



Nesfatin-1, Dopamine, and NADPH levels in Infertile Women with Polycystic Ovary Syndrome: Is There a Relationship Between Their Levels and Metabolic and Hormonal Variables

Enas Ahmed Hamed ^{1*}, Hayam Gaber Sayyed ¹, Ahmed Mohamed Abbas ², Mohamed Maher Abdel Gaber ³, Hassnaa Mahmoud Abd El Aleem ¹

1- Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt

2- Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Assiut, Egypt

3- Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

Abstract

Background: Polycystic ovary syndrome (PCOS) is commonest endocrine disease occurring in women of reproductive age. This study conducted to clarify altered concentrations of Nesfatin-1, nicotinamide adenine dinucleotide phosphate (NADPH), and dopamine in PCOS women and controls. Also, to assess their role in PCOS pathophysiology and their correlation with measured biochemical parameters.

Methods: In this observational study, 60 PCOS patients and 24 controls included. Medical history was recorded and full examinations were done. Serum concentrations of lipid profile, fasting blood glucose (FBG), fasting insulin (FSI), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, testosterone, progesterone, estradiol, Nesfatin-1, dopamine, and NADPH were measured by ELISA kits. Values were analyzed using unpaired t-test and Pearson Chi-square test. The $p < 0.05$ was considered statistically significant.

Results: In this study, there was significantly elevated waist hip ratio (WHR) and body mass index (BMI) in PCOS patients versus controls ($p < 0.0001$ and $p = 0.014$). There was significant increase in FSH, LH, prolactin, estradiol, testosterone, Nesfatin-1, and dopamine ($p = 0.021$, $p = 0.015$, $p < 0.0001$, $p < 0.0001$, $p = 0.006$, $p = 0.017$, $p < 0.0001$) and decrease of NADPH ($p < 0.0001$) in PCOS patients. There were significant positive correlations between Nesfatin-1, prolactin, and dopamine levels. Also, there was significant positive correlation between dopamine and BMI, FSI, FSH, LH, estradiol, and prolactin levels; however, significant negative correlations observed between NADPH and BMI, FSI, estradiol, and prolactin levels.

Conclusion: Elevated serum Nesfatin-1 concentrations and their association with hyperprolactinemia indicate that they have a role in PCOS pathophysiology. Moreover, elevated dopamine and decreased NADPH concentrations could play role in PCOS pathogenesis.

Keywords: Dopamine, Infertility, Nesfatin-1, Nicotinamide adenine dinucleotide phosphate, Polycystic ovary syndrome, Prolactin.

To cite this article: Ahmed Hamed E, Gaber Sayyed H, Abbas AM, Maher Abdel Gaber M, Mahmoud Abd El Aleem H. Nesfatin-1, Dopamine, and NADPH levels in Infertile Women with Polycystic Ovary Syndrome: Is There a Relationship Between Their Levels and Metabolic and Hormonal Variables. *J Reprod Infertil.* 2022;23(3):160-168. <https://doi.org/10.18502/jri.v23i3.10006>.

Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder of women at reproductive age occurring among 5–15% of

female population. It is characterized by hyperandrogenemia, irregular ovulation, and decreased fertility and is associated with metabolic disorders

* Corresponding Author:
Enas Ahmad Hamed,
Professor, Department of
Medical Physiology, Assiut
University Hospital, Assiut,
Egypt
E-mail:
Eah3a2010@aun.edu.eg,
eah3a2010@yahoo.com

Received: Jun. 20, 2021
Accepted: Nov. 20, 2021

such as hyperinsulinemia, Insulin Resistance (IR), obesity, and chronic low-grade inflammation (1). Till now, PCOS pathophysiology is unclear. Specific genetic patterns that affect the formation, regulation, and actions of sex hormones and insulin receptors seem to contribute to disease progression (2).

