617 Effects of different doses of curcumin on apoptosis, mitochondrial oxidative stress and calcium influx in DBTRG glioblastoma cells

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630 Levels of paraoxonase, high-density lipoprotein and total sialic acid in patients with polycystic ovary syndrome

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AIM AND SCOPES
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Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer’s Disease, Parkinson’s Disease.
Abstract

Paraoxonase (PON1) enzyme has an important role in various pathological processes, including inflammatory response, neurological disease and cardiovascular disease, and recently also in ovarian dysfunction. We aimed to evaluate the relation of high density lipoprotein cholesterol (HDL) levels and PON1 activity and total sialic acid status (TSA) levels in serum of patients with polycystic ovary syndrome (PCOS) and healthy individuals.

Twenty PCOS patients (PCOS group) and 20 healthy nonhyperandrogenic women (control group) were studied in the current study. Levels of HDL, TSA, and activity of PON1 were measured in serum of PCOS patients.

Levels of PON1 activity and level of HDL were significantly lower in the PCOS group than in the control group. TSA levels were higher in the PCOS group than the control group. There was also a significant correlation between the parameters and syndrome initiation.

In conclusion, patients with PCOS had low values of PON1 and HDL, although they have high TSA levels. It might be hypothesized that elevated serum TSA, HDL and PON1 may be associated with increased cardiovascular risk in PCOS and/or menstrual irregularities associated with this syndrome.

Keywords: Paraoxonase activity; Polycystic ovary syndrome; Total sialic acid; HDL

Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine and metabolic disorders of reproductive aged women, depending on the deterioration of the interaction between the central nervous system, pituitary gland, ovaries, adrenal gland and other tissues. Diagnostic criteria were established by the European Society for Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) in 2003 based on the
extensive studies during the last decades, which is the so-called Rotterdam Consensus Criteria (Balen et al., 1995; The Rotterdam ESHRE/ASRM-Sponsored group.

PCOS is a chronic illness and can adversely affect the quality of life of women. Initiator factors have not fully understood although that can be evaluated as a disease which is caused by the interaction of genetic and environmental factors. The syndrome occurs heterogeneous complexity effect on 6–14% of women population (Boomsma et al, 2006).

The insulin secretion impairment, insulin resistance, neuroendocrine disorders and increased luteinizing hormone secretion, changes in excessive cortisol metabolism, and increased synthesis of androgen may play a role in the pathogenesis of PCOS. Increase in insulin and androgen hormones retards the growth of follicles by overcoming paracrine signaling. The presence of a large number of agents responsible for the development of PCOS and its involvement in a continuous cycle makes it difficult to understand the pathogenesis (Radosh, 2009, Altug Sen et al., 2011; Abdel-Wahab et al., 2002).

PCOS is a syndrome which is usually associated with insulin resistance, dyslipidemia, obesity, cardiovascular diseases, psychiatric, neurological disorders, gynecological cancers and oxidative stress, though the pathogenesis mechanism has not been well defined (Crosignani and Nicolosi, 2001). A lot of investigations have revealed that oxidative stress level is significantly increased in patients with PCOS compared with the normal, when oxidative status is evaluated by circulating markers, such as paraoxanase (PON1), malondialdehyde, superoxide dismutase, and glutathione peroxidase. Oxidative stress reflects an imbalance between production and scavenging of reactive oxygen/nitrogen species (ROS/RNS), and excess ROS accumulated in vivo would induce cell, protein, and lipid damage (Bickerton et al., 2005; Bayram et al., 2012).

PCOS metabolic comorbidities might be explained by the presence of a circle of vicious reflections. Oxidative stress can be an important factor in the initiation of atherosclerosis, PCOS and chronic inflammatory diseases. It is thought that high levels of oxidative agents in the formation of cardiovascular disease play a role in the lipoproteins being oxidized in PCOS patients. Oxidative stress, which is defined as the degeneration of the balance between oxidant and antioxidant mechanisms for oxidant parameters, plays an important role in etiopathogenesis of infertility in women (Dincer et al., 2001). The protection against these radicals is enzymatically mediated by superoxide dismutase, glutathione peroxidase, catalase and PON1 (Dursun et al., 2006).

