Circulating C-Terminal Propeptide of Type I Collagen (CICP) Levels in Women with Polycystic Ovary Syndrome

Polikistik Over Sendromlu Kadınlarda Dolaşımdaki Tip 1 Kolajen C-Terminal Propeptid Düzeyleri

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Geliş Tarihi/*Received:* 08.01.2011
Kabul Tarihi/*Accepted:* 09.08.2011

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ABSTRACT Objective: Collagen type I is abundant in the outer layers of capsular stroma and theca externa in the ovary. C-terminal propeptide of Type I collagen (CICP) is the measurable form of type I procollagen in serum. Circulating CICP levels are indicative of collagen production. This study was designed to determine the serum levels of CICP and tissue inhibitor of metalloproteinase-1 (TIMP-1) levels in women with Polycystic Ovary Syndrome (PCOS). **Material and Methods:** This study included twenty-five women with PCOS in the study group and twenty healthy women in the control group. Serum lipid sub-fractions, fasting glucose and insulin, hormone (gonadotropins, androgens), CICP and TIMP-1 levels were measured. Homeostasis model assessment (HOMA-IR) was used to estimate insulin resistance. **Results:** Serum luteinizing hormone (LH) and fasting insulin levels, LH/follicule stimulating hormone (FSH) ratio, free androgen index (FAI) and HOMA-IR values were higher in patients with PCOS compared with healthy women. A significant increase in CICP level was observed in subjects with PCOS, and TIMP-1 level was found to be significantly decreased. HOMA-IR value was positively correlated with CICP level, but inversely with TIMP-1 level. The best cut-off values for CICP and TIMP-1 were >49.94 ng/mL (sensitivity 92.6% and specificity 65%) and <275.99ng/ml (sensitivity 92.6% and specificity 40%) respectively. **Conclusion:** Elevated circulating CICP levels may be associated with thickened tunica albuginea in women with PCOS. However, the exact role of CICP in the pathogenesis of the disease remains to be elucidated.

Key Words: Collagen Type I; polycystic ovary syndrome; procollagen type I carboxy terminal peptide; tissue inhibitor of metalloproteinase-1

ÖZET Amaç: Tip 1 kolajen overde kapsüler stroma ve teka eksternanın dış tabakalarında yoğundur. Tip 1 kolajen C-terminal propeptit [C-terminal propeptide of Type I collagen (CICP)], serumdaki tip 1 prokolajenin ölçülebilir şeklidir. Dolaşımdaki CICP düzeyleri kolajen üretiminin işaretidir. Bu çalışma, Polikistik Over Sendromu (PKOS) olan kadınlardaki serum CICP düzeylerini ve metalloproteinaz-1'in doku inhibitorü [tissue inhibitor of metalloproteinase-1 (TIMP-1)] düzeylerini saptamak için tasarlanmıştır. **Gereç ve Yöntemler:** Bu kontrollü klinik çalışmada PKOS'li yirmi beş kadın çalışma grubu ve sağlıklı yirmi kadın kontrol grubu olarak değerlendirildi. Serum lipit alt grupları, açlık glikoz ve insülin, hormon (gonadotropinler, androjenler), CICP ve TIMP-1 düzeyleri ölçüldü. İnsülin direncini değerlendirmek için homeostaz model değerlendirmesi (HOMA-IR) kullanıldı. **Bulgular:** Serum luteinizan hormon (LH) ve açlık insülin düzeyleri, LH/folikül stimüle edici hormon (FSH) oranı, serbest androjen indeksi (free androgen index-FAI) ve HOMA-IR değeri, PKOS'li hastalarda sağlıklı kadınlara kıyasla daha yüksekti. PKOS'li bireylerde serum CICP düzeyinde anlamlı bir yükselme gözlendiğinde, serum TIMP-1 düzeyi anlamlı olarak azalmış bulundu. HOMA-IR değeri CICP düzeyi ile paralellik gösterirken, TIMP-1 düzeyi ile ters bir ilişki sergilemekteydi. CICP ve TIMP-1 için en iyi eşik değerleri sırasıyla >49,94 ng/ml (duyarlılık %92,6, özgüllük %65) ve <275,99 ng/ml (duyarlılık %92,6 ve özgüllük %40) olarak tespit edildi. **Sonuç:** PKOS'li kadınlarda dolaşımdaki artmış CICP düzeyleri tunika albugineanın kalınlaşması ile ilişkili olabilirse de, CICP'nin hastalığın patogenezindeki kesin rolü halen bilinmemektedir.