Nesfatin-1 is a newly detected peptide that originates from nucleobindin-2 (NUCB-2) precursors. It is primarily found in the central nervous system (CNS) (as cerebral cortex and hypothalamus) and then expressed in peripheral systems (pancreas, pituitary gland, adipose tissue, and gastrointestinal system) (3). Nesfatin-1 and its binding locations are expressed in reproductive organs of males (4) and females (5). Nesfatin-1 plays an essential role in regulating food intake, gastrointestinal motility, glucose homeostasis, arterial blood pressure, stress, and reproductive functions (6). In mice, the interaction of Nesfatin-1 with a putative G-coupled receptor promotes glucose-induced insulin production by boosting Ca^{2+} flow via L-type channels (7). In addition, Nesfatin-1 causes anorexia by reducing the number of meals and lengthening the time between meals (8). So, Nesfatin-1 concentrations in PCOS, which usually coexist with obesity and IR, were measured in previous research, and some studies found it to be low (9), while others found it to be high (10). Aside from the role of Nesfatin-1 in the metabolic process and its link to PCOS, it has been proven that excessive oxidative stress leads to the destruction of cellular structures and numerous metabolic activities, which could be linked to the genesis of a variety of disorders, including PCOS. Also, oxidative stress development may have an adverse effect on oocyte quality and maturation (11). Nicotinamide is essential for removing cellular reactive oxygen species (ROS) by peroxidase systems and glutathione reductase as it is needed for glutathione regeneration as a reducing equivalent. Nicotinamide adenine dinucleotide phosphate (NADPH) is generated from NADP^+ . The major origin of NADPH is pentose phosphate pathway and the enzyme of glucose-6-phosphate dehydrogenase. It is the most potent intracellular second messenger for Ca^{+2} release, serving as an electron carrier in a variety of processes by being alternatively oxidized NADP^+ and reduced NADPH (12). NADPH provides reducing equivalents for oxidation-reduction reaction involved in cellular protection against ROS toxicity, allowing regeneration of glutathione (GSH) (13). Dopamine (DA) is a cat-

echolamine neurotransmitter found in the brains and peripheral organs of many species (14). The physiological effects of DA are mediated through interactions with specific DA receptors, which are G protein-coupled receptor sites; there are five subtypes of receptors in mammals (15). Unlike NADPH, DA is a relatively unstable molecule. It undergoes auto-oxidation, which may attribute to defects of functions found in PCOS patients via ROS production, causing apoptosis of granulosa cells (GCs). Apoptosis is closely related to follicular atresia (16).

The aim of the present study was to identify the concentrations of Nesfatin-1, NADPH, and dopamine in PCOS infertile women. Also, an attempt was made to study correlations between their levels with other measured biochemical parameters in PCOS infertile women and controls.

Methods

Patients and Methods: This observational study was carried out in the inpatient and outpatient clinic of Assiut Women Health Hospital, Egypt, between June 2019 and June 2020. Patients recruited were definitely diagnosed with PCOS (17). The research was approved by the Institutional Review Board of Assiut University Hospital, Assiut, Egypt (approval no. 17100712) according to the Declaration of Helsinki. Informed consent was obtained from every participant before inclusion and after explaining the research aim to them.

Sample size calculation: Sample size was calculated based on the primary outcome "difference in mean Nesfatin-1 concentration between patients and controls" that was assessed by Deniz et al. (9). Nesfatin-1 concentration in the control group was 2.22 with standard deviation (SD) of 1.14. Considering a 50% reduction in mean Nesfatin-1 concentration in PCOS patients as significant, a sample size of 24 women in each group was needed with alpha error of 0.05 and 95% statistical power. In this study, 60 women with PCOS were included to obtain reliable results.

Subjects: In total, eighty-four women were included. Women were subdivided into two groups; the control group (n=24) included healthy fertile women recruited from the outpatient clinic of the aforementioned hospital. Healthy women included individuals who used no medications and had no specific manifestations, without any pathology based upon clinical examination and laboratory investigations.

PCOS group (n=60) included women with proven PCOS. Inclusion criteria of PCOS patients were age more than 18 and less than 40 years. PCOS diagnosis was based on 2003 ESHRE/ASRM diagnostic criteria (18). Exclusion criteria were the age of less than 18 years or more than 40, other endocrinology disorders as thyroid diseases, brain disorders as pituitary or brain masses or tumors, and chronic disorders as hepatic disease, renal, and hematologic disorders of patients. Women who used oral contraceptives, anti-androgenic chemicals, glucocorticoids, and anti-obesity drugs, as well as patients unwilling to participate in the study were also excluded.

Upon interview of all recruited women before the examination, their history including age (years), menarche age, marriage duration, date of last menstrual cycle, parity, abortions, gravidity, number of living children, and menstrual cycle regularity, history of previous treatments and comorbidities was recorded. Complete general, physical, and gynecological examinations were made. Measurement of weight (kilograms), height (meters), waist and hip circumference (centimeters), as well as vital signs as pulse rate and blood pressure were measured for all participants.

BMI was calculated. Waist circumference (WC) was measured midway between iliac crest and lowermost rib margin utilizing tape at the end of the normal expiration. Hip circumference (HC) was estimated around the point with the greatest circumference over buttocks utilizing tape, with feet fairly close together (about 12-15 cm apart); this position ensures equal weight distribution. Waist hip ratio (WHR) was calculated by dividing WC by HC.

