

Serum Levels of CCN3 Protein in Iranian Women with Polycystic Ovary Syndrome

Akram Vatannejad ^{1*}, Fatima Zahra Frouni ², Reza Fedaei ³, Azma Kheirollahi ⁴, Soheila Amaripour ⁵, Maryam Shoham Nashtaei ^{6*}, Nazam Maradi ⁷, Hossein Montakhab-Yeganeh ^{8*}, Fozeh Farahi ⁹, Farsh Jafarizadeh ¹⁰

¹ Department of Comparative Biomedicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

² Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

³ Sleep Disorders Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁴ Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

⁵ Department of Infertility, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

⁶ Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

⁷ Liver and Digestive Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

⁸ Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

⁹ Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

¹⁰ Emergency Department, Shariati Foyadshahr Hospital, Tehran, Iran

Abstract

Background: Polycystic ovary syndrome (PCOS) is a heterogeneous condition that encompasses several cardiometabolic and endocrinological disorders. Studies have shown that its pathogenesis aligns with several underlying mechanisms associated with recurrent pregnancy loss (RPL). Cellular communication network (CCN)-3 protein is a well-studied adipokine involved in tumorigenesis, organogenesis, inflammation, fibrosis, and glucose metabolism. The purpose of the current study was to determine the association of CCN3 levels with a number of parameters involved in PCOS pathogenesis.

Methods: This is a case-control study including 120 PCOS patients (60 cases with RPL; PCOS-RPL and 60 cases with infertility; PCOS-Inf and 60 healthy controls). Circulating levels of homocysteine and high-sensitivity C-reactive protein (hs-CRP), homocysteine, and CCN3 were measured using ELISA kits.

Results: Circulating levels of CCN3 were significantly elevated in PCOS-RPL and PCOS-Inf subgroups when compared to the control group (7.61 ± 3.03 and 6.85 ± 3.34 vs. 3.12 ± 0.82 , $p < 0.001$). Serum CCN3 positively correlated with fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR) in the control group ($p < 0.05$) and PCOS group ($p < 0.001$). Moreover, CCN3 was significantly associated with PCOS (OR 4.808, 95%CI [2.744-8.423], $p < 0.001$).

Conclusion: According the results of this study CCN3 may be involved in the pathogenesis of PCOS. However, future studies are needed to evaluate the possibility of utilizing CCN3 in the diagnosis and treatment of the disease.

Keywords: Abortion, C-reactive protein, CCN3, Homocysteine, Metabolic syndrome, Nephroblastoma overexpressed protein, Polycystic ovary syndrome.

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Introduction

Polycystic ovary syndrome is a complex endocrine-metabolic syndrome affecting approximately 10% of women of reproductive age

(1). It is characterized by polycystic ovaries on ultrasound, clinical and/or biochemical hyperandrogenism, and/or oligo- or anovulation. PCOS

is often associated with visceral adiposity, cardiometabolic diseases, and impaired fecundity such as Recurrent Pregnancy Loss (RPL) and infertility (2, 3). In fact, adiposity, insulin resistance, chronic low-grade inflammation, and hyperandrogenism create a vicious cycle, where each factor amplifies and exacerbates the severity of the others (4).

Adipose tissue is a crucial endocrine organ, releasing adipokines that modulate appetite and energy expenditure, along with insulin sensitivity. The aberrant release of these adipokines contributes to the development of insulin resistance and other metabolic disorders. For instance, PCOS patients exhibit such aberrations with reduced levels of adiponectin, C1q/tumor necrosis factor α -related protein (CTRP)-3, -5, -11, -13, and metastatin-like protein (Mleum) and elevated levels of leptin, and CTRP6 (5-11). In cases of recurrent implantation failure, an impaired balance between adiponectin and leptin is evident, characterized by abrogated endometrial leptin expression and downregulation of adiponectin receptor (AdipoR) expression (12).

Nephrinblastoma overexpresses NOV (also known as CCN3 or IGFBP9), a proto-oncogene that encodes a 48-kDa protein associated with the extracellular matrix (ECM) (13-15). CCN3 is a member of the CCN family of proteins, which also includes Cyr61/CCN1 (cysteine-rich protein 61), CTGF/CCN2 (connective tissue growth factor), Nov/CCN3, WISP-1/CCN4 (WNT1-inducible signaling pathway protein 1), and other related proteins (16). These regulatory proteins play diverse biological roles in cell proliferation, chemotaxis, angiogenesis, adhesion, and ECM (17).