Anahtar Kelimeler: Kolajen tip I; polikistik over sendromu; prokolajen tip I karboksi terminal peptide; metaloproteinaz-1' in doku inhibitörü

doi: 10.5336/medsci.2011-22566

Turkiye Klinikleri J Med Sci 2012;32(1):88-93

Polycystic ovary syndrome (PCOS) is a common disorder affecting approximately 7% of women at reproductive age and it is characterized by oligo-amenorrhea, enlarged cystic ovaries and cystogenesis, signs of androgen overproduction, disordered gonadotropin secretion, and reduced fertility.[1-3] Insulin resistance (IR) plays a crucial role in the pathogenesis of PCOS.[4-6]

Follicular growth, ovulation, corpus luteum formation, and corpus luteum regression are associated with cyclical ovarian tissue remodeling. The collagenous components in the basement membrane layer of ovaries comprise type I, III and IV collagen.[7] Collagenolytic activity increases throughout ovarian cycle.[8] The basement membrane layer becomes thinner, but it remains intact.[9] Collagen type I is abundant in the outer layers of capsular stroma and theca externa in the ovary. C-terminal propeptide of Type I collagen (CICP) is the measurable form of type I procollagen in serum. Circulating CICP levels are indicative of collagen production. The rupture of follicular wall needs a proteolytic enzyme system. This system includes matrix metalloproteinases (MMPs), which have more than 26 members. MMPs and tissue inhibitor of metalloproteinases (TIMPs) regulate the extracellular matrix (ECM) reorganization.[10] The ovarian cycle is characterized by cyclic remodeling of ECM around the follicles.[11] The ECM comprises the proteinaceous and nonproteinaceous components and provides architectural support to the ovarian cells. MMPs degrade especially proteinaceous components. TIMPs inhibit activities of MMPs. There is a moderate correlation between TIMP and MMP levels and insulin resistance.[12] Insulin resistance may contribute to the development of thickened, fibrotic tunica albuginea and cystic ovaries by enhancing both ovarian theca and granulosa cell function in women with PCOS.[5,13]

During the last decade, ovarian follicular enzymes have gained attention to understand the mechanisms underlying anovulation. This study was designed to determine the serum levels of C-terminal propeptide of Type I collagen (CICP) and tissue inhibitor of metalloproteinase-1 (TIMP-1) levels in women with PCOS.

MATERIAL AND METHODS

SUBJECTS

Patients with PCOS (n= 25) aged between 17-35 years and healthy control subjects (n= 20) aged between 18-37 years were enrolled in this study. The healthy controls were checked by medical history, physical and pelvic examinations, and entire blood chemistry. This study was approved by the local medical ethics committee and all participants gave informed consent before the onset of study.

The diagnosis of PCOS was based on Rotterdam consensus criteria on PCOS by two of the following three features: i) clinical and/or biochemical signs of hyperandrogenism, ii) oligo- or anovulation and iii) polycystic ovaries.[14] Common findings of the PCOS group were; 12 or more subcapsular follicles by transvaginal ultrasound examination, clinical hyperandrogenism with the presence of acne and hirsutism (Ferriman-Gallwey score of ³8), and oligomenorrhea (≤6 menses/year).[15] Exclusion criteria included consuming alcohol and/or smoking, infectious diseases, use of medications known to alter insulin secretion or action, and lipoprotein metabolism, hypertension, family history of cardiovascular disease, and endocrinopathies including diabetes, Cushing's Syndrome or androgen secreting tumors, late-onset 21-hydroxylase deficiency, thyroid dysfunction, and hyperprolactinemia.