PON1 was first found as an enzyme that hydrolyzes aromatic carboxylic esters, organophosphates and nerve gas. PON gene family consists of PON1, PON2 and PON3. Each has antioxidant properties and has about 65% of the same amino acid content. These genes are encoded in human chromosome 7. PON1 and PON3 are mostly synthesized in the liver and transported in the plasma due to HDL (Fenkci et al., 2007; Liu and Zhang, 2012). Many non-genetic factors influence the activity of this enzyme. The PON1 enzyme exhibits arylesterase (ARE) and homocysteine thiolactonease activity, respectively, using phenyl acetate and homocysteine thiolactone as a substrate. The PON1 enzyme involved in HDL structure acts as an antioxidant by detoxification of lipid peroxides. Hyperlipidemia is one of the common metabolic disorders in PCOS. Researches have shown that PON1 inhibits lipoproteins from being oxidized. The presence of PON1 enzyme activity, which is involved in HDL structure, will prevent the secondary development of oxidative stress with PCOS cases (Dursun et al., 2006; Liu and Zhang, 2012). The effect of oxidative stress on etiology of PCOS and effect of PON1 in women with PCOS are still debate; there are a few studies with conflicting results about relationship between PON1 and PCOS (Teiber et al., 2007).

Sialic acids have considerable efficacy in fertilization, tumor growth, immunological reactions, determination of apoptosis-containing cell life, signal transduction between membranes, microbial and non-microbial inflammation, and many other biological cellular and pathological events. The change in sialic acid levels is thought to play a role in the initiating mechanisms of PCOS (Atiomo et al., 2003; Suer Gokmen et al., 2006).

In the current study, we evaluated the relationship PON1, HDL and TSA values in patients with PCOS patients for clarifying role of the values in etiology of PCOS.
Materials and Methods

Patients
Twenty women between the ages of 18 to 40 years with PCOS, and 20 healthy women as the control group, were included in the study. Study participants were enrolled from patients who attended the outpatient clinics of the gynecology and obstetrics department in our institution. Twenty volunteer women between 18-40 years of age who had healthy, normal menstrual cycle, no hirsutism, no biochemical and clinically hyperandrogenemia findings, no polycystical appearance on USG, body mass ≤ 25 kg / m² were included in the control group. Age, weight, height were noted and body mass indices (BMI) were calculated according to the body weight(kg) /height(m²) formula. Pelvic transabdominal or transvaginal ultrasonography was performed for each subject.

Blood sampling
Blood samples were taken from all subjects between 08:00-09:00 A.M. after 12 hours of fasting on the third day of the menstrual cycle for measurements. The blood samples were centrifuged at 4000 x g for 10 min, the serum samples were separated by a pasteurized pipette and stored at -80°C.

PON1, HDL and TSA Analyses
PON1 activity was analyzed according to the methods of Eckerson et al. (1983) and Gulcu (2003). The paraoxon (0, 0-diethyl-O-p-nitrophenylphosphate) used as substrate is based on the measurement of absorbance at 412 nm spectrophotometrically at 25°C of p-nitrophenol which is hydrolyzed by PON1. PON1 activity was calculated as U/L, taking into account the molar absorption coefficient determined for p-nitrophenol and the dilutions made in the experiment. The enzyme activity which converts 1 μmol paraoxon to para-nitrophenol in 1 minute for PON1 activity is described as Unit (U).

TSA analyses were performed by using methods previously defined by Katopodis et al. (1982) and Plucinsky et al. (1986). HDL levels were analyzed in an automated analyzer (Huma Star 600, Germany) using commercial kits.

Statistical analyses
Statistical analysis was performed using the Statistical Package for Social Science (SPSS) 11.5 (Inc., Chicago, Illinois, USA) programme. Data were represented as mean±standard deviation (SD). Data were analyzed by using Mann-Whitney U test and correlation analyses. In all examinations, a p-value of <0.05 was considered statistically significant.

Results

Results of demographic values
There were no statistically significant difference between control and patients groups regarding to age, body mass index, gravida and parity.

Results of HDL
Results of serum HDL in control and patients with are shown in Figure 1. The levels of HDL were significantly (p≤0.001) lower in the PCOS group than in the control group.

Figure 1. HDL level measurement in control and patients with PCOS (mean±SD and n=20)
Results of TSA

Results of serum TSA in control and patients with are shown in Figure 2. The levels of TSA were significantly (p≤0.001) lower in the PCOS group than in the control group.

Figure 2. TSA level measurement in control and patients with PCOS (mean±SD and n=20)

Results of PON1 activity

Results of serum PON1 activity in control and patients with are shown in Figure 3. The activity of PON1 was significantly (p≤0.001) lower in the PCOS group than in the control group.

Figure 3. TSA level measurement in control and patients with PCOS (mean±SD and n=20)
Results of correlation between HDL and PON1

We also investigated the correlation of serum HDL levels and PON1 activity in the two groups and their correlation with characteristics of the groups themselves. In patients with PCOS, PON1 activity was negatively correlated with total sialic acid levels. Remarkably, HDL levels were positively correlated with the follicle number, prolactin and Ferriman–Gallwey score.

Discussion

Many studies addressed relationship between oxidative stress and PCOS (Atiomo et al., 2003). Our results confirm the relationship and PCOS is related to an increase in ROS production in PON1. Studies in the literature show that ARE activity is low in patients with PCOS. In the study conducted by Fenkci et al. (2008) HDL levels were found to be significantly higher in the control group than the group of patients with PCOS (Apridonidze et al., 2005; Valkenburg et al., 2008).

