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Serum zonulin level is not elevated in patients with polycystic ovary syndrome without metabolic syndrome

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Abstract

Aim Polycystic ovary syndrome (PCOS) is a complex disorder with gynecological, metabolic and carcinogenic effects. Increased intestinal permeability is related with obesity, insulin resistance, type 1 and 2 diabetes mellitus. The existence of such a relationship between PCOS and intestinal permeability has come to an end. Zonulin can change intestinal permeability, and this effect is reversible. We studied the relation between zonulin and the hormonal and metabolic parameters of PCOS.

Method A total of 45 women with PCOS and 17 healthy women were included in the study. Histories were taken from all the participants, body mass indexes were calculated, and biochemical tests and suprapubic over ultrasonography were made. Zonulin was studied with enzyme-linked immunosorbent assay.

Results Serum zonulin levels were similar between PCOS and control groups ($p=0.893$). In all participants, there were negative correlations between zonulin and the total cholesterol, LDL-cholesterol, triglycerides and non-HDL-cholesterol (respectively, $p=0.00$, 0.018, 0.004, 0.002), there were boundary correlations with age and total cholesterol/HDL-cholesterol (respectively, $p=0.052$ and 0.058). No statistically significant was detected in the PCOS group except negative correlation between zonulin and age ($p=0.046$), boundary correlation between zonulin and total cholesterol/HDL-cholesterol ($p=0.064$).

Conclusion PCOS patients did not have metabolic syndrome. Zonulin was not higher in PCOS then controls, and it had only negative relation with age. The negative relation between zonulin and some metabolic parameters in all participants was not detected in PCOS group. So zonulin is not a useful molecule for the diagnosis of PCOS without metabolic syndrome.

Keywords Polycystic ovary syndrome · Zonulin · Metabolic syndrome

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinological pathology in women of reproductive age. It is characterized by hyperandrogenism and chronic anovulation. Diagnosis and treatment are important in terms of causing endometrial cancer with metabolic and cardiovascular diseases as well as sexual dysfunction [1, 2].

Obesity is commonly seen in PCOS, and gut permeability is increased in obesity. Other pathological conditions with increased gut permeability, different than obesity, are insulin resistance, irritable bowel syndrome and chronic fatigue syndrome [3]. These are also common in women with PCOS. Therefore, increased gut permeability in the pathogenesis of PCOS may play a role [3].

Zonulin is the equivalent of zonula occludens toxin of vibrio cholera in eukaryotic cells, and it intensifies permeability by reversibly affecting intestinal tight ligaments. The level of this protein has been shown to increase in insulin

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resistance, obesity, type 1 and type 2 DM and celiac disease [4–6]. The relationship with PCOS is under investigation recently. The aim of this study was to determine the relationship between serum zonulin levels and gynecological and metabolic parameters in PCOS patients.

Materials and method

Sixty two premenopausal women were informed and incorporated into the study. Forty five of these women were diagnosed with polycystic over syndrome; other 17 women were included as control group. None of the participants used any medicine which affecting metabolic, gynecological and hormonal parameters. None of them had a systematic disease. All volunteer patients were included in the study by excluding from other factors causing hyperandrogenism [congenital adrenal hyperplasia (CAH), Cushing Syndrome, drugs, androgen-producing tumors, etc.]. The body mass index and the Ferriman–Galley score were calculated for patients suffering from hirsutism. They were questioned whether they suffered from smoking, menstrual irregularity, acne, seborrhea and androgenic alopecia. 1 mg dexamethasone suppression was tested to exclude Cushing's syndrome. CAH was excluded with performing ACTH stimulation test in the participants with high baseline 17 hydroxy progesterone (17-OH P).

Blood samples were drawn from all participants after 8 h of fasting, and these samples were centrifuged and conserved − 80 °C. Blood samples necessary for hormonal parameters were drawn in 3–5 days of menstruation from women with regular menses. Patients with menstrual irregularities gave these at the first application.

