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Evaluation of Serum Nesfatin-1 Levels in Women with Polycystic Ovarian Syndrome and Estimating Its Correlation with Body Mass Index and Clinico-metabolic Profile

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is a diverse condition marked by irregular menstruation, hyperandrogenism, and recurrent anovulation. An incidence of 6% to 20% of PCOS in women of reproductive age has been reported. Nesfatin-1 is a potent anorexigenic peptide having antihyperglycemic effects and is associated with energy balance and homeostasis, glucose metabolism, obesity, and probably gonadal functions. Nesfatin-1 is related to obesity, insulin resistance, and appetite. Nesfatin-1 is associated with insulin resistance, body mass index, diabetic inflammatory stimulation, hypertension, and PCOS. This study aims to evaluate the serum Nesfatin-1 levels in women with Polycystic Ovarian Syndrome and correlate with Body Mass Index, clinical and metabolic profile.

Materials and Methods: This will be a prospective Hospital-based observational study conducted at AVBRH in the Department of Obstetrics and Gynecology. A total of 96 women of reproductive age (15-45 years) will be enrolled. Detailed history of the menstrual cycle, obstetric history, background, medical and family history, and any primary care and inquiries will be documented. The general and systemic examination will be done to note any clinical evidence of

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hyperandrogenisms like acne, alopecia, acanthosis nigricans or hirsutism. The Ferriman-Gallwey Score (FGS) will be calculated. The ELISA method will be used for the calculation of N1. Serum Nesfatin-1 values will be correlated with clinical and metabolic profiles divided in the lean versus obese PCOS group. Data will be entered into a predetermined, pretested proforma and analyzed with appropriate statistical tests.

Expected Results: The levels of Serum Nesfatin in PCOS patients are expected to be abnormal. We will measure sensitivity, specificity, positive and negative predictive value, and efficacy. We will analyze the difference of results between lean PCOS and Obese PCOS and association with the clinico-metabolic profile.

Keywords: Polycystic ovarian syndrome; nesfatin-1; obesity; insulin resistance; anovulation; oligo-ovulation; dysmenorrhoea.

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a diverse condition marked by irregular menstruation, hyperandrogenism, and recurrent anovulation. Various studies have quoted an incidence of 6% to 20% of PCOS in women of reproductive age group [1]. Symptoms of PCOS usually occur during the early puberty years. Irregular menstrual cycles, anovulation, and acne are characteristics of both average pubertal growth and PCOS. It is challenging to know the inciting factors due to intricate interwoven pathophysiology. Numerous pathways can be associated with the pathophysiology of PCOS. It is believed that the syndrome is defined by complex correlations between hypothalamic-pituitary-ovarian or hypothalamic-pituitary-adrenal axis activities and metabolic disturbances, such as insulin resistance (IR), obesity, and compensatory hyperinsulinemia [2].

Rotterdam criteria are commonly used for PCOS diagnosis, involving at least 2 of the following criteria: chronic anovulation, hyperandrogenism, and polycystic ovaries [3]. Young adults with symptoms of androgen overload and amenorrhea/oligomenorrhea are considered at risk for PCOS" well until a conclusive diagnosis of PCOS is established. The management includes educational interventions, healthy living, and therapeutic interventions that target their symptoms [4].

As a meticulously synchronized and tightly regulated network, the female HPO is the main pathway liable for species reproduction and competence of reproduction [1]. The hormonal and neural stimuli (external stimuli) and internal signals generate responses from the HPO axis. The chronic hyperandrogenism observed in PCOS is associated with abnormal hypothalamic-pituitary feedback, LH hypersecretion, premature granulosa cell luteinization, aberrant oocyte maturation, and premature arrest of activated primary follicles [5].

In women with PCOS, increased insulin levels and resistance to insulin (IR) are commonly observed, and about 40 to 60% of PCOS women are overweight or obese [6]. A correlation between insulin levels and PCOS severity also appears to exist [7]. Nesfatin-1 is a potent anorexigenic peptide having antihyperglycemic effects and is associated with energy balance and homeostasis, glucose metabolism, obesity, and probably gonadal functions [8]. Nesfatin 1 (N1) is a nucleobindin2 (NUCB2) derivative that is encoded by the NUCB2 gene. It has 82 amino acids and is a recently discovered peptide [9]. Nesfatin-1 is formed in the brain stem, forebrain, midbrain nuclei, pituitary, hypothalamus, peripheral adipose tissue, gastric mucosa, pancreatic endocrine beta cells, etc [10]. Nesfatin-1 is related to obesity, insulin resistance and appetite and it is found serum levels of Nesfatin-1 in gestational DM patients and type 2 diabetes mellitus are low. Several studies have shown that N1 is associated with insulin resistance, body mass index (BMI), diabetic inflammatory stimulation, hypertension, and PCOS [5]. Regulation of the reproductive system, such as the initiation of puberty and gonadotropin secretion, can also be impaired [7].