Methodology: Five ml of overnight fasting venous blood samples were drawn from cubital vein on 3–5 days of follicular phase from each participant. The levels of triglyceride (TG) (19), total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) were evaluated in samples of fasting venous blood by routine laboratory investigations. Low density lipoprotein cholesterol (LDL-C) concentrations were calculated according to Friedewald equation:

$$\text{LDL-C} = \text{TC} - [\text{HDL-C} + \text{TG}/5].$$

Fasting glucose concentration, estradiol, progesterone, testosterone, prolactin, FSH, and LH were determined by routine laboratory investigations. Enzyme-linked immunosorbent assay (ELISA) kits were utilized to measure serum concentration

of fasting insulin (FSI) (Sinogeneclon Biotech Co., China, catalog number #SG-10161), Nesfatin-1 (Sinogeneclon Biotech Co., China., catalog number #SG-13208), dopamine (Elabscience, USA, catalog number #E-EL-H1231), and nicotinamide adenine dinucleotide phosphate (Elabscience, USA, catalog number #E-EL-H-2221). Homeostasis model of insulin resistance index (HOMA-IR) was calculated using the following equation:

$$[\text{fasting glucose (mmol/L)} \times \text{fasting insulin (l U/ml)}] / 22.5].$$

Patients were considered to have insulin resistance with HOMA-IR >2.5 (20).

Statistical analysis: Values were analyzed using SPSS vs. 20 (IBM Corp., USA). Results are presented as mean±SD or frequency (%) for parametric and non- parametric tests, respectively. Normality of data distribution was checked by Shapiro-Wilk Test. Unpaired student's t-test was used for parametric values with normal distribution and Mann-Whitney U test was utilized for parametric values with abnormal distribution in order to compare continuous variables between the two groups. Pearson Chi-square test was applied for comparing categorical values. Pearson's correlation tests were utilized to determine relations between measured parameters. The $p < 0.05$ was considered statistically significant.

Results

The study results revealed that body weight, BMI, WC, HC, and WHR ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, and $p = 0.014$) were significantly elevated, while height ($p < 0.0001$) was significantly decreased in PCOS group versus controls (Table 1). There were significant increases in FBG, FSI, and HOMA-IR in PCOS patients versus controls ($p < 0.0001$). Values of TC, TG, and LDL-C were elevated, while HDL-C was significantly decreased in PCOS patients versus controls ($p < 0.0001$ for all) (Table 2).

Compared with controls, in PCOS patients, there were significant elevation in serum values of FSH ($p = 0.021$), LH ($p = 0.015$), prolactin ($p < 0.0001$), estradiol ($p < 0.0001$), free testosterone ($p = 0.006$), Nesfatin-1 ($p = 0.017$), and dopamine ($p < 0.0001$), while there was significant decrease in NADPH serum concentrations ($p < 0.0001$) (Table 3).

Serum prolactin value revealed significant positive correlations with BMI ($r = 0.362$, $p = 0.001$), WHR ($r = 0.250$, $p = 0.022$), FSI ($r = 0.311$, $p = 0.004$), FSH ($r = 0.240$, $p = 0.028$), LH ($r = 0.308$, $p = 0.004$),

Table 1. Demographic characteristics of the controls and PCOS patients

Groups/ Characteristics	Control group (n=24)	PCOS group (n=60)	p-value
Age (years)	30.13±3.26	28.42±4.34	0.086
Weight (kg)	67.42±3.39	79.30±11.32	0.0001
Height (m)	1.63±0.03	1.59±0.04	0.0001
BMI (kg/m ²)	25.43±1.44	31.32±4.80	0.0001
Waist (cm)	89.31±2.48	98.15±6.81	0.0001
Hip (cm)	112.50±2.84	120.27±4.92	0.0001
WHR (%)	0.80±0.01	0.82±0.04	0.014

PCOS: Polycystic Ovary Syndrome, BMI: Body Mass Index, WHR: Waist Hip Ratio

Table 2. Biochemical assay in control and the PCOS groups

Variables	Control group (n=24)	PCOS group (n=60)	p-value
FBG (mmol/l)	5.65±0.54	6.40±0.79	0.0001
FSI (mIU/l)	5.05±0.17	5.58±0.64	0.0001
HOMA-IR	1.27±0.12	1.58±0.23	0.0001
TC (mg/dl)	174.38±12.60	230.00±26.14	0.0001
TG (mg/dl)	132.00±16.30	184.35±14.85	0.0001
HDL-C (mg/dl)	55.99±8.93	40.38±10.21	0.0001
LDL-C (mg/dl)	100.65±8.48	145.14±27.86	0.0001

PCOS: Polycystic Ovary Syndrome, FBG: Fasting Blood Glucose, FSI: Fasting Serum Insulin, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol

Table 3. Hormonal values of the controls and the PCOS groups

Variables	Control group (n=24)	PCOS patients (n=60)	p-value
FSH (mIU/ml)	4.52±0.81	5.27±1.49	0.021
LH (mIU/ml)	3.88±0.99	4.73±1.53	0.015
Prolactin (ng/ml)	7.28±1.92	17.26±6.52	0.000
Progesterone (ng/ml)	1.32±0.46	1.60±0.82	0.122
Estradiol (pg/ml)	41.03±11.34	61.52±16.75	0.0001
Free testosterone (nmol/l)	1.06±0.30	1.36±0.49	0.006
Nesfatin-1 (pg/ml)	316.10±59.87	362.37±85.06	0.017
NADPH (ng/ml)	7.24±3.88	4.55±2.05	0.0001
Dopamine (pg/ml)	29.80±9.91	67.92±14.23	0.0001

PCOS: Polycystic Ovary Syndrome, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, PRL: Prolactin, NADPH: Nicotinamide Adenine Dinucleotide Phosphate.

and estradiol ($r=0.545$, $p<0.0001$). Serum testosterone revealed significant positive association with estradiol ($r=0.372$, $p<0.0001$). Serum Nesfat-

in-1 showed significant positive associations with prolactin ($r=0.338$, $p=0.002$) and dopamine ($r=0.407$, $p<0.000$). Serum dopamine had significant direct correlations with BMI ($r=0.395$, $p<0.0001$), FSI ($r=0.265$, $p=0.015$), FSH ($r=0.272$, $p=0.012$), LH ($r=0.269$, $p=0.013$), estradiol ($r=0.516$, $p<0.0001$), and prolactin ($r=0.542$, $p<0.0001$). Serum NADPH had significant negative associations with BMI ($r=-0.250$, $p=0.022$), FSI ($r=-0.224$, $p=0.040$), estradiol ($r=-0.307$, $p=0.005$), and prolactin ($r=-0.281$, $p=0.010$) (Table 4).

Discussion

In the present study, elevated serum Nesfatin-1, elevated dopamine, and decreased NADPH concentrations in PCOS infertile women indicated that they have a role in PCOS pathophysiology. In this study, there were significant elevations in BMI and WHR which are often associated with obesity especially central obesity in PCOS patients versus controls. These findings were in line with Keen et al. (21) and Abdelazim et al. (22) outcomes who reported that prevalence of obesity was 27% and 35.5%, and overweight was 53% and 44.5% in PCOS women. Lazúrová et al. (23) reported that significant elevation in BMI and WC was associated with elevation in serum-free testosterone and free androgen indices in overweight PCOS patients. Moreover, Kirmizi et al. (24) found that elevated WHR and BMI in PCOS patients contributed to inflammation and were independent risk factors affecting sexual dysfunction.

Furthermore, IR is another problem that may be found along hyperandrogenism in PCOS cases which is in agreement with findings reported by Deniz et al. (9) and Ademoglu et al. (10). The results of this study revealed higher FBG, FSI, and HOMA-IR in PCOS patients. IR, found in PCOS women, could be explained by the decline in hepatic extraction of insulin that contributed to their hyperinsulinemia. In muscles, serine phosphorylation of insulin receptor and of insulin receptor substrate 1 (IRS1) was elevated leading to defect in insulin signaling (18). Also, IR and hyperinsulinemia might stimulate pituitary reaction to gonadotropin releasing hormone (GnRH), culminating in elevated LH and androgen secretions, which could disturb normal hypothalamic-pituitary-gonadal (HPG) axis functions (25). Increased insulin levels are considered as factors affecting excessive androgen production in ovary and theca cells, resulting in follicular atresia and dominant

Table 4. Correlations between different measured parameters

Parameters	Prolactin	Testosterone	Nesfatin-1	NADPH	Dopamine
BMI					
	r=0.362 p=0.001	r= -0.034 p=0.761	r=0.004 p=0.970	r= -0.250 p=0.022	r=0.395 p<0.0001
WHR					
	r=0.250 p=0.022	r= -0.118 p=0.283	r= -0.057 p=0.608	r= -0.134 p=0.226	r=0.159 p=0.147
FSI					
	r=0.311 p=0.004	r=0.082 p=0.458	r=0.118 p=0.286	r= -0.224 p=0.040	r=0.265 p=0.015
HOMA-IR					
	r=0.182 p=0.097	r=0.130 p=0.238	r= -0.011 p=0.922	r= -0.148 p=0.179	r=0.154 p=0.163
FSH					
	r=0.240 p=0.028	r= -0.026 p=0.817	r= -0.058 p=0.600	r= -0.125 p=0.258	r=0.272 p=0.012
LH					
	r=0.308 p=0.004	r=0.212 p=0.052	r=0.103 p=0.349	r= -0.092 p=0.406	r=0.269 p=0.013
Estradiol					
	r=0.545 p<0.0001	r=0.372 p<0.0001	r=0.096 p=0.386	r= -0.307 p=0.005	r=0.516 p<0.0001
Prolactin					
	-	r=0.204 p=0.063	r=0.338 p=0.002	r= -0.281 p=0.010	r=0.542 p<0.0001
Testosterone					
	-	-	r=0.041 p=0.708	r= -0.186 p=0.091	r=0.141 p=0.200
Nesfatin-1					
	-	-	-	r=0.068 p=0.538	r=0.407 p<0.0001
NADPH					
	-	-	-	-	r= -0.213 p=0.052