In all participants, fasting plasma glucose (FPG), fasting plasma insulin, total cholesterol, triglyceride, high density lipoprotein (HDL-C) cholesterol, low density lipoprotein (LDL-C), C-reactive protein (CRP), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, total testosterone, 17 hydroxy progesterone (17-OH P), dehydroepiandrosterone sulfate (DHEAS), prolactin and thyroid stimulating hormone (TSH) levels were measured. Serum zonulin levels (ng/ml) were studied by enzyme-linked immunosorbent assay (Elabscience Biotechnology Co., Texas, ABD). The assay sensitivity was 0.781–50 ng/ml pg/ml; coefficient of variation was < 10%.

Insulin resistance was calculated by the HOMA-IR method (fasting plasma glucose (mg/dl) × insulin (mU/ml)/405); triglyceride/HDL-C ratio, total cholesterol/HDL-C ratio, HDL-C/LDL-C ratio were detected. The cholesterol retention fraction (CRF) was calculated by using the LDL-C–HDL-C/LDL-C formula.

Bilateral ovary ultrasonography was applied to all participants by the same radiologist, using suprapubic approach, and over size and the appearance of the ovaries were evaluated.

Statistical studies were analyzed in the IBM SPSS for Windows 22.0 program. Numerical variables were summarized by the mean ± standard deviation or median (min–max). Categorical variables were stated by number and percentage. Whether there were differences in terms of categorical variables among groups were researched by Chi-square test or Fisher's exact test. Whether the numerical variables showed a normal distribution or not was analyzed by Kolmogorov–Smirnov test, and the homogeneity of the variances was analyzed by Levene test. In case parametric assumptions could be provided, differences between two independent groups were analyzed by *t* test. In case parametric test assumptions could not be provided, Mann–Whitney *U* test was used. Spearman's correlation coefficient was used to show whether there was a relationship between numerical variables. Significance level was taken as $p < 0.05$.

Results

A total of 62 premenopausal women were included in our study. PCOS was detected in 45 patients. According to the distribution analysis, age, F–G score, APG, total cholesterol, LDL-C, HDL-C, non-HDL-C, total cholesterol/HDL-C ratio, corrected calcium, phosphorus, uric acid, FSH variables showed normal distribution; other variables did not show. The groups were compared with the mean ± standard deviation for the normally distributed variables, and the median (minimum–maximum) values for those who did not show normal distribution (Table 1). LH, LH/FSH ratio, total testosterone, androstenedione and DHEAS levels were significantly higher in the PCOS group than the control group. There was no significant difference between the groups in other parameters.

The zonulin levels were similar between the two groups, and no significant difference was found ($p = 0.893$). Correlation analysis was performed between zonulin and other variables in all participants and the PCOS group. In all participants, negative correlation was detected between zonulin and total cholesterol, LDL-C, triglyceride and non-HDL-C, also boundary negative correlation between zonulin and age and total cholesterol/HDL-C was determined. Negative correlation with age and boundary negative correlation with total cholesterol/HDL were stated in PCOS group (Table 2).

Discussion

Our study is the second study investigating the relationship between zonulin and PCOS in the literature. The first study was conducted by Zhang et al. [3]. According to the first