Another research was observed that oxidative stress can increased in mice genetically deficient in PON1, HDL could not protect LDL from oxidation and consequently atherosclerosis developed. It is also reported that PON1 activity is low in atherosclerotic patients under increased oxidative stress. In support of this view, PON1 activity was also found to be low in PCOS patients with a high risk of atherosclerosis and cardiovascular disease. In this study, PON1 activity was found to be low in PCOS patients, consistent with the literature. Liver PON1 mRNA expression has been reported to be influenced by some genetic and environmental factors. Particularly, androgens and proinflammatory mediators are decreased liver PON1 mRNA expression (Valkenburg et al., 2008).

In study of Bin Ali et al. (2003), PON1 activity was found to be low in male mice and a 170% increase was observed hepatic PON1 mRNA expression after castration. In support of this view, Dursun et al. (2006) was reported a negative correlation between PON1 activity and androgen hormones in PCOS women. While oxidative stress disrupts insulin action, decreasing PON1 activity may contribute to the formation of insulin resistance (Legro et al., 2002). In knowledge from latest research, there is relation between PON activities in female infertility. The findings were reported as PON1 and arylesterase activity was positively associated with embryo cell cleavage rate (Apridonidze et al., 2005; Khan, 2007).

Our study findings and literature findings are evaluated together, oxidative stress can be considered one of the mechanisms responsible for PCOS like a high cardiovascular risk. Oxidative stress is defined as an imbalance derived from excessive formation of oxidants in the presence of limited antioxidant defenses. In several studies; excessive oxidant formation of substrates were claimed as the main etiological factors on PCOS, and it was associated with dyslipidemia, elevated metabolic syndrome-type 2 diabetes and increased oxidative stress risks. Contrary to this process; HDL shows anti-inflammatory and antioxidant effects (Fauser et al., 2008).

In recent years, the decreased antioxidant and increased oxidant activity have been reported in women having PCOS. PON1, which support a protection from oxidative and peroxidative transformation has been identified by proteomic analysis. In a recently published systematic review and meta-analysis, it has been reported that the concentrations of several promoters and by-products of oxidative stress such as sialic acid was significantly increased, and some circulating antioxidant markers, such as PON1 activity and HDL levels, were decreased in patients with PCOS compared with those controls (Talbott et al., 2001; Piskinpasa and Yildiz, 2005).

The lipid findings of women with PCOS differ from the lipid species in the characteristics of the populations. Some studies have reported a significant decrease in HDL levels compared to body weight comparative controls in PCOS (Rajkhowa et al., 1997). In some studies (Robinson et al., 1996), there was no significant difference between women with PCOS and control groups in terms of HDL cholesterol. Results of Randeva et al. (2002) study suggested that women with PCOS generally have a decrease in HDL levels while an increase in triglyceride and VLDL cholesterol levels. In addition, there was an increase in triacylglycerol levels in obese women affected by this syndrome therefore, it is reported that lipoprotein levels in these cases are comparable to patients with type 2 diabetes. Aye and Malek (2012) have reported similarities to women with the metabolic syndrome and diabetes mellitus due to features such as low HDL cholesterol at 2/3 and hypertriglyceridemia at 1/3. Women with PCOS showed
that the likelihood of dyslipidemia was 1.8 times higher if there was a familial hyperlipidemia story (Fenkci et al., 2007; Meyer et al., 2007; Turan et al., 2015).

Serum TSA levels have been reported to be a risk factor for cardiovascular and cerebrovascular disease. Higher serum sialic acid levels have also been detected in diabetes mellitus, in either type. Hangloo et al. (1990) demonstrated that TSA levels did not change with varying age and sex. In contrast, Lindberg et al. (1991) reported in a study including approximately ten thousand subjects that TSA levels were similar between ages 25 and 44 years but afterwards progressively increased up to 74 years of age. Similarly, Crook et al. stated that, in healthy cases, TSA levels in the elderly population were higher than those in the younger subjects, and this was explained by atherosclerosis which increased with age and thus TSA was a risk factor for cardiovascular disease. Pömniö et al. (1999) showed that serum TSA levels did not increase with age in men, but in women levels increased with age, especially during the menopausal years. We also found that serum TSA levels were higher in patients with PCOS (Turan et al., 2015; Zheng et al., 2015).

In conclusion, patients with PCOS had high level of PON1. It might be hypothesized that elevated serum TSA, HDL and PON1 may be associated with increased cardiovascular risk in PCOS and/or menstrual irregularities associated with this syndrome.

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Conflict of Interests
The authors declare, which they have no conflict of interest.

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