While most of the research on CCN3 has focused on its role in tumorigenesis, fibrosis, and inflammation, its specific function in PCOS remains unclear (15, 18-31). However, CCN3 has been linked to metabolic disorders such as insulin resistance, obesity, dyslipidemia, and inflammatory conditions (32, 33), all of which have been identified as hallmarks of PCOS. Also, the association of CCN3 with a number of disorders including diabetes mellitus (DM), non-alcoholic steatohepatitis (NASH), cardiomyopathy, multiple and systemic sclerosis, rheumatoid arthritis (RA), and obstructive sleep apnea (OSA) has been reported (32, 34-41). Notably, studies have shown that CCN3 plays a role in placental angiogenesis during pregnancy (42, 43). Intriguingly, cumulus cells of infertile PCOS women show downregula-

tion in ECM proteins and cell adhesion molecules (44).

The purpose of the current research was to investigate the serum levels of CCN3 in women with PCOS, specifically examining its relationship with metabolic disturbances, such as insulin resistance, lipid metabolism, and reproductive complications including RPL and infertility. The findings from this study could provide valuable insights into the role of CCN3 in PCOS pathogenesis and its potential as a diagnostic marker or therapeutic target.

Methods

Study design, setting, and participants: This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Hormozgan University of Medical Sciences (IR.HUMS.REC.1393.415). Subjects were selected from the Obstetrics and Gynecology Department of Avicenna Fertility Center, Tehran, Iran, with healthy controls also recruited from the same center. An informed written consent form was obtained from all subjects. It is important to note that this study is in line with our previous report on PCOS and adipokines; therefore, some data have been utilized for the second time in the current study (10, 45, 46).

This is a case-control study consisting of 60 women with polycystic ovary syndrome and recurrent pregnancy loss (PCOS-RPL), 60 infertile women with PCOS (PCOS-Inf), and 60 healthy controls, all aged between 20 and 40 years (10, 45, 46). PCOS patients were those diagnosed according to the 2003 Rotterdam Criteria, after the exclusion of conditions such as hyperprolactinemia, thyroid diseases, premature ovarian failure, congenital adrenal hyperplasia, Cushing's syndrome, and adrenal tumors (47). Patients assigned to the PCOS-RPL subgroup were those who experienced more than two consecutive miscarriages earlier than the 20th week of gestation (48). Infertile patients were defined as those unable to conceive following one year of unprotected intercourse as a result of PCOS pathogenesis. The control group included fertile females with regular menstrual cycles and the absence of clinical or biochemical hyperandrogenism. Subjects who were smokers, pregnant, lactating, diagnosed with gynecological or obstetric disorders, taking hormonal therapy, or receiving medications for the past six months including glucocorticoids, prescription weight loss drugs, estrogens and anti-

androgens were excluded. Subjects diagnosed with viral, bacterial, or inflammatory diseases as well as those with cardiovascular disorders, thyroid disorders, or diabetes mellitus were also excluded.

Anthropometric and Biochemical measurements: Anthropometric data and medical histories were obtained from all subjects. Body mass index was calculated as weight divided by height squared (Kg m^{-2}). Blood samples were obtained from all subjects following overnight fasting at the follicular phase of their menstrual cycle. Serum samples were aliquoted in batches and frozen at -70°C . Fasting blood glucose (FBG), lipid profile (including serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)), fasting insulin, free testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and hs-CRP were measured as previously described (10, 45, 46). Insulin resistance was measured using the HOMA-IR, defined as $[(\text{FBG (mg/dl)}) \times (\text{fasting insulin (}\mu\text{U/ml)})] / 405$ (49).

Adipokine measurement: Serum levels of CCN3 protein were measured using a commercial ELISA kit (MyBioSource, USA). The intra- and inter-assay coefficients of variations (CV) of CCN3 were 6.2% and 6.7%, respectively. Circulating levels of adiponectin were assessed using the ELISA technique, as previously described (10, 45, 46).

