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Original Article

Obstetrics and Gynaecology Section

Serum Copeptin as a Biomarker of Polycystic Ovarian Syndrome and its Correlation with Metabolic Syndrome Components: A Cross-sectional Analytical Study

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ABSTRACT

Introduction: Approximately 5 to 10% of women of reproductive age suffer from the prevalent endocrine illness known as Polycystic Ovarian Syndrome (PCOS). Copeptin, irrespective of age and weight, has been found to have significant associations with cardiometabolic parameters. Studies on the diagnostic and prognostic significance of copeptin and its correlation with components of metabolic syndrome in PCOS are scarce, particularly in the Indian context.

Aim: To assess the utility of copeptin as a diagnostic marker of PCOS and to evaluate the correlation of serum copeptin levels with metabolic syndrome components in women with PCOS.

Materials and Methods: This cross-sectional analytical study was conducted at Father Muller Medical College Hospital, Mangalore, Karnataka, India, from June 2022 to September 2023. A total of 60 subjects with PCOS were selected through convenient sampling and divided into two groups: Group 1-subjects with PCOS having metabolic syndrome, and Group 2-subjects with PCOS but not having metabolic syndrome. Blood samples for serum copeptin were taken under aseptic precautions, and levels were analysed using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (Biovendor, USA) following the manufacturers' instructions. The copeptin ELISA kit had an assay range of 0-100 pmol/L and results were expressed in ng/mL. Serum insulin levels were measured using specific Electro-chemiluminescence immunoassays. Levels of total cholesterol, High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG) were determined with enzymatic colorimetric assays by spectrophotometry. Low-Density Lipoprotein Cholesterol (LDL-C) was calculated using the Friedewald formula. Insulin resistance was calculated using the Homeostasis Model Assessment Insulin Resistance Index (HOMA-IR). Statistical analysis was done by using descriptive statistics. A comparison was done by student's unpaired t-test. The chi-square test and Pearson correlation test were used for categorical data. The Statistical Package for Social Sciences (SPSS) version 24.0 was used for analysis.

Results: The mean age of the study participants was 24.24±4.721 years, ranging from 15 to 43 years. The mean age of patients with metabolic syndrome was 23.96±6.3 years, while those without metabolic syndrome was 24.40±3.52 years. The mean Body Mass Index (BMI) was 31.17±5.38 in those with metabolic syndrome and 23.2±4.7 in those without (p=0.0001). The Waist-to-Hip Ratio (WHR) of Group 1 was significantly higher than Group 2 (p=0.001). The two groups did not differ significantly with regard to serum copeptin level, i.e., 7.386±4.58 in Group 1 and 8.66±6.03 in Group 2 (p=0.736). Serum copeptin levels showed a significant correlation with fasting serum insulin (0.006) and Homeostatic Model Assessment - Insulin Resistance (HOMA-IR) (0.012).

Conclusion: Serum copeptin cannot be used as an independent marker for the diagnosis of metabolic syndrome in PCOS patients but may indicate other prognostic factors.

Keywords: Copeptin, Metabolic syndrome, Polycystic ovarian syndrome

INTRODUCTION

Increased plasma concentrations of Arginine Vasopressin (AVP) have been linked to the development of type 2 diabetes, metabolic syndrome, chronic renal, and cardiovascular illnesses, according to recent research [1]. However, AVP's short half-life of 16 to 20 minutes in plasma, small size, and poor stability, which make direct measurement challenging, limit its therapeutic utility as a biomarker [2]. Copeptin, the stable and physiologically inactive C-terminal portion of pro-vasopressin, is co-secreted in equimolar levels with AVP, making it a suitable and practical surrogate marker for AVP in clinical settings [3]. The range of copeptin plasma concentration in healthy adults is 1 to 13.8 pmol/L, with an average of 4.2 pmol/L [4]. There is notable variation in the concentration of copeptin across genders, with lower values in females [5,6].