Table 1 Demographic features, metabolic and hormonal values

	Control group ($n=17$)	PCOS group ($n=45$)	p value
Age	23.18 ± 3.95	22.51 ± 3.80	0.584
BMI (kg/m^2)	25 (20.20–44.96)	24.03 (19.05–39.10)	0.290
FPG (mg/dl)	88 ± 6.32	88 ± 8.02	0.992
Creatinine (mg/dl)	0.77 (0.6–0.91)	0.77 (0.65–1.18)	0.305
Uric acid (mg/dl)	4.68 ± 0.76	4.42 ± 0.67	0.399
Calcium (mg/dl)	9.30 ± 0.43	9.34 ± 0.30	0.720
Phosphorus (mg/dl)	3.45 ± 0.50	3.27 ± 0.41	0.268
Total cholesterol (mg/dl)	172.50 ± 39.80	171.87 ± 32.51	0.954
LDL-C (mg/dl)	106.43 ± 29.52	97.68 ± 27.13	0.312
HDL-C (mg/dl)	51.75 ± 11.06	53.96 ± 10.26	0.495
Triglyceride (mg/dl)	93 (41–202)	90 (50–381)	0.896
Non-HDL-C (mg/dl)	124.56 ± 31.74	117.90 ± 32.36	0.502
Total cholesterol/HDL-C	3.42 ± 0.83	3.29 ± 0.86	0.621
Triglyceride/HDL-C	1.75 (0.65–5.77)	1.63 (0.8–10)	0.810
LDL-C/HDL-C	0.46 (0.3–0.86)	0.58 (0.32–1.41)	0.294
CRF	0.53 (0.14–0.70)	0.41 (−0.41–0.68)	0.294
CRP (mg/l)	2 (0.2–14)	1 (0.2–11)	0.600
Fasting insulin (mIU/ml)	7.52 (3.31–24.91)	7.54 (2.57–41.36)	0.705
HOMA-IR	1.68 (0.7–4.49)	1.71 (0.47–7.25)	0.906
FSH (mIU/ml)	5.29 ± 1.91	6.03 ± 2.27	0.240
LH (mIU/ml)	6.24 (1–18.53)	9.68 (2–39.11)	0.014*
LH/FSH ratio	0.903 (0.41–3.75)	1.63 (0.33–5.43)	0.014*
E_2 (pg/ml)	73.25 (15–216)	49.5 (14.28–304)	0.166
Total testosterone (ng/dl)	0.55 (0.23–0.74)	0.78 (0.03–5.49)	0.001*
17-OH P (ng/dl)	1.085 (0.5–2.76)	1.32 (0.62–6.97)	0.121
Androstenedione (ng/dl)	1.43 (1–3.51)	2.35 (0.43–5.79)	0.033*
DHEAS (mcg/dl)	284.75 (147–505.3)	359.55 (106.9–819)	0.040*
Prolactin (ng/ml)	12.14 (5.36–25)	12.79 (6.27–37.95)	0.881
TSH (mU/l)	1.56 (0.69–4.43)	1.88 (0.69–5.82)	0.473
Zonulin (ng/ml)	43.528 (9.84–72.38)	42.889 (1.568–90.337)	0.893

BMI body mass index, *PCO* polycystic over, *FSH* follicle stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *17-OH P* 17 hydroxy progesterone, *DHEAS* dihydroepiandrostenodian sulfate, *TSH* thyroid stimulating hormone, *FPG* fasting plasma glucose, *HOMA-IR* homeostatic model values for insulin resistance, *LDL-C* low density lipoprotein-cholesterol, *HDL-C* high density lipoprotein-cholesterol, *non-HDL-C* lipoproteins other than HDL, *CRF* cholesterol retention fraction, *CRP* C-reactive protein, *OPG* osteoprotegerin

*Statistically significant p values

study, different results were obtained in our study. First, zonulin level was similar between the control group and the PCOS group. Second, there was no association with HOMA-IR. Finally, negative correlations between FPG, lipid parameters and zonulin were not observed in the PCOS group.

In recent years, a large number of studies have been carried out on the structure and function of tight bonds between cells. Detection of zonulin has pointed out intestinal paracellular permeability [7]. This protein increases the permeability of intestinal tight bonds rapidly and reversibly [4]. The zonulin system is responsible for the movement of fluid, macromolecules, and leukocytes between the blood vessels and the intestinal lumen [8], and the protection of the proximal small intestine mucosa against the microorganisms' colonization [9]. Small bowel infections are involved in the pathogenesis of allergic, autoimmune and inflammatory diseases by weakening the intestinal barrier. When the small intestine is exposed to enteric bacteria, zonulin is secreted. The zonulin-induced paracellular pathway allows the washing of microorganisms, thus preventing bacterial colonization [9].