Statistical methods: Statistical analyses were conducted using SPSS Statistics software 20.0 (IBM, USA). Kolmogorov-Smirnov test was used to check for normality. Continuous variables were presented in mean and standard deviation (SD). Statistical difference between PCOS and control group was assessed using Student's t-test. The statistical differences among the PCOS group, PCOS-Inf subgroup, PCOS-RPL subgroup, and control group were assessed using a one-way ANOVA, followed by Bonferroni correction for post hoc tests. The logarithms of nonparametric variables were calculated to approximate normal distribution. The correlation between circulating CCN3 with other variables was assessed using Pearson's correlation test. Multinomial logistic regression was performed to assess the association of serum CCN3 with PCOS risk. Finally, receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off level

of circulating levels of CCN3 that differentiate PCOS from controls. All tests were conducted as two-tailed, with statistical significance determined by a p-value of less than 0.05.

Results

Characteristics of the study population: The characteristics of the study population are presented in table 1. There was no significant difference between the control group and the PCOS group in terms of age, BMI, FBG, TG, LDL-C, and HDL-C. Regarding the glucose profile, the PCOS subgroups had significantly higher levels of fasting insulin and HOMA-IR when compared to the healthy controls ($p < 0.05$). Moreover, the PCOS subgroups had significantly elevated levels of TG when compared to the control group ($p < 0.05$). Regarding the hormonal profile, the PCOS-Inf subgroup exhibited significantly higher levels of LH and lower levels of FSH ($p < 0.05$) when compared to the healthy non-PCOS subjects. These results were similar in the PCOS-RPL subgroup but did not reach statistical significance. However, both subgroups showed significantly higher levels of free testosterone ($p < 0.001$) when compared to the control group. These findings were reported in our previous publications (10, 45, 46).

Serum levels of cardiovascular biomarkers and adipokines: The serum levels of homocysteine and hs-CRP were significantly higher in the PCOS group, PCOS-Inf, and PCOS-RPL subgroups when compared to the control group ($p < 0.001$ and $p = 0.0043$, respectively) (Table 1). On the other hand, circulating levels of adiponectin were considerably reduced in the PCOS group and subgroups when compared to their respective controls ($p < 0.001$). These findings were also reported in our previous publications (10, 45, 46). Nevertheless, serum levels of CCN3 were significantly elevated in PCOS group, PCOS-Inf, and PCOS-RPL subgroups ($7.23 \pm 3.00 \text{ pg/ml}$, $6.85 \pm 2.54 \text{ pg/ml}$, and $7.61 \pm 3.03 \text{ pg/ml}$, respectively, $p < 0.001$) (Figure 1).

Association of serum CCN3 with clinical parameters: The associations between serum CCN3 levels and the clinical and biochemical parameters of the control and PCOS groups are outlined in table 2. In the control group, serum levels of CCN3 significantly correlated with fasting insulin levels ($r = 0.243$, $p < 0.05$) and HOMA-IR ($r = 0.262$, $p < 0.05$); similarly, it directly correlated with fasting insulin levels ($r = 0.496$, $p < 0.001$) and HOMA-IR ($r = 0.493$, $p < 0.001$) in the PCOS group.