Data were expressed as correlation coefficient (r) and significance. PCOS: Polycystic Ovary Syndrome, BMI: Body Mass Index, WHR: Waist Hip Ratio, FSI: Fasting Serum Insulin, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, NADPH: Nicotinamide Adenine Dinucleotide Phosphate

follicle selection (26). In hyperandrogenism, follicle growth is arrested, resulting in follicular atresia, and elevated androgen values that led to long-term insulin resistance (27). Furthermore, Ng et al. (28) found that obesity and hyperinsulinemia in PCOS women would further elevate androgen concentrations through lowering sex hormone binding globulin concentrations. Genetic change of melatonin receptor 1B in PCOS may also impair insulin secretion (29).

Furthermore, this study showed a significant elevation in serum concentrations of TC, LDL-C, and TG and a significant decline in HDL-C serum

values in PCOS patients versus controls that indicated abnormal lipid profiles in PCOS patients. Dyslipidemia is a frequent metabolic issue that affects up to 70% of PCOS women (30). Kiranmayee et al. and Javaid et al. (31, 32) reported dyslipidemia in PCOS women and attributed the incidence to hyperandrogenism and IR. Increased androgen concentrations increased hepatic lipase (27) activity that breaks down phospholipids on HDL surface causing conversion of HDL-2 to smaller denser HDL-3. HDL-3 being a better substrate for liver increases HDL clearance (32). Also, hyperandrogenism decreased LDL-C catabol-

ic removal (28).

Consistent with findings of Malini and George (33), the present study revealed significant elevations in serum concentrations of FSH and LH in PCOS patients. In partial agreement with the present study, Abdelazim et al. (22) showed a significant increase in LH and an insignificant increase in FSH and they claimed that GnRH pulse frequency of LH was rapid over GnRH pulses of FSH because there was a decline in GnRH sensitivity to inhibition by ovarian hormones, particularly progesterone (34).

The current study demonstrated significant elevation in serum values of estradiol and testosterone in PCOS patients. This rising in androgen concentration could be explained by hyperinsulinemia that alters the hypothalamic function leading to steady elevation in LH level which further stimulates testosterone and estradiol production as proposed by Malini and George (33). Furthermore, Nath et al. (35) suggested that combination of raised values of adrenal androgen and obesity resulted in increased formation of extra-glandular estrogen by peripheral aromatization that had direct impact on LH release. Consequently, this increased LH produced hyperplasia of theca cells and ovarian stroma and elevated androgen formations that provides more substances for aromatization and this vicious circle continues.

The results of this research revealed increase in serum prolactin concentrations in PCOS women which is directly associated with BMI, WHR, fasting insulin, FSH, LH and estradiol indicating that hyperprolactinemia might contribute to obesity, IR, hyperinsulinemia, and gonadal dysfunction in PCOS patients. Hyperprolactinemia was explained by Bouckennooghe et al. (36) who reported that macrophages that infiltrated adipose tissue as a result of inflammation and hyperglycemia found in PCOS might increase the extrapituitary pool of prolactin. This hyperprolactinemia has a role in metabolic disturbance. Andersen and Glinborg (37) reported that hyperprolactinemia might be linked with increased risk of metabolic syndrome and probably become a metabolic risk assessment tool in PCOS cases. Additionally, Auriemma et al. (38) reported that prolactin reduced glucose tolerance and induced insulin resistance in both non-obese and obese patients.

The present research revealed elevated serum value of Nesfatin-1 which was directly associated with prolactin. The findings of this study were partially in accordance with results of Ademoglu

et al. (10) who proposed that elevated Nesfatin-1 value might be related to Nesfatin-1 resistance due to defect in Nesfatin-1 receptors and post receptor signaling in target organs. Sahin et al. (17) suggested that high serum Nesfatin-1 values in obese subjects and patients with IR might be a defensive action in comparison to hyperglycemia and obesity. On the contrary, Deniz et al. (9) revealed the decline of Nesfatin-1 serum concentrations in PCOS patients. These low concentrations were claimed to be responsible for IR and metabolic syndrome as Nesfatin-1 stimulates glucose induced insulin secretion by promoting Ca^{+2} influx through L-type channels in mouse islet beta-cells of pancreas (39). The direct association between Nesfatin-1 and prolactin demonstrated in this study might be due to Nesfatin-1 expression in pituitary gland and its effects on hypothalamic pituitary gonadal axis at different levels (6). Könczöl et al. (40) reported that Nesfatin-1 colocalizes with prolactin-releasing peptide producing neurons, in both adrenal medullary A1 and A2 catecholamine cell groups which mediate visceral sensory information toward hypothalamic paraventricular nucleus. Moreover, Könczöl et al. (41) reported that Nesfatin-1 was co-expressed with prolactin-releasing peptide. Bouckennooghe et al. (36) reported that adipose tissue (which secretes Nesfatin-1) also secreted extra-pituitary prolactin with more secretion in patients with inflammations and hyperglycemia.