The actions of Nesfatin-1 on obesity, energy dynamics, glucose metabolism, and low delayed puberty-related levels indicate that Nesfatin-1 may be important for ovarian function and reproduction under normal and pathological conditions [5]. Studies have suggested that ovarian estrogen and progesterone may regulate the pituitary expression of Nesfatin-1 [5].

A microenvironment made up of several regulatory proteins, hormones and metabolites

provides the follicular fluid that supports oocyte development and maturation [11-15]. The results of FF incorporation on oocyte maturation have suggested that Nesfatin-1 levels could be linked with ovarian dysfunction in PCOS patients, such as oligo/anovulation, polycystic ovaries, etc [10]. Some researchers investigated the role of Nesfatin-1 in the production of PCOS, and an increased level of Nesfatin-1 was intimately connected with the development of PCOS [10]. In contrast, others have measured lower levels of Nesfatin-1 that may contribute to PCOS development [16]. Therefore, there are contrasting findings with respect to serum Nesfatin-1 levels in PCOS [17]. Thus, the present study is being planned to study the serum Nesfatin-1 levels in diagnosed cases of polycystic ovarian syndrome and evaluate its association in the said disorder.

1.1 Rationale

Nesfatin 1- has been shown to regulate appetite and energy homeostasis and has an association with insulin regulation and glucose metabolism, which has a pivotal role in the development of PCOS [28-21]. However, dilemma exists in its specific role. It has been shown to suppress feeding and increase insulin secretion by pancreatic beta islet cells, independent of the leptin pathway. As a novel therapeutic agent, nesfatin-1 is now illuminated, particularly for the treatment of obesity and diabetes mellitus [22-26]. Once we evaluate the role of Nesfatin 1 in PCOS we may pave a way to use Nesfatin- 1 as a therapeutic intervention in PCOS.

1.2 Aim

This study aims to evaluate serum Nesfatin-1 levels in women with Polycystic Ovarian Syndrome and correlate with Body Mass Index, clinical and metabolic profile.

1.3 Objectives

1. To study clinical and metabolic profile in women with PCOS.
2. To study the serum levels of Nesfatin 1 in women with PCOS.
3. To correlate Nesfatin levels with clinical and metabolic profile.
4. To compare the serum Nesfatin 1 levels in the lean versus obese PCOS group.

2. METHODOLOGY

Nesfatin 1 kit will be purchased with the help of intramural institutional grant and the study subject will not have to bear the expenses of this test.

2.1 Research Question

1) Are Nesfatin-1 serum levels abnormal in women with polycystic ovarian syndrome?
2) Is the difference significant in the Serum levels of Nesfatin 1 in lean versus obese women with polycystic ovarian syndrome?
3) Is there a correlation between Nesfatin levels and clinico-metabolic profile?

2.2 Study Design

Prospective Hospital Based Observational Study.

2.2.1 Duration of study

2 years.

2.3 Study Site

Department of Obstetrics & Gynecology, AVBRH, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha.

2.4 Study Population

Women of reproductive age group (15-45 years) seeking care at an outpatient unit of Obstetrics &Gynecology, in the, AVBRH hospital, Sawangi, Meghe, Wardha fulfilling inclusion and exclusion criteria study.

2.5 Sample Size

PCOS prevalence in India ranges from 3.7% to 22.5 % (2) .The sample size is estimated based on the observation that 8-10 % of women of reproductive age group at study hospital, visiting outpatient OBGY unit will be diagnosed with PCOS. Following the 95% confidence interval assumption with a 6 % margin of error, the sample size was calculated using the sample size formula.

$n=z^2 1-\alpha/2 \times P \times (1-P)/d^2$
where $z^2 1-\alpha$ is the level of significance at 95 % confidence interval =1.96
P= 10 %=0.1
D= desired error of margin=5%=0.06

Power of the test=80 %
$n = 1.96^2 x 0.1x(1-0.1)/0.06^2$
=96.04

A sample size of 96 will be considered in the study.

2.6 Inclusion Criteria

PCOS will be diagnosed according to criteria defined by the PCOS Consensus Workshop Group in Rotterdam, commonly known as the Rotterdam criteria, sponsored by the Rotterdam European Society for Human Reproduction and the American Society for Reproductive Medicine [3].

The criteria require at least 2 of the following 3 to label a woman as PCOS.

1) Anovulation and oligo-ovulation, defined by the presence of amenorrhea or oligomenorrhea.
2) The presence of at least one of the three features suggestive of clinical hyperandrogenism: acne, hirsutism, and androgenic alopecia, or Biochemical hyperandrogenism, identified as >60 ng/dL (>2.08 nmol/L) serum testosterone level.
3) Either of the ovaries containing follicles more than 12 in number or measuring 2-9mm in diameter and having volume >10ml measured ultrasonographically.