Unusual changes in intestinal permeability are involved in the pathogenesis of many diseases. These include Type 1 DM, celiac disease, multiple sclerosis, and autoimmune diseases such as rheumatoid arthritis. The deterioration in the intestinal permeability leads to passage of the antigens from

Table 2 Correlation analysis between zonulin and other parameters

	Zonulin (ng/ml)			
	All participants ($n=62$)		PCOS group ($n=45$)	
	R	*P*	*R*	*P*
Age	−0.248	0.052*	−0.299	0.046*
BMI (kg/m^2)	−0.195	0.210	−0.315	0.090
FSH (mIU/ml)	+ 0.089	0.491	−0.073	0.633
LH (mIU/ml)	+0.007	0.958	−0.011	0.941
LH/FSH ratio	−0.061	0.642	−0.024	0.876
E2 (pg/ml)	−0.134	0.303	−0.092	0.555
Total Testosterone (ng/dl)	−0.028	0.834	−0.100	0.533
Prolactin (ng/ml)	+0.023	0.858	+0.163	0.285
TSH (mU/l)	−0.217	0.090	−0.189	0.214
17-OH P (ng/dl)	−0.126	0.347	−0.099	0.532
Androstenedione (ng/dl)	+0.179	0.250	+0.234	0.230
DHEAS (mcg/dl)	+0.127	0.349	0.196	0.225
FPG (mg/dl)	−0.263	0.039*	−0.266	0.078
Fasting Insulin (mIU/ml)	−0.158	0.220	−0.242	0.109
HOMA-IR	−0.173	0.178	−0.269	0.074
Total cholesterol (mg/dl)	−0.488	0.00*	−0.077	0.613
LDL-C (mg/dl)	−0.341	0.018*	−0.078	0.612
Triglyceride (mg/dl)	−0.406	0.004*	−0.223	0.141
HDL-C (mg/dl)	−0.190	0.197	+0.024	0.875
Non-HDL-C (mg/dl)	−0.429	0.002*	−0.117	0.443
Total cholesterol/HDL-C	−0.276	0.058*	−0.332	0.064*
Triglyceride/HDL-C	−0.252	0.084	−0.318	0.076
LDL-C/HDL-C	+0.191	0.193	0.250	0.167
CRF	−0.191	0.193	−0.250	0.167
CRP (mg/l)	−0.267	0.147	−0.231	0.372

BMI body mass index, *PCO* polycystic over, *FSH* follicle stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *17-OH P* 17 hydroxy progesterone, *DHEAS* dihydroepiandrostenodian sulfate, *TSH* thyroid stimulating hormone, *FPG* fasting plasma glucose, *HOMA-IR* homeostatic model values for insulin resistance, *HDL-C* high density lipoprotein-cholesterol, *LDL-C* low density lipoprotein-cholesterol, *non-HDL-C* lipoproteins other than HDL, *CRF* cholesterol retention fraction, *CRP* C-reactive protein, *OPG* osteoprotegerin

*Statistically significant *p* values

the intestinal lumen to the blood, and in individuals with genetic sensitivity, the immune system produces an immune response to a target organ or tissue. Intestinal permeability also affects cancer and infection pathogenesis. Serum zonulin levels were found to be high in squamous cell oral cancer, lung cancer, liver and pancreatic cancers. In people with genetic predisposition, intestinal barrier function triggers cancer, inflammation, and autoimmunity after exposure to environmental factors, with the activation of the zonulin pathway [7].

In obese individuals, inflammation in the small intestine leads to changes in intestinal permeability. Furthermore, serum zonulin level was found to be increased in correlation with BMI, waist–hip ratio, FPG, fasting insulin, fasting triglycerides and interleukin 6 (IL-6). IL-6 is a cytokine associated with obesity and increases zonulin expression [10]. Zonulin gene was found to coincide with the pre-haptoglobulin 2 gene [11]. The study of the pre-haptoglobulin 2 gene is also under the control of IL-6 [12]. In people with glucose intolerance, serum zonulin levels were strongly associated with insulin resistance and obesity [11]. Also, in patients with normal glucose tolerance, zonulin has been shown to be associated with high uric acid, HbA1c, serum interleukin-6 and low HDL [11].