The subjects in the control group had regular menstrual cycles (cyclic uterine bleedings with duration of 4-5 days and a frequency of 25-34 days/month) and none of them met any exclusion criteria mentioned above.

BIOCHEMICAL ANALYSIS

Venous blood samples were drawn in the morning after at least 10 hours of fasting on the study day (on cycle, days 3-5 after spontaneous or progesterone-induced menses in the PCOS and control groups). Serum fasting glucose (F.Glc), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL) levels were determined by Hitachi 917 Clinical Chemistry Analyzer using diagnostic kits supplied by Roche Diagnos-

tics. Low-density lipoprotein cholesterol (LDL) levels were calculated by using the Friedewald's formula. Fasting insulin levels were measured by Hitachi E170 Analyzer using diagnostic kits supplied by Roche Diagnostics. Sex hormone-binding globulin (SHBG) immunometric assay was performed using a solid phase competitive chemiluminescence immunoassay (Immulite 2000, DPC Biosystems, CA, USA). Follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone levels were determined by using diagnostic kits (Bayer Corporation, Tarrytown, NY, USA) in Advia Centaur Immunoassay System.

Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (mIU/L) x glucose (mmol/L)/22.5.[16] Individuals with HOMA-IR> 2.7 were considered insulin resistant.

Free androgen index (FAI) was defined here as 100 times the molar ratio of total testosterone to SHBG [FAI= 100 x total testosterone (nmol/L)/ SHBG (nmol/L)].

TIMP-1 and CICP levels were determined by using commercially available human immunoassay kits supplied by Biosource International, Inc. (Catalog number: KHC1492/KHC1491, California, USA) and Quidel Corporation (Catalog number: 8003, San Diego, USA). The results were expressed as ng/ml for both TIMP-1 and CICP.

ANTHROPOMETRIC MEASUREMENTS

All anthropometric measurements were performed by the same physician on the day blood specimen were collected. Waist and hip circumferences (cm) were obtained and body mass index (BMI) (Body weight (kg)/height m^2) and waist-to-hip ratio (WHR) were computed.

STATISTICAL ANALYSIS

Data were analyzed by using the SPSS (Statistical Package for the Social Science) version 17.0. The data were expressed as means ± standard deviation (SD). Since many variables had a non-gaussian distribution with significant skewness, statistical analysis was performed by a non-parametric test, Mann-Whitney U test. Correlations between variables were calculated by the Pearson's correlation coefficient. Stepwise multiple regression analysis was also run introducing serum CICP level as dependent variable and the other parameters having +/- correlations as independent variables. After addition of each new independent variable to the equation, all previously entered independent variables were checked to see whether they maintained their level of significance. Previously entered independent variables were retained in the regression equation only if their removal would have caused a significant reduction in R^2. In addition, the cut-off values, area under curve (AUC) values, sensitivity, and specificity were calculated by the receiver operating characteristic (ROC) curve technique with 95% confidence intervals (CI) for CICP and TIMP-1. Statistical significance was set as $p< 0.05$.

RESULTS

There were no statistically significant differences in age, BMI, waist measurements, waist/hip ratio, serum lipid parameters and FSH levels between the groups. However FAI, total testosterone, HOMA-IR, serum LH and fasting insulin levels, and LH/FSH ratios were considerably higher in patients with PCOS compared with healthy women. A significant increase in CICP level was observed in subjects with PCOS, and TIMP-1 level was found to be significantly decreased (Table 1 and 2).

HOMA-IR was positively correlated with CICP (r= 0.38, p= 0.021). However, TIMP-1 was negatively associated with HOMA-IR (r= -0.33, p= 0.045). CICP was positively correlated with LH/FSH ratio (r= 0.36, p= 0.027). FAI was positively associated with total testosterone (r= 0.74, p= 0.0001), LDL (r= 0.32, p= 0.031), fasting insulin (r= 0.58, p= 0.0001), HOMA-IR (r= 0.52, p= 0.0001), and negatively with SHBG (r= -0.65, p= 0.0001). FAI was not significantly associated with CICP and with TIMP-1. Although, as seen above, the degrees of relationships between many variables were small, stepwise multiple regression analysis revealed that HOMA-IR and LH/FSH ratio were strong predictors of serum CICP levels (Table 3).