The role of the AVP system in controlling human metabolic homeostasis has received more attention recently. Numerous elements of the metabolic syndrome, including dyslipidemia, insulin resistance, glucose intolerance, hyper-insulinemia, hypertension, and abdominal obesity, have been linked to high levels of circulating plasma copeptin [7]. Enhorning S et al., showed a strong link between elevated copeptin and a higher frequency of Non-Alcoholic Fatty Liver Disease (NAFLD) in a population-based study with mixed ethnicities. Copeptin also showed a negative correlation with HDL and a positive correlation with raised HbA1c, insulin, BMI, HOMA-IR, and waist circumference [8].

There is a link between copeptin and the probability of elevated HOMA-IR ≥2.5, according to a case-control research involving PCOS women [9]. Patients with PCOS, particularly those who are obese, have been found to have elevated serum copeptin levels and a favourable association between serum copeptin concentrations and cardiometabolic markers such as total testosterone, HOMA-IR, WHR, BMI, and hirsutism score. This suggests that copeptin may be helpful in identifying future cardiovascular risk in PCOS patients [9].

Copeptin, like AVP and neurophysin II, is generated from the precursor pre-vasopressin. Copeptin is thought to be an accurate and feasible clinical surrogate for AVP in disorders involving the homeostasis of bodily fluids [10]. It has been found that there is a strong positive correlation between copeptin and AVP levels in both healthy individuals and patients with different cardiovascular illnesses [11].

Studies on the diagnostic and prognostic significance of copeptin and its correlation with components of the metabolic syndrome in PCOS are scarce, particularly in the Indian context. In the present study, the authors aimed to assess the utility of copeptin as a diagnostic marker of PCOS and the correlation of serum copeptin levels with metabolic syndrome components in women with PCOS.

MATERIALS AND METHODS

This was an observational study conducted at Father Muller Medical College Hospital, Mangalore, Karnataka, India, from June 2022 to September 2023. The research was approved by the Institutional Ethics Committee (FMMCIEC/CCM/513/2022). Voluntary informed consent was obtained from all participants. A total of 60 subjects with PCOS were selected via convenient sampling after calculating the sample size using the following formula: $N=S\alpha^2\ s^2/d^2$, where $S\alpha=1.96$ at a 95% confidence level, and s=standard deviation, and d=relative precision=10% of the mean Dehydroepiandrosterone (DHEAS) level (µg/dL).

Sample size calculation: For DHEAS, d=10% of 275.65, and S=15.45. With a 95% confidence level and 90% power, the sample sise was calculated as 60. DHEAS was chosen instead of copeptin level as the two groups were divided based on the presence or absence of metabolic syndrome in the patients with PCOS.

Inclusion criteria: Patients diagnosed with PCOS as per Rotterdam's criteria (presence of any two of the following: clinical or biochemical hyperandrogenism, clinical evidence of oligo-anovulation, Polycystic appearing-ovarian morphology on ultrasound, and belonging to the age group of 19-40 years were included in the study and allocating to two groups [12].

Group-1 included 30 individuals with PCOS and metabolic syndrome, while Group-2 included subjects with PCOS but without metabolic syndrome.

Exclusion criteria: Individuals with a history of chronic smoking and systemic illnesses such as diabetes mellitus, hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumours, thyroid disorders, Cushing syndrome, infectious diseases, hypertension, and hepatic or renal dysfunction were excluded from the study. Patients with a history of usage of medications like oral contraceptive agents, anti-lipidemic drugs, hypertensive medications, and insulin-sensitising drugs within three months before enrollment were also excluded.

Metabolic syndrome criteria were based on the International Diabetes Federation (IDF) criteria [13]: abdominal obesity (>88 cm in women), raised concentration of Triglycerides (TGs) (≥150 mg/dL), reduced concentration of High Density Lipoprotein (HDL) cholesterol (<40 mg/dL (1.03 mmol/L) in women or raised blood pressure (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg), and high fasting plasma glucose concentration (≥100 mg/dL).