Also, in this study, there were elevated serum DA concentrations in PCOS patients. DA was positively associated with BMI, FSH, LH, estradiol, Nesfatin-1, and prolactin indicating the association between PCOS and higher sympathetic activity. These results were in line with the findings of Saller et al. (42), Musalı et al. (43), and Ferrero et al. (44) who found increased DA concentrations in follicular fluid of PCOS women along with increased enzymes levels involved in DA metabolic breakdown. These higher DA concentrations may be in agreement with sympathetic hyperinnervation of ovary in PCOS (32). The elevated DA concentrations increased free radical's production which in turn reduced adenosine triphosphate values and cell viability and were associated with impaired oocyte maturation and fertilization, poor embryo quality, and decline in pregnancy rates. However, Chaudhari et al. (45) found decreased DA concentrations along with reduced dopamine 2 receptors in brain tissues of PCOS rats. They attributed low DA concentrations to

the increase in LH concentrations in PCOS.

In this study, there was a decrease in serum NADPH concentrations that were inversely associated with BMI, fasting insulin, estradiol, and prolactin levels. In PCOS, NADPH oxidation by NADPH oxidase led to superoxide formation, ROS generation, and oxidative stress (46). Zuo et al. (47) suggested that oxidative stress had a role in IR and obesity among PCOS patients. Moreover, Nuñez-Calonge et al. (11) reported that reduced antioxidant level in PCOS has an adverse effects on oocyte quality and maturation.

There were some limitations in this study. Small sample size of PCOS patients is one of them and larger study populations with PCOS can be selected including obese and non-obese cases. Another limitation is evaluating only the effects of receptors of Nesfatin-1, dopamine, and nicotinamide level on the ovaries. In fact, more detailed molecular studies of ovaries and their receptors including Nesfatin-1, dopamine, and nicotinamide should be conducted. Lack of follow-up is the third limitation of this study; a longitudinal study with follow-up of PCOS patients is required to confirm the role of Nesfatin-1, dopamine, and nicotinamide and their correlations with each other in the PCOS patients.

Conclusion

In conclusion, this study demonstrated that elevated serum Nesfatin-1, dopamine, and decreased NADPH concentrations have a role in the pathogenesis of PCOS. Direct association between Nesfatin-1 and prolactin might indicate the presence of Nesfatin-1 resistance as a defense mechanism that needs further research. Elevated serum dopamine concentration could be due to an increase in sympathetic activity in PCOS cases and its direct association with BMI, gonadotropins, and prolactin contributed to impaired folliculogenesis through ROS production, IR, and obesity. Moreover, decrease in serum nicotinamide values in PCOS cases and their inverse associations with BMI, FSI, and prolactin proved that nicotinamide could be used in management of PCOS cases via its effect on reduction of ROS production.

Acknowledgement

The authors would like to acknowledge Research Grants' Office of Faculty of Medicine, Assiut University, Assiut, Egypt for the fund received for completion of this study (Fund # 12019-03-26-001-R1).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Regidor PA, Mueller A, Sailer M, Gonzalez Santos F, Rizo JM, Egea FM. Chronic inflammation in PCOS: the potential benefits of specialized proresolving lipid mediators (SPMs) in the improvement of the resolutive response. *Int J Mol Sci.* 2021;22(1):384.
2. Daghestani MH. Rs1799817 in INSR associates with susceptibility to polycystic ovary syndrome. *J Med Biochem.* 2020;39(2):149-59.
3. Prinz P, Goebel-Stengel M, Teuffel P, Rose M, Klapp BF, Stengel A. Peripheral and central localization of the nesfatin-1 receptor using autoradiography in rats. *Biochem Biophys Res Commun.* 2016;470(3):521-7.
4. Gao X, Zhang K, Song M, Li X, Luo L, Tian Y, et al. Role of nesfatin-1 in the reproductive axis of male rat. *Sci Rep.* 2016;6:32877.
5. Kim J, Yang H. Nesfatin-1 as a new potent regulator in reproductive system. *Dev Reprod.* 2012;16(4):253-64.
6. Dore R, Levata L, Lehnert H, Schulz C. Nesfatin-1: functions and physiology of a novel regulatory peptide. *J Endocrinol.* 2017;232(1):R45-R65.
7. Nakata M, Manaka K, Yamamoto S, Mori M, Yada T. Nesfatin-1 enhances glucose-induced insulin secretion by promoting Ca²⁺ influx through L-type channels in mouse islet β -cells. *Endocrine J.* 2011;58(4):305-13.
8. Alp E, Görmüş U, Güdücü N, Bozkurt S. Nesfatin-1 levels and metabolic markers in polycystic ovary syndrome. *Gynecol Endocrinol.* 2015;31(7):543-7.
9. Deniz R, Gurates B, Aydin S, Celik H, Sahin I, Baykus Y, et al. Nesfatin-1 and other hormone alterations in polycystic ovary syndrome. *Endocrine.* 2012;42(3):694-9.
10. Ademoglu EN, Gorar S, Carliloglu A, Dellal FD, Berberoglu Z, Akdeniz D, et al. Plasma nesfatin-1 levels are increased in patients with polycystic ovary syndrome. *J Endocrinol Invest.* 2014;37(8):715-9.
11. Nuñez-Calonge R, Cortés S, Gonzalez LMG, Kireev R, Vara E, Ortega L, et al. Oxidative stress in follicular fluid of young women with low response compared with fertile oocyte donors. *Reprod Biomed Online.* 2016;32(4):446-56.
12. Su P, Bretz JD, Gunaratne GS, Marchant JS, Walseth TF, Slama JT. Chemo-enzymatic synthesis of adenine substituted nicotinic acid adenine dinuc-