Table 1. Clinical features of the study population

Variables	Control group (n=60)	PCOS group (n=120)	PCOS-inf subgroup (n=60)	PCOS-RPL subgroup (n=60)	p-value ^a
Age (years)	28.58±4.47	28.64±4.21	28.57±4.18	28.72±4.43	0.979
BMI (kg/m ²)	21.44±2.15	21.87±2.43	21.62±1.47	24.23±5.38	0.325
Fasting (mg/dL)	91.76±9.38	91.15±10.04	91.75±12.00	90.22±10.00	0.761
Fasting insulin (μU/mL)	5.37±1.85	5.22±1.48 [*]	5.14±1.88 ^{**}	5.31±1.88 [*]	0.801
HOMA-IR	4.79±1.44	4.23±0.85 ^{**}	4.33±0.81 ^{**}	4.20±1.00 ^{**}	0.305
FG (mg/dL)	124.83±29.80	136.14±59.13	126.57±58.25	145.72±69.94	0.054
TC (mg/dL)	164.24±40.58	176.10±56.22 [*]	172.18±55.52 ^{**}	180.14±56.79 [*]	0.024
LDL-C (mg/dL)	95.65±30.61	102.84±29.71	102.60±29.46	102.86±31.12	0.349
HDL-C (mg/dL)	46.79±7.25	44.80±8.80	43.89±8.32	45.49±11.26	0.221
FFH (μU/L)	6.49±2.48	7.39±4.56	6.55±2.81 ^{**}	6.55±5.94	0.081
LH (μU/L)	6.79±2.68	7.81±4.88	6.80±3.12 ^{**}	6.82±2.25	0.002
Free testosterone (pg/mL)	1.54±0.35	3.14±1.19 ^{**}	3.06±0.82 ^{**}	1.27±1.29 [*]	<0.001
LH to FSH ratio	0.87±0.42	1.23±0.78 ^{**}	1.45±0.89 ^{**}	1.09±0.68	<0.001
hs-CRP (mg/L)	2.46±0.91	4.04±1.28 ^{**}	4.14±1.21 ^{**}	3.94±1.31 ^{**}	<0.001
Hemoglobin (g/dL)	14.79±3.73	12.61±3.99 ^{**}	12.40±3.74 [*]	12.82±7.39 [*]	0.048
Adiponectin (μg/mL)	5.72±1.48	2.79±1.43 ^{**}	2.70±1.34 ^{**}	2.86±1.53 ^{**}	<0.001
CCN3 (pg/mL)	1.12±0.82	7.22±2.40 ^{**}	6.85±2.94 ^{**}	7.41±3.03 ^{**}	<0.001

Parameters data are presented as mean ± standard deviation.

a. Comparison between control group vs. PCOS group. b. Comparison between control group vs. PCOS-inf subgroup. c. Comparison between control group and PCOS-RPL subgroup.

* p<0.05 is of statistical significance. A p-value obtained from ANOVA analysis.

PCOS: Polycystic Ovary Syndrome, BMI: Body Mass Index, FPG: Fasting Blood Glucose, TC: Total Cholesterol, LDL-C: Low-Density Lipoprotein Cholesterol, HDL-C: High-Density Lipoprotein Cholesterol, LH: Luteinizing Hormone, FSH: Follicle-Stimulating Hormone, hs-CRP: High-Sensitivity C-reactive protein.

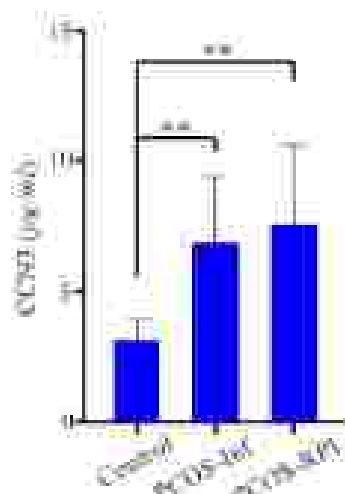


Figure 1. Serum levels of CCN3 in PCOS subgroups and control group.

PCOS: Polycystic Ovary Syndrome, RPL: Recurrent Pregnancy Loss, PCOS-inf: Infertile PCOS, CCN3: Cellular Communication Network.

Association of serum CCN3 with PCOS risk: Logistic regression analysis was performed to determine the association between circulating levels of CCN3 and PCOS. The results indicated a sig-

Table 2. Correlation of circulating CCN3 with anthropometric, hormonal, and biochemical variables

Variables	Control group (n=60)	PCOS group (n=120)
Age (years)	0.148	0.070
BMI (kg/m ²)	0.061	0.003
FPG (mg/dL)	0.553	0.136
FG (mg/dL)	0.173	0.101
TC (mg/dL)	0.175	0.143
LDL-C (mg/dL)	0.146	0.151
HDL-C (mg/dL)	0.147	0.014
FFH (μU/L)	0.093	0.016
LH (μU/L)	0.096	0.009
Free testosterone (pg/mL)	0.080	0.043
Fasting insulin (μU/mL)	0.208 [*]	0.005 ^{**}
HOMA-IR	0.262 [*]	0.493 ^{**}
hs-CRP (mg/L)	0.166	0.003
Hemoglobin (g/dL)	0.001	0.078
Adiponectin (μg/mL)	0.142	0.002

Pearson correlation analysis were performed to determine if an association exists between the variables. * p<0.05, ** p<0.01.