TABLE 1: Clinical features and clinical chemistry parameters for healthy controls and women with PCOS.

Variable	Healthy Controls (n= 20)	PCOS (n= 25)	p
Age, years	24.50 ± 2.43	23.28 ± 4.89	0.102
BMI (kg/m^2)	25.18 ± 4.67	23.32 ± 3.72	0.192
Waist (cm)	72.91± 15.30	73.64 ± 10.02	0.554
Waist/Hip ratio	0.73 ± 0.07	0.74 ± 0.06	0.378
Fasting Glucose (mg/dl)	87.75 ± 7.65	88.88 ± 8.32	0.432
Fasting Insulin (mIU/l)	8.35 ± 2.36	14.25 ± 4.64	0.0001[a]
HOMA-IR	1.79 ± 0.57	3.08 ± 1.04	0.0001[a]
Total Cholesterol (mg/dl)	159.91 ± 31.38	176.96 ± 29.30	0.192
HDL-Cholesterol (mg/dl)	50.06 ± 6.65	48.03 ± 11.68	0.170
LDL-Cholesterol (mg/dl)	115.36 ± 52.71	109.32 ± 27.78	0.962
Triglycerides (mg/dl)	97.16 ± 39.52	87.24 ± 31.76	0.471
TIMP-1 (ng/ml)	273.64 ± 11.35	235.97 ± 5.48	0.042[a]
CICP (ng/ml)	51.51 ± 3.53	61.85 ± 2.29	0.013[a]

[a]p<0.05 statistically significant.

BMI, body mass index; CICP, C-terminal propeptide of type I collagen; HOMA-IR, Homeostasis Model Assessment; HDL-Cholesterol, high-density lipoprotein cholesterol; LDL-Cholesterol, low-density lipoprotein cholesterol; PCOS, polycystic ovary syndrome; TIMP-1, tissue inhibitor of metalloproteinase-1.

TABLE 2: Steroid levels for both healthy controls and women with PCOS.

Variable	Healthy Controls (n=20)	PCOS (n=25)	p
FSH (mIU/ml)	4.99 ± 1.60	4.45 ± 1.49	0.227
LH (mIU/ml)	5.38 ± 1.68	10.09 ± 5.93	0.001[a]
LH/FSH ratio	1.12 ± 0.34	2.21 ± 0.86	0.0001[a]
Total testosterone (nmol/l)	1.22 ± 0.2	2.48 ± 0.1	0.0001[a]
SHBG (nmol/l)	73.2 ± 6.1	20.5 ± 1.4	0.0001[a]
FAI	1.6 ± 0.1	12.1 ± 0.7	0.0001[a]

[a]p< 0.05 statistically significant.

FAI, free androgen index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin.

The sensitivity, specificity and cut-off values for serum CICP and TIMP-1 levels were shown in Table 4. The sensitivity and specificity curves of these parameters were shown in Figure 1. There was no significant difference between the AUC values of CICP and TIMP-1 (0.784 versus 0.691; p = 0.30, Table 4, Figure 1).

TABLE 3: Stepwise multiple regression analysis, serum CICP level as dependent variable.

	Beta	p
LH/FSH ratio	0.441	0.001[a]
HOMA-IR	3.183	0.007[a]

[a]p< 0.05 statistically significant.

CICP, C-terminal propeptide of type I collagen; FSH, follicle-stimulating hormone; LH, luteinizing hormone; HOMA-IR, Homeostasis Model Assessment.

DISCUSSION

The ECM supplies a particular microenvironment by regulating activities of the cells and it also provides binding proteins, cytokines, and some growth factors.[11] MMPs and TIMPs are essential to control the turnover of ECM.[17,18] Most follicles (approximately 99%) become atretic; a small percentage of follicles undergo ovulation.[19] Follicular atresia seems to be the consequence of apoptosis and tissue remodeling. The concept that PCOS is associated with high rate of follicular atresia is not well established, even though defective cellular control mechanism in PCOS may cause lack of ovulation.[20,21] Interestingly, a decreased rate of atresia was demonstrated in culture of follicles from polycystic ovaries.[22]

TABLE 4: Sensitivity and specificity values of serum CICP and TIMP-1 levels.