Method of data collection and analysis: Subjects diagnosed with PCOS as per the Rotterdam criteria, who visited the outpatient department of Obstetrics and Gynaecology, were enrolled in the study after obtaining consent. Height and weight were measured with subjects wearing light clothing without shoes. BMI was calculated by dividing weight by the square of height (kg/m^2). Waist circumference was measured at the narrowest level between the costal margin and iliac crest, and hip circumference was measured at the widest level over the buttocks with the subjects standing, after which the Waist-to-Hip Ratio (WHR) was calculated. Blood pressure was measured in the right arm with the subject in a sitting position.

A blood sample for serum Copeptin was taken under aseptic precautions; fasting blood samples (2 mL) were collected into tubes containing Ethylene Diamine Tetra-Acetic Acid (EDTA). The blood sample was centrifuged at 1600 × g for 15 minutes, plasma was separated, and stored at -80°C until the assessment of copeptin. Copeptin levels in the serum samples were analysed using a commercially available ELISA kit (Biovendor, USA) following the manufacturer's instructions. The assay range of the copeptin ELISA kit was 0-100 pmol/L and the results were expressed in ng/mL.

Biochemical evaluation: After overnight fasting, venous blood samples were obtained for fasting plasma glucose levels (using the glucose oxidase/peroxidase method). Serum insulin was measured using specific Electro-chemiluminiscence immunoassays.

Levels of total cholesterol, High Density Lipoprotein-Cholesterol (HDL-C), and TG were determined using enzymatic colorimetric assays by spectrophotometry. LDL-C was calculated using the Friedewald formula. Insulin resistance was calculated using the HOMA-IR formula, which is calculated as fasting plasma glucose (mmol/L)×fasting serum insulin (mU/mL)/22.5. The cut-off value for HOMA-IR was set at 2.7 [12].

STATISTICAL ANALYSIS

This was done by using descriptive statistics. Comparisons were made using either the Student's unpaired t-test or Pearson correlation test as per the normality of the data. The Chi-square test was utilised for qualitative data. The statistical package SPSS version 24.0 was used for the analysis. A p-value of <0.05 was considered significant.

RESULTS

Out of the total 60 study participants, one did not return with the requested laboratory investigations; asked for, hence data from only 59 participants were included. Among the 59 study participants, 22 (37.2%) had metabolic syndrome. The mean age of the study participants was 24.24±4.721 years, ranging between 15 to 43 years. The mean age of patients with metabolic syndrome was 23.96±6.3 years, while those without it was 24.40±3.52 years. The mean BMI was 31.17±5.38 in those with metabolic syndrome and 23.2±4.7 in those without it (p=0.0001).

Among the study participants, 22 had metabolic syndrome, while 37 did not meet the criteria for metabolic syndrome. Of the participants with metabolic syndrome, two had normal weight, 10 were overweight, six had Grade-I obesity, and four had Grade-II obesity. Among those without metabolic syndrome, five were underweight, 17 had normal weight, 11 were overweight, and four had Grade-I obesity.

The mean Copeptin value was 7.38±4.58 (ng/mL) in patients with metabolic syndrome and 8.66±6.03 (ng/mL) in patients without metabolic syndrome [Table/Fig-1].

Serum copeptin levels showed a significant correlation with fasting serum insulin (p=0.006) and HOMA-IR (p=0.012) [Table/Fig-2].