PCOS: Polycystic Ovary Syndrome, BMI: Body Mass Index, FPG: Fasting Blood Glucose, FG: Fasting Glucose, TC: Total Cholesterol, LDL-C: Low-Density Lipoprotein Cholesterol, HDL-C: High-Density Lipoprotein Cholesterol, LH: Luteinizing Hormone, FSH: Follicle-Stimulating Hormone, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

Table 3. Logistic regression analysis of the association between serum levels of CCN3 with PCOS

Model	Beta coefficient	Standard error	Wald statistic	Odds ratio (95%CI)	p-value
1	1.878	0.286	38.121	4.808 [2.744—8.423]	<0.001
2	1.592	0.291	29.361	4.918 [2.768—8.720]	<0.001

Model 1: not adjusted; Model 2: adjusted to age and BMI

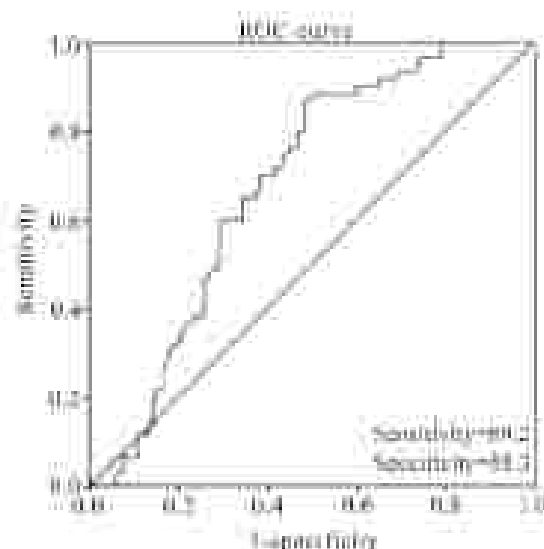


Figure 3. Receiver operating characteristic (ROC) curve analysis for predicting polycystic ovary syndrome (PCOS) using serum CCN3

AUC, Area Under the Curve

nificant association with the presence of PCOS (OR 4.808, 95%CI [2.744—8.423], $p<0.001$). The adjustment for the potential confounders including age and BMI did not affect the significance of the results (Table 3). Finally, ROC curve analysis was performed to evaluate the potential of CCN3 in predicting the occurrence of PCOS disease. The optimal cut-off point was 3.94 $\mu\text{g/ml}$, with a sensitivity of 89.2 and a specificity of 88.3. The area under the curve (AUC) was 0.680 (95%CI [0.603—0.758], $p<0.001$) (Figure 2).

Discussion

Metabolic inflexibility is a hallmark of the pathophysiology of PCOS, with mitochondrial dysfunction contributing to impaired fat oxidation, ectopic fat accumulation, and lipotoxicity. These processes exacerbate insulin resistance and hyperandrogenism which are the key features of the syndrome (50, 51). This study demonstrates for the first time that elevated serum concentrations of CCN3 are independently associated with

PCOS, providing evidence of its potential role in the disease's metabolic and endocrine dysfunction.

In this paper, the elevated levels of CCN3 in PCOS patients revealed an independent association with the pathogenesis of the disease, partly through insulin resistance. This was evidenced by the direct correlation between CCN3, fasting insulin levels, and HOMA-IR. Several reports highlight the role of CCN3 in glucose metabolism. *In vivo* studies showed that streptozotocin (STZ)-induced T1DM mice models exhibited reduced levels of CCN3, and an elevation occurred following insulin or anti-CCN2 antibody treatment (52, 53). Paradis et al. reported that CCN3 disrupted β -cell proliferation and insulin secretion (54). Elevated CCN3 levels in diabetic patients have been linked to glycemic parameters, including HOMA-IR and HbA_{1c}, suggesting a broader role in insulin-resistant states (32, 55). CCN3 levels predicted the presence of T1DM at levels exceeding 5.77 $\mu\text{g/ml}$, highlighting its possible role in the pathogenesis of the disease (32). Quite the contrary, in a South Indian population, Srinivas et al. reported a significant decline in CCN3, along with remarkable elevation in transforming growth factor (TGF)- β 1 and CCN2 levels, in diabetic patients suffering from diabetic foot ulcer and chronic kidney disease (CKD) when compared to those with no concurrent complications. The authors proposed that high blood glucose levels and inflammatory stimulus resulted in the elevated levels of TGF- β 1, which in turn, downregulated the expression of CCN3 and increased the activity of CCN2 (56). Intriguingly, CCN3 treatment abrogated and/or reversed glomerular fibrosis in BTRR ob/ob mice model of human obesity and diabetic nephropathy progression (57). The discrepancy might be related to the different stages of DM investigated in the articles. Similar to findings in gestational diabetes mellitus (GDM), elevated CCN3 levels in PCOS might contribute to insulin resistance, possibly through pathways involving glucose transport and inflammatory sig-

