmetaşik, Güneş H, Sen H, Ahmetvural, Kubilayukin, Plasma levels of nesfatin-1 in patients with polycystic ovary syndrome. Acta Medica Mediterranea. 2014;30:201-4.
13. Deniz R, Gurates B, Aydin S. Nesfatin-1 and other hormone alterations in polycystic ovary syndrome. Endocrine. 2012;42(33):694-9.

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