- leotide phosphate (NAADP) analogs. *Bioorg Med Chem*. 2021;30:115901.
13. Kim SH, Kim H, Lee JH, Park JW. Oxalomalate suppresses metastatic melanoma through IDH-targeted stress response to ROS. *Free Radic Res*. 2019;53(4):418-29.
 14. Parillo F, Maranesi M, Mignini F, Marinelli L, Di Stefano A, Boiti C, et al. Evidence for a dopamine intrinsic direct role in the regulation of the ovary reproductive function: In vitro study on rabbit corpora lutea. *PLoS One*. 2014;9(8):e104797.
 15. Beaulieu JM, Borrelli E, Carlsson A, Caron MG, Civelli O, Espinoza S, et al. Dopamine receptors (version 2019.4) in the IUPHAR/BPS guide to pharmacology database. *IUPHAR/BPS guide to pharmacology CITE*. 2019;2019(4):1-28.
 16. Lai Q, Xiang W, Li Q, Zhang H, Li Y, Zhu G, et al. Oxidative stress in granulosa cells contributes to poor oocyte quality and IVF-ET outcomes in women with polycystic ovary syndrome. *Front Med*. 2018;12(5):518-24.
 17. Sahin FK, Sahin SB, Ural UM, Cure MC, Senturk S, Tekin YB, et al. 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.
 18. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers*. 2016;2:16057.
 19. Masjedi F, Keshtgar S, Agah F, Karbalaee N. Association between sex steroids and oxidative status with vitamin D levels in follicular fluid of non-obese PCOS and healthy women. *J Reprod Infertil*. 2019;20(3):132-42.
 20. Singh Y, Garg M, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. *J Clin Res Pediatr Endocrinol*. 2013;5(4):245-51.
 21. Keen MA, Shah IH, Sheikh G. Cutaneous manifestations of polycystic ovary syndrome: A cross-sectional clinical study. *Indian Dermatol Online J*. 2017;8(2):104-10.
 22. Abdelazim IA, Alanwar A, AbuFaza M, Amer OO, Bekmukhambetov Y, Zhurabekova G, et al. Elevated and diagnostic androgens of polycystic ovary syndrome. *Prz Menopauzalny*. 2020;19(1):1-5.
 23. Lazúrová I, Lazúrová Z, Figurová J, Ujházi S, Dravecká I, Mašlanková J, et al. Relationship between steroid hormones and metabolic profile in women with polycystic ovary syndrome. *Physiol Res*. 2019;68(3):457-65.
 24. Kirmizi DA, Baser E, Onat T, Caltekin MD, Yalvac ES, Kara M, et al. Sexual function and depression in polycystic ovary syndrome: Is it associated with inflammation and neuromodulators? *Neuropeptides*. 2020;84:102099.
 25. Tao X, Chen L, Cai L, Ge S, Deng X. Regulatory effects of the AMPK α -SIRT1 molecular pathway on insulin resistance in PCOS mice: an in vitro and in vivo study. *Biochem Biophys Res Commun*. 2017;494(3-4):615-20.
 26. Kucur C, Kucur SK, Gozukara I, Seven A, Yuksel KB, Keskin N, et al. Extended high frequency audiometry in polycystic ovary syndrome. *Sci World J*. 2013;2013:482689.
 27. Cameron JL, Jain R, Rais M, White AE, Beer TM, Kievit P, et al. Perpetuating effects of androgen deficiency on insulin resistance. *Int J Obes (Lond)*. 2016;40(12):1856-63.
 28. Ng NYH, Jiang G, Cheung LP, Zhang Y, Tam CHT, Luk AOY, et al. Progression of glucose intolerance and cardiometabolic risk factors over a decade in Chinese women with polycystic ovary syndrome: a case-control study. *PLoS Med*. 2019;16(10):e1002953.
 29. Spinedi E, Cardinali DP. The polycystic ovary syndrome and the metabolic syndrome: a possible chronobiotic-cytoprotective adjuvant therapy. *Int J Endocrinol*. 2018;2018:1349868.
 30. Lath R, Shendye R, Jibhkate A. Insulin resistance and lipid profile in polycystic ovary syndrome. *Asian J Biomed Pharm Sci*. 2015;5(47):30.
 31. Kiranmayee D, Kavya K, Himabindu Y, Sriharibabu M, Madhuri GLJ, Venu S. Correlations between anthropometry and lipid profile in women with PCOS. *J Hum Reprod Sci*. 2017;10(3):167-72.
 32. Javaid S, Ahmed U, Lone KP, Ikram N. Plasma dopamine levels in Polycystic Ovary Syndrome women. *Pakistan J Physiol*. 2019;15(3):49-52.
 33. Malini NA, George KR. Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS)–Clinical based case control study. *Gen Comp Endocrinol*. 2018;260:51-7.
 34. Moffett RC, Naughton V. Emerging role of GIP and related gut hormones in fertility and PCOS. *Peptides*. 2020;125:170233.
 35. Nath CK, Barman B, Das A, Rajkhowa P, Baruah P, Baruah M, et al. Prolactin and thyroid stimulating hormone affecting the pattern of LH/FSH secretion in patients with polycystic ovary syndrome: a hospital-based study from north east India. *J Fam Med Prim Care*. 2019;8(1):256-60.
 36. Bouckenoghe T, Sisino G, Aurientis S, Chinetti-