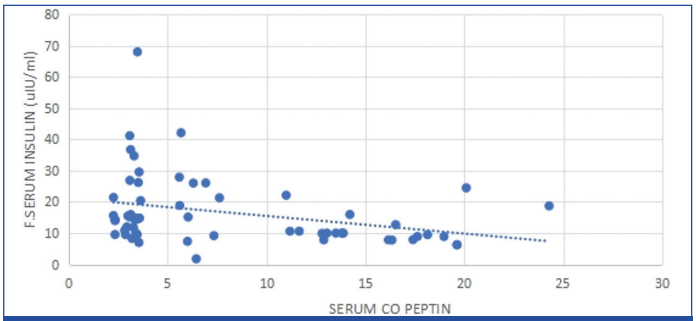
Parameters	Group-1: PCOS with metabolic syndrome (n=22)	Group-2: PCOS without metabolic syndrome (n=37)	Significance
Age (years)	23.30±6.34	24.40±3.51	p=0.313
BMI (kg/m^2)	31.17±5.3	23.90±4.7	**p<0.001**
Waist HIP Ratio	0.87±0.04	0.825±0.05	**p=0.001**
Serum copeptin (ng/mL)	7.386±4.58	8.66±6.03	p=0.736
Systolic blood pressure (mmHg)	122.27±15.18	113.96±8.634	**p=0.016**
Diastolic blood pressure (mmHg)	83.182±10.42	74.864±8.36	**p=0.04**
Fasting plasma glucose (mg/dL)	95.636±9.604	91.27±8.54	p=0.079
Fasting serum insulin (uIU/mL)	22.17±14.515	91.279±8.54	**p=0.008**
Homeostatic Model Assessment - Insulin Resistance (HOMA-IR)	5.37±4.02	2.99±1.664	**p=0.01**
Total cholesterol (mg/dL)	191.36±33.15	170.75±23.17	**p=0.020**
Triglycerides (TG) (mg/dL)	161.18±33.70	111.81±42.78	**p=0.001**
HDL-cholesterol (mg/dL)	44.382±7.84	53.37±15.03	**p=0.004**
LDL-cholesterol (mg/dL)	124.73±26.58	101.476±20.78	**p=0.001**

[Table/Fig-1]: Comparison study variables in Polycystic Ovarian Syndrome (PCOS) subjects with and without metabolic syndrome (Values are mean±median). Unpaired t-test was used

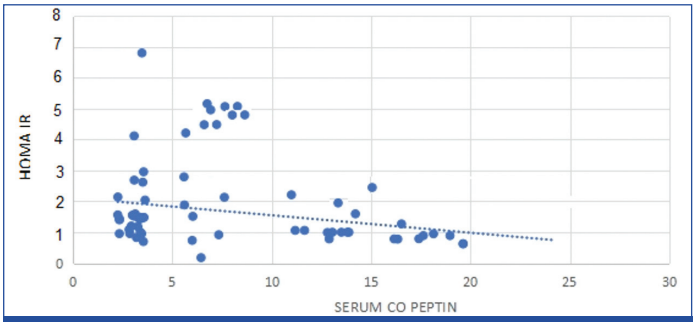
	Serum copeptin	
	Correlation coefficient (r)	p-value
Copeptin-BMI (kg/m^2)	-0.2	0.13
Copeptin-Waist hip ratio	-0.188	0.153
Copeptin-Systolic blood pressure	-0.081	0.542
Copeptin-Diastolic blood pressure	-0.168	0.203
Copeptin-Fasting plasma glucose	-0.106	0.424
Copeptin-Fasting serum insulin	-0.353	**0.006**
Copeptin- HOMA-IR	-0.325	**0.012**
Copeptin-Serum total cholesterol	-0.255	0.052
Copeptin-Serum Triglycerides (TG)	-0.075	0.573
Copeptin-Serum HDL cholesterol	0.059	0.659
Copeptin-Serum LDL cholesterol	-0.082	0.536

[Table/Fig-2]: Correlation of serum copeptin level with metabolic syndrome components in women with PCOS.
Pearson correlation test; p<0.05 considered significant

There was a significant negative moderate correlation between serum copeptin and serum insulin levels, and between serum copeptin and HOMA-IR [Table/Fig-3,4]. The accuracy, sensitivity, and specificity of copeptin as a biomarker for PCOS were low and not significant [Table/Fig-5,6].



[Table/Fig-3]: Correlation of between serum copeptin and serum insulin among women with PCOS.
r=-0.353



[Table/Fig-4]: Correlation of between serum copeptin and HOMA-IR among women with PCOS.