naling. Conversely, in the context of GDM, Zheng and Chen reported that resveratrol improved glucose uptake and insulin sensitivity in mice model and insulin-resistant adipocytes by upregulating miR-23a-3p expression and downregulating CCN3 expression via the activation of PI3K/Akt pathway. These effects were also observed following CCN3 knockdown (58). Furthermore, in a recent study, Wang et al. investigated the levels of CCN3 in pregnant women suffering from GDM and its relation with glucose homeostasis during pregnancy. The authors found that women with GDM exhibited higher levels of CCN3 compared to healthy pregnant females, suggesting its involvement in the pathogenesis of GDM. CCN3 overexpression in GDM mice model augmented glucose intolerance by upregulating glucose transporter (GLUT)-3 and affecting mammalian target of rapamycin (mTOR) pathway (59). Therefore, elevated levels of CCN3 are associated with insulin-resistant states including T2DM, GDM, and in this context, PCOS. These findings underscore the potential of CCN3 as a biomarker for diagnosing or predicting metabolic complications in PCOS, enabling earlier interventions to mitigate insulin resistance and associated comorbidities.

Dysfunctional adipose tissue in PCOS contributes to chronic low-grade inflammation and metabolic dysregulation (4). Increased CCN3 levels, driven by hypoxia and inflammatory cytokines in adipose tissue, may exacerbate these dysfunctions by promoting mitochondrial stress and impairing adiponectin production. Excess adiposity leads to adipocyte hyperplasia and hypertrophy, resulting in tissue hypoxia and the subsequent activation of hypoxia-inducible factor (HIF)-1 α , along with the release of pro-inflammatory adipokines such as tumor necrosis factor alpha (TNF- α) and CCN3. These changes contribute to major metabolic dysfunctions inducing insulin resistance, inflammation, and oxidative stress (34, 35, 60, 61). Circulating levels of CCN3 strongly correlate with BMI and fat mass. Morbidly obese patients had significantly higher levels of CCN3 when compared to lean individuals (39), and drastic weight loss resulted in a significant decline in CCN3 levels post-surgery (55). Despite the current evidence, no correlation was found between CCN3 levels and BMI, adiponectin levels, or lipid parameters. Several studies demonstrated the profound role of CCN3 in energy metabolism by promptly responding to glycolytic inhibition and glucose

starvation in chondrocytes, adipocytes, and fibroblasts (62-64). Martinerie et al. reported that inhibiting CCN3 resulted in an upregulation in the expression of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 α , thereby improving adipocyte differentiation, counteracting diet-induced obesity (DIO), reducing adipose tissue inflammation, and ameliorating insulin resistance (34). Trepiana et al. suggested that the negative correlation between CCN3 levels and adipogenic genes might be the result of a protective mechanism against the obesogenic diet (65). In obesity, CCN3 expression is upregulated leading to mitochondrial dysfunction and reduced adiponectin levels (65). This suggests a link between CCN3 and metabolic dysfunction in conditions characterized by excess adiposity, such as PCOS. Reports showed that elevated levels of CCN3 significantly correlate with metabolic disorders, including NASH, cardiomyopathy, and renal dysfunction (60, 66, 67). Targeting CCN3 pathways could potentially mitigate insulin resistance and inflammation in these conditions. In a recent study, Shan et al. reported that adipose-specific overexpression of PGC-1 α in DIO mice resulted in an improvement in HO-1, mitochondrial biogenesis, fasting glucose levels, blood pressure, and fibrosis. Moreover, browning of fat tissue was evident with the upregulation of UCP1, FGF21, and p-AMPK, and the downregulation of inflammatory cytokines, CCN3 expression, and TGF- β (68). Similar results were observed following the silencing of CCN3 (69). This indicates the pivotal role of CCN3 in adipogenesis and mitochondrial dysfunction, partly via the PGC-1 α -HO-1 signaling pathway. Intriguingly, HO-1 levels are significantly reduced in women with PCOS (30, 71). The elevated levels of CCN3, combined with reduced levels of HO-1, may represent another factor contributing to the pathogenesis of PCOS. Future studies are needed to provide a better insight on the pharmacological induction of HO-1 to reduce CCN3 levels as a potential therapeutic approach in PCOS.