Thus, Inclusion criteria for study would be

1. Consenting women of reproductive age group (15-45 years).
2. Women with PCOS diagnosis according to Rotterdam criteria.

2.7 Exclusion Criteria

1. Pregnant Women.
2. Women with the following endocrine disorders: Congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, 21-hydroxylase deficiency, hyperprolactinemia, diabetes, and a history of gestational diabetes.
3. Women of chronic conditions such as chronic kidney failure, cardiovascular, hepatic, hematologic, hypertension, and cancer.
4. Women using oral contraceptives pills, antiandrogens, glucocorticoids, antihypertensives, antidiabetics, and anti-obesity drugs.
5. Women who smoke cigarettes, chew tobacco, or consume alcohol.
6. Women using nutritional supplements.

3. METHODS

Women fulfilling selection criteria will be explained about the nature of the study, and written informed consent in the local vernacular language (Marathi) will be obtained on a structured proforma before enrollment. Women willing to participate will be informed about the Biochemical test (Serum Nesfatin-1) and PCOS with the help of an information sheet that will be provided to them.

The study subjects will be recruited from the women visiting gynecology clinics and presenting with menstrual irregularities like irregular menses, delayed menses, hypomenorrhea (reduced menstrual bleeding), weight gain, excessive hair growth on the body, acne, alopecia, etc. History and clinical examination of these women will be carried out to note whether they fulfill the study criteria, and accordingly, they will be further evaluated. Demographic details will be gathered, including age (years), schooling, profession, residential area, marital status, menarche age, last menstrual period, gravity, parity, abortion, number of live children, regularity of the menstrual cycle, etc. A detailed history of the menstrual cycle, obstetric history, background, medical and family history, and any primary care and inquiries will be documented, and people with chronic diseases such as hypertension, diabetes, renal diseases, and malignancies will be omitted from the study. The general and systemic examination will note any clinical evidence of hyperandrogenisms like acne, alopecia, acanthosis nigricans, or hirsutism.

After a general physical and gynecological examination, the Ferriman-Gallwey Score (FGS), height (cm), weight (kg), and waist circumference (WC) (cm) would be calculated. BMI will be calculated according to BMI=body weight (kg)/square height (m2). Women with BMI <23 (kg)/square height (m^2) will be classified as lean PCOS, and those with BMI >=23 (kg)/square height (m^2) will be considered as obese PCOS.

In 11 areas, the FGS system will be used to test hair development. The lack of terminal hair growth will be graded as zero, and optimal

growth will be rated as 4+. It would consider a cumulative score of 8 or higher as hirsutism.

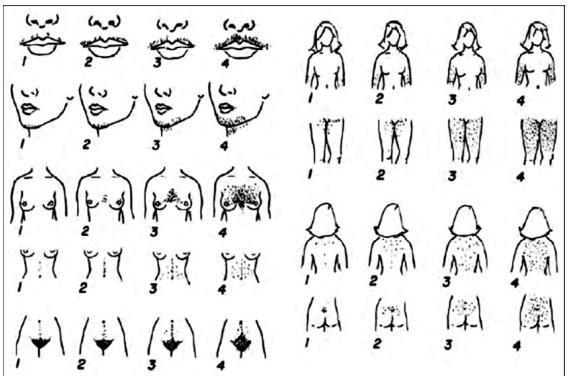

Fig. 1. Modified Ferriman-Gallwey Score

The size of the uterus (mm), the myometrial structure, the thickness of the endometrium (mm), the size of the ovary (mm), the number of follicles (number), and their diameter (mm) will be calculated. PCOS will be diagnosed according to Rotterdam criteria [3].

3.1 Clinical, Biochemical, and Hormonal Measurements

Morning blood venous samples will be withdrawn at around 9-10 is (fasting state) on day 3^{rd}-5^{th} of a spontaneous or progesterone-induced menstrual cycle.

The photometric assays of the Abbott Architect C16000 analyzer will be used to calculate the levels of fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and low density lipoprotein cholesterol (LDL). Fasting serum insulin (FSI), FSH, luteinizing hormone (LH), prolactin (PRL), dehydroepiandrosterone sulfate (DHEAS), total testosterone (TT) and thyroid stimulating hormone (TSH) serum levels will be calculated using the Abbott Architect i2000 system of chemiluminescent microparticle enzyme immunoassay (CMIA). The radioimmunoassay system will be used to assess serum 17-hydroxyprogesterone (17-OHP) and free testosterone (FT) [5].

All study subjects will undergo a 75g oral glucose tolerance test (OGTT). The following formula will be used to measure the Homeostasis Model Evaluation as an insulin resistance index (HOMA-IR). [9]: HOMA-IR = FPG (mmol/L) × FSI (mU/mL)/22.5.