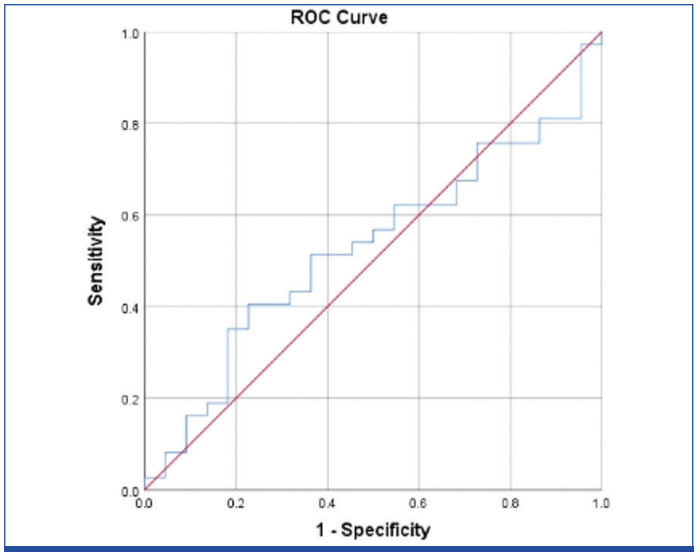
	Area under the curve	Accuracy	Sensitivity	Specificity	Cut-off value	p-value
Copeptin	0.527	0.627	1.00	0.00	1.25	0.730

[Table/Fig-5]: Sensitivity and specificity of copeptin as a biomarker of Polycystic Ovarian Syndrome (PCOS).
Pearson correlation test; p<0.05 considered significant

DISCUSSION

In the present study, the authors observed that 37.2% of the subjects with PCOS had metabolic syndrome. There was a significant correlation between serum copeptin levels and serum insulin and insulin resistance among PCOS patients with metabolic syndrome. The accuracy, sensitivity, and specificity of copeptin as a biomarker for PCOS were found to be low and not significant.

In a research done by Aly AE et al., it was found that in PCOS women with positive insulin resistance (>2.5), plasma copeptin levels



[Table/Fig-6]: ROC curve of copeptin as a biomarker of Polycystic Ovarian Syndrome (PCOS).

were significantly higher compared to healthy controls and PCOS women with insulin resistance <2.5 [11]. The authors suggested that copeptin could be a useful marker of insulin resistance among PCOS patients. They also found that serum copeptin levels were significantly higher in the obese PCOS group compared to non-obese individuals and healthy controls and proved that plasma copeptin cut-off value for detecting insulin resistance in PCOS with 88% sensitivity and 36% specificity, with an AUC of 0.88 [13], which is not in agreement with the present study.

In the present study, when comparing serum copeptin levels in patients with metabolic syndrome and those without, the authors concluded that serum copeptin values were not statistically significant. Widecka J et al., in their case-control study involving 150 PCOS women, concluded that copeptin is associated with insulin resistance in PCOS patients, but due to low sensitivity, it cannot be considered as a marker of insulin resistance [14]. Similarly, a study by Saleem U et al., revealed a cross-sectional association between plasma copeptin and measures of insulin resistance and metabolic syndrome [15].

The present study showed copeptin levels were not significantly raised in patients with metabolic syndrome. Therefore, copeptin cannot serve as an independent marker for metabolic syndrome in patients with PCOS. Overall, it is evident from the study that circulating copeptin levels serve as a biomarker of insulin resistance and metabolic derangements in patients with PCOS [16].

Limitation(s)

Some of the limitations in the present study include the lack of a control group, the inability to establish the temporal changes in plasma copeptin and its relationship with pre-existing metabolic risk factors or the development of future complications. Additionally, the authors did not measure psychosocial stress in participants or their plasma cortisol levels, which is the final mediator of a perturbed HPA axis.

Furthermore, the authors reported insulin resistance based on HOMA-IR values. Although the gold standard for establishing insulin resistance is the Euglycaemic-hyperinsulinemic clamp, this elaborate procedure is not suitable as a screening method.

CONCLUSION(S)

The serum copeptin cannot be used as an independent marker for the diagnosis of metabolic syndrome in PCOS patients, as copeptin measurements in plasma have very low sensitivity. However, due to the limited availability of studies on serum copeptin in PCOS patients in the Indian context, further research in a larger cohort is warranted.

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