- Gbaguidi G, Kerr-Conte J, Staels B, et al. Adipose tissue macrophages (ATM) of obese patients are releasing increased levels of prolactin during an inflammatory challenge: a role for prolactin in diabetes? *Biochim Biophys Acta*. 2014;1842(4):584-93.
37. Andersen M, Glintborg D. Metabolic syndrome in hyperprolactinemia. *Front Horm Res*. 2018;49:29-47.
 38. Auriemma RS, De Alcubierre D, Pirchio R, Pivonello R, Colao A. Glucose abnormalities associated to prolactin secreting pituitary adenomas. *Front Endocrinol (Lausanne)*. 2019;10:327.
 39. Ayada C, Toru Ü, Korkut Y. Nesfatin-1 and its effects on different systems. *Hippokratia*. 2015;19(1):4-10.
 40. Könczöl K, Bodnár I, Zelena D, Pintér O, Papp RS, Palkovits M, et al. Nesfatin-1/NUCB2 may participate in the activation of the hypothalamic-pituitary-adrenal axis in rats. *Neurochem Int*. 2010;57(3):189-97.
 41. Könczöl K, Pintér O, Ferenczi S, Varga J, Kovács K, Palkovits M, et al. Nesfatin-1 exerts long-term effect on food intake and body temperature. *Int J Obes (Lond)*. 2012;36(12):1514-21.
 42. Saller S, Kunz L, Berg D, Berg U, Lara H, Urra J, et al. Dopamine in human follicular fluid is associated with cellular uptake and metabolism-dependent generation of reactive oxygen species in granulosa cells: implications for physiology and pathology. *Hum Reprod*. 2014;29(3):555-67.
 43. Musalı N, Özmen B, Şükür YE, Ergüder Bİ, Atabekoğlu CS, Sönmezer M, et al. Follicular fluid norepinephrine and dopamine concentrations are higher in polycystic ovary syndrome. *Gynecol Endocrinol*. 2016;32(6):460-3.
 44. Ferrero H, Díaz-Gimeno P, Sebastián-León P, Faus A, Gómez R, Pellicer A. Dysregulated genes and their functional pathways in luteinized granulosa cells from PCOS patients after cabergoline treatment. *Reproduction*. 2018;155(4):373-81.
 45. Chaudhari N, Dawalbhakta M, Nampoothiri L. GnRH dysregulation in polycystic ovarian syndrome (PCOS) is a manifestation of an altered neurotransmitter profile. *Reprod Biol Endocrinol*. 2018;16(1):37.
 46. González F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids*. 2012;77(4):300-5.
 47. Zuo T, Zhu M, Xu W. Roles of oxidative stress in polycystic ovary syndrome and cancers. *Oxid Med Cell Longev*. 2016;2016:8589318.