Chronic low-grade inflammation is a hallmark in the pathogenesis of PCOS. It occurs as a consequence of metabolic and inflammatory cells residing in metabolic organs, such as the liver and skeletal muscle, in response to excess energy load, resulting in metabolic dysregulation and insulin resistance (72). PCOS and RPL individuals exhibit elevated levels of inflammatory cytokines including hs-CRP and homocysteine (4, 73-75).

However, no correlation was found between CCN3 and these cytokines. Nevertheless, CCN3 is governed by several inflammatory cytokines including TNF- α , IL-1 β , and TGF- β and is known to regulate the expression of other cytokines and chemokines (76). Pakradouni et al. suggested that CCN3-induced chemokine secretion might facilitate immune cell recruitment and in turn, influence adipose tissue remodeling and systemic insulin resistance (55). The relationship between CCN3 and inflammation seems to be contradictory. On one hand, high levels of CCN3 positively correlate with CRP in patients with metabolic disorders and rheumatoid arthritis (38, 55). CCN3 knockdown attenuated inflammation and apoptosis in human alveolar epithelial cells by inhibiting TGF- β /p-5mad and NF- κ B signaling pathways (77). On the other hand, CCN3 levels were drastically reduced in the atherosclerosis mouse model and individuals with abdominal aortic aneurysm (AAA). Intriguingly, the AAA phenotype of CCN3-knockout mice showed more aggressive inflammatory infiltration, ECM deterioration, smooth muscle cell loss, higher matrix metalloproteinase (MMP) activity, and heightened reactive oxygen species (ROS) production (78, 79). Moreover, Shi et al. found that CCN3 deficiency worsened atherosclerotic plaque burden by enhancing lipid accumulation within the macrophages (33). On the other hand, CCN3 overexpression abrogated inflammation and dyslipidemia in atherosclerosis and ameliorated AAA development via the ERK1/2 signaling pathway (78, 79). This discrepancy highlights the role of CCN3 in the inflammatory process. The lack of correlation of CCN3 with hs-CRP or homocysteine does not nullify the possibility of its relationship with the inflammatory aspect of PCOS. The association of CCN3 with the previously discussed inflammatory disorders indicates its potential involvement in PCOS, potentially mediated by more dominant pro-inflammatory cytokines.

Despite the novelty of the present study, there are some limitations. First, CCN proteins interact extensively with one another, exhibiting a range of overlapping or inhibitory functions. For instance, CCN2-knockout mice show an upregulation in CCN3 expression (30); CCN3-overexpression in atherosclerosis models significantly downregulates CCN1 and CCN2 expression (73); CCN3-overexpression or its administration significantly reduces CCN2 activity in mesangial cells and renal cortex, respectively (57, 81); and anti-

CCN2 antibody treatment elevates an otherwise downregulated CCN3 in NASH model (33). Therefore, analyzing CCN1 and CCN2 would be of high interest to understand how these proteins interact with one another in the context of PCOS. Second, BMI was used as a tool to assess body composition in this study; however, more accurate methods such as body composition analysis and functional body composition assessment would be more beneficial. Third, the findings may be specific to the Iranian population, which limits their generalizability. Finally, the methodology employed in the current study constrains the results to correlational analysis, thereby lacking definitive evidence of causation. Additional research is warranted to establish causal relationships.

Conclusion

Based on the findings of the current study, there is an independent association between CCN3 levels and PCOS, as well as a positive correlation with insulin resistance. Elevated serum CCN3 levels in PCOS patients may represent a compensatory response to underlying metabolic dysregulations, highlighting its potential as a biomarker for early detection. Additionally, CCN3 could serve as a therapeutic target for managing insulin resistance and associated metabolic dysfunctions in PCOS. However, the aforementioned potential warrants further research and clinical exploration.

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Conflict of Interest

Authors declare no conflict of interest.

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