The prevalence of obesity in PCOS suggests that impaired intestinal barrier function may have a role in the pathogenesis of PCOS due to PCOS patients' susceptibility to glucose intolerance, insulin resistance and hyperlipidemia [13, 14]. Zhang et al. [3] made the first study investigating the possible relationship between zonulin and PCOS. According to this study, zonulin levels were found to be higher in PCOS group and it was higher in patients with heavier menstrual dysfunction. Both obese PCOS and non-obese PCOS patients were found to have higher zonulin levels than obese and non-obese controls. There was a strong correlation between serum zonulin level and BMI, triglyceride, FPG and HOMA-IR. Even BMI-corrected analyzes showed higher zonulin levels in the PCOS group. Insulin resistance is a pathology seen in both obese and non-obese patients with PCOS [15]. In this study, the positive correlation between insulin resistance and zonulin, even in non-obese patients, suggested that insulin resistance is the link between zonulin and PCOS. Disorders of gut permeability lead to disruption of the intestinal barrier, transmission of infectious agents and dietary antigens; these activate the immune system, so cytokines such as IL-6 and TNF-alpha also cause insulin resistance [16]. Since cytokines such as IL-6 also control zonulin production, increased gut permeability leads to insulin resistance [12].

In our study, different results were obtained compared to the above-mentioned study. First of all, there was no significant difference between the control group and the PCOS group regarding anthropometric and metabolic values. BMI of both groups is similar and consist of non-obese patients. There was no difference between the groups in terms of FPG, lipid profile, fasting insulin, HOMA-IR, uric acid and CRP. The most important metabolic parameter, insulin resistance value (HOMA-IR) was similar (Table 1). Contrary to reference 11, there was no correlation between zonulin and serum uric acid, HbA1c and low HDL-C [11]. However, there was a poor negative correlation between lipid parameters and zonulin in all participants.

Based on these results, we can say that, in non-obese PCOS patients without insulin resistance, abnormalities in gut permeability are not expected and thus zonulin level does not

increase. It is also possible to say the opposite. That is, insulin resistance is not triggered if there is integrity in the gut permeability. As this result was also obtained in the control group, the positive relationship—observed previous studies—between insulin resistance and zonulin was found in our study indirectly.

There are limitations of our study. The sample size of our study, which includes 62 people in total, can be considered insufficient. Perhaps the small number of the control group may have affected the results. However, the hospital where the study was conducted was closed. The researchers participating in the study now work in different hospitals. Therefore, the study could not be expanded. Even so, PCOS patients were included in the study without any treatment and newly diagnosed. The absence of enough obese subjects prevented us from observing the relationship between zonulin and obesity. Metabolic parameters were unexpectedly close to normal, unlike many studies in the literature [17, 18]. Although insulin resistance is very important in the pathogenesis of PCOS, it is not necessary in every patient. Insulin resistance is seen in 50–75% of PCOS patients [19, 20]. As a result, zonulin elevation is not expected in PCOS patients without metabolic syndrome and especially without insulin resistance.

Author contributions ZC contributed to project development, data management, manuscript writing, and data analysis. AK was involved in project development, data collection, and kit (zonulin) running. BC, OB, and MC collected the data. TT was involved in data collection and kit running. DB contributed to project development and manuscript editing.

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Compliance with ethical standards

Conflict of interest Zeynep Cetin, Arzu Kosem, Bulent Can, Ozden Baser, Merve Catak, Turan Turhan and Dilek Berker declare that they have no conflict of interest.

Ethical approval The local ethics committee of University of Health Sciences Ankara Numune Education and Research Hospital approved this study with ID: 1190/2017. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study does not contain any study animals. This is a sectional study.

Informed consent Informed consent was obtained from all individual participants included in the study.

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