	Cut-off value	Sensitivity (%)	Specificity (%)	AUC	95%CI	p
CICP (ng/ml)	>49.94	92.6	65	0.784	0.640 – 0.891	0.0001[a]
TIMP-1 (ng/ml)	<275.99	92.6	40	0.691	0.539 – 0.817	0.02[a]

[a]p<0.05 statistically significant.

AUC; area under curves; CI, confidence intervals; CICP, C-terminal propeptide of type I collagen; TIMP-1, tissue inhibitor of metalloproteinase-1.

Four different types of TIMP have been identified (TIMP 1-4).[23,24] TIMP-1 may contribute to luteal development and stimulation of steroidogenezis.[25,26] Especially, TIMP-1 acts as a growth factor and provides an appropriate microenvironment, and stimulates progesterone production.[25,26] Reduced serum TIMP-1 levels may be in favor of apoptosis. Increased preovulatory collagenolytic activities in the ovarian follicles may be regulated by TIMP-1.[27] The MMP/TIMP ratio may assign some ovarian functions such as proliferation and apoptosis.[28] Insulin resistance may contribute to the inappropriate microenvironment of follicles by decreasing TIMP-1 level in PCOS. In the current study, decreased serum TIMP-1 levels were observed in women with PCOS, and TIMP-1 was negatively associated with HOMA-IR.

Collagen is synthesized as procollagen. It consists of mature collagen with extension peptides at both the amino and carboxyl termini. Some proteases cleave these peptides. Collagen type I is abundant in the outer layers of capsular stroma and theca externa in ovary.[29] The syntheses of collagen type I decreases continuously from the preovulatory stage to the postovulatory stage.[30] CICP is the measurable form of the type I procollagen with extension peptide at the carboxyl termini in serum. Circulating CICP levels are indicative of collagen production. In this study, we determined the increased basal CICP level in women with PCOS and it was positively correlated with HOMA-IR. It will be reasonable to presume that insulin resistance may be responsible for the development of thickened fibrotic tunica albuginea by increasing collagen type I production in ovarian stroma and theca externa in women with PCOS. However, this presumption exceeds the scope of the current study and may be a subject for further investigations.

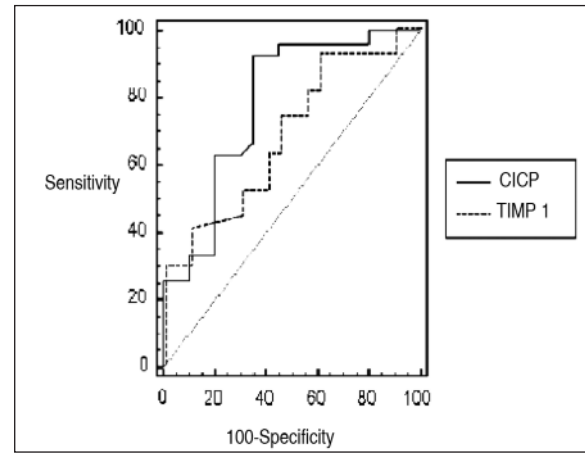

FIGURE 1: Sensitivity and specificity curves for serum CICP and TIMP-1 levels.

Being a study to develop a nomogram, this study concludes that cut-off values of >49.94 for CICP and <275.99 for TIMP-1 are statistically significant. However, the changes in CICP and TIMP-1 levels do not differ whether these parameters are involved primarily or secondarily in the pathogenesis of PCOS. If further pathological and/or molecular studies prove that these changes are secondary to the development of PCOS, those cases outside the defined minimum and maximum ranges would be explained by secondary involvement in the process. On the other hand, a primary involvement may implicate that these parameters would be included in Rotterdam Criteria for diagnosis of PCOS.

In conclusion, elevated circulating CICP levels may be associated with thickened tunica albuginea in women with PCOS, nevertheless the exact role of CICP in the pathogenesis of the disease remains to be elucidated.

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