3.2 Measurement of Nesfatin 1 (N1)

The ELISA method will be used for the calculation of N1. We will use the commercially available human N1 ELISA kit (Biotechnica Info lab, Bangalore). The ELISA process protocol will be performed in compliance with the instructions given by the manufacturer. Absorbance will be measured using the ELISA reader at a wavelength of 450 µm and the N1 levels will be reported as pg/mL. For the nesfatin-1 assay, the detection limit (LOD) would be 0.25ng/mL.

Data will be entered into a predetermined, pretested proforma. Study subjects will be classified as Lean PCOS and Obese PCOS for analysis. Further management of patient will be done without any intervention from study and according to expertise and discretion of consulting Gynaecologist.

3.3 Statistical Analysis

Data will be entered into the spreadsheet and analysed using version 18 of SPSS Program. The outcomes will be represented as mean ± SD. For naturally distributed data, the independent sample t-test will be used and the Mann-Whitney U test will be used to compare continuous variables for abnormally distributed data, while the Chi-square test will be used to compare categorical variables such as FGS. To measure the associations between variables, the correlation test by Pearson will be used. For subgroup analysis, a two-way Anova test would be used. A *p* value of less than 0.05 will be considered as statistically significant.

4. EXPECTED OUTCOME/RESULTS

The levels of Serum Nesfatin in PCOS patients are expected to be abnormal. We will measure sensitivity, specificity, positive and negative predictive value and efficacy. We will analyze the difference of results between lean PCOS and Obese PCOS and association with clinico-metabolic profile.

5. DISCUSSION

Medical or metabolic hyperandrogenism, amenorrhea or oligomenorrhea combined with chronic anovulation are the classical characteristics of PCOS [1]. The current opinion is that it is deemed acceptable to use the Rotterdam criterion for adult women [3].

PCOS diagnosed women have inherent Insulin Resistance regardless of the degree of obesity and the severity of androgen levels [5]. IR is manifested also by lean PCOS women; increasing body mass index (BMI) amplifies IR. In contrast to normal-weight girls, normal-weight adolescent girls with PCOS have IR peripherally, elevated liver fat, and dysfunctional muscle mitochondria [4].

Latest research has shown that when delivered peripherally, nesfatin-1 decreases food consumption in rodents. The presence of nesfatin-1 in the nervous system and in peripheral tissues, including pancreatic beta cells, suggests that nesfatin-1 could be implicated in the regulation of the release of insulin from pancreatic beta cells [5]. It can also be concluded that Nesfatin-1 and PCOS have a partnership depending upon the knowledge that there exists a similar relationship of Nesfatin 1 or PCOS with BMI, obesity and IR. There is only one literature review on nesfatin-1 levels in patients with PCOS where circulating nesfatin-1 levels have been found to be lower in patients compared to controls. In the above stated research, it was concluded that they did not know if the decline in levels of nesfatin-1 was caused by IR or was attributed to other biochemical factors. They speculated that lower levels of nesfatin-1 can play a role in PCOS development [9].

The assessment of circulating Nesfatin-1 is alleged to support the possible role of this peptide as a peripheral follicular well-being marker. Nesfatin-1 could also be implicated in our underestimation of the uncertain facets of follicular growth in PCOS patients [10,9]. Another explanation for lowered levels of nesfatin-1 may be the substantially greater hyperinsulinemia observed in PCOS cases. In mouse islet beta-cells, nesfatin-1 increases glucose-induced insulin secretion by encouraging Ca2+ influx through L-type channels [9].

Despite peripheral IR, insulin causes hyperandrogenaemia through increasing androgen production through its action on ovary theca cells through an insulin-like growth factor (IGF-1). This is one of the main pathways contributing to the production of PCOS [5]. The fact that despite the peripheral IR in PCOS, insulin induces ovarian effects means that insulin will operate in different organs through other receptors or secondary precursors. The signal system of nesfatin-1 in the ovarian tissue may contain both ligand and receptor components, N1 might have a role in both normal and abnormal conditions of ovary. Low levels of nesfatin-1 in women with PCOS by their effect on the extremely responsive hypothalamic-pituitary-gonadal axis may be implicated in the development of the syndrome [9]. A number of related studies were reported [27-29]. Jungar et. al. reported on clinical picture of PCOS patients in a peri urban tertiary care hospital of Central India [30]. Deshpande and Phatak reported a rare case of bilateral multiple ovarian dermoids with uterine fibroid and ectopic kidney [31]. Related studies on obesity and related aspects were also reported by Wagh et. al. [32], Acharya et. al. [33] and Sawal et. al [34].

6. CONCLUSION

Conclusion will be drawn after obtained result.

CONSENT AND ETHICAL APPROVAL

Ethical approval from the Institutional Ethics Committee (IEC) and written informed consent from all registered women will be obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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