ORIGINAL ARTICLE

Decreased serum paraoxonase and arylesterase activities in patients with rosacea

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Abstract

Background Recent evidence suggests that oxidative stress may be an important phenomenon in the pathophysiology of rosacea. Paraoxonase-1 (PON1) is an antioxidant enzyme with three activities: paraoxonase, arylesterase and diazoxonase. In this study, we evaluated serum paraoxonase and arylesterase activities, and serum lipid hydroperoxide (LOOH) levels in patients with rosacea in comparison to healthy controls.

Material and method The study included 39 rosacea patients and healthy controls, consisting of 40 age- and sex-matched healthy volunteers. Serum paraoxonase and arylesterase activities were measured using paraoxon and phenylacetate substrates. Serum LOOH levels were measured with the ferrous ion oxidation-xylenol orange assay.

Results In rosacea group mean serum paraoxonase and arylesterase activities were 74.54 ± 38.30 U L^{-1} and 141.29 ± 22.27 kU L^{-1} respectively, which were significantly lower than controls ($P = 0.010$, 0.005; respectively). Mean serum LOOH level of rosacea group was 8.17 ± 1.91 μmol L^{-1} which was significantly higher than controls ($P = 0.009$). There were no statistically significant differences between the clinical subtypes of the disease, menopause situation or ocular involvement with the respect to the serum paraoxonase and arylesterase activities and LOOH levels (all; $P > 0.05$).

Conclusions Serum PON1 enzyme activities have decreased significantly in rosacea. These findings support that decreased PON1 activity and increased oxidative stress may play a role in the pathogenesis of rosacea. Further studies are needed to elucidate the role of PON1 activity in the pathophysiology of rosacea.

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Conflicts of interest

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Introduction

Rosacea is one of the most common chronic inflammatory dermatological diseases, most often affecting the facial skin and the eyes. It is characterized by midfacial erythema, telangiectasia, inflammatory papules and pustules with intermittent episodes of exacerbation and remission.[1] The pathophysiology of rosacea has been poorly understood.[2] There is some agreement that inflammation plays a central role in rosacea, although, it has primarily been considered to be a cutaneous vascular disorder. This inflammation is linked to other processes through a variety of mechanisms, including ultraviolet (UV) damage, vascular changes and oxidative tissue damage.[3,4]

Oxidative stress constitutes the basis for many inflammatory skin diseases.[5] Oxidative stress results from an imbalance between pro-oxidant and antioxidant defences, in favour of pro-oxidants.[5,6] Some findings show that oxidative stress may be an important phenomenon in the pathophysiology of rosacea. Imbalances in the oxidant/antioxidant system, such as the accumulation of serum peroxide, diminishing of serum total antioxidative potential and low tissue superoxide dismutase (SOD) activities in advanced cases, have recently been demonstrated in patients with rosacea.[7,8]

Serum lipid hydroperoxide (LOOH) level is a reliable marker of lipid peroxidation and an oxidative stress indicator.[9]

Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated antioxidant enzyme with three activities: paraoxonase, arylesterase and dyazoxonase that protect against such peroxidation.[10] PON1 plays an important role in the protection against atherosclerosis by preventing the oxidative modification of serum lipoproteins, and hydrolyzing lipid peroxide accumulation.[11,12] In addition, human PON1 contributes to the anti-atherogenic effect of HDL, and its activity has been shown to reduce metabolic syndrome, atherosclerosis and diabetes mellitus, in which oxidative stress plays a major role.[11,12]

Several studies have suggested that serum PON1 activity may play an important role in the pathogenesis of various dermatological diseases.[13–15] However, to the best of our knowledge, there is no data concerning serum paraoxonase and arylesterase activities in patients with rosacea. The objective of this study was to investigate the role of antioxidant systems by measuring the enzyme activities of serum paraoxonase and arylesterase, and the serum levels of LOOH in patients with rosacea.

Materials and methods

This prospective case–control study was conducted in the Departments of Dermatology at Kecioren Research and Training Hospital (Ankara, Turkey) and Yuzuncu Yil University (Van, Turkey) between November of 2011 and April of 2012.

A total of 39 patients with rosacea (22 female, 17 male, mean age: 47.58 ± 12 years) and 40 control subjects (20 female, 20 male, mean age: 46 ± 13 years) were included in this study.

Rosacea was diagnosed using clinical findings and histological examinations. According to the standard classification of rosacea proposed by the National Rosacea Society in 2002,[16] the disease is classified into four different subtypes: erythematotelangiectatic rosacea (subtype I), papulopustular rosacea (subtype II) and phymatous rosacea (subtype III). Patients who had only ocular rosacea (subtype IV), without skin involvement, were not included in this study, but we have noted the patients with whom ocular involvement was accompanying. According to this classification, the study included 11 patients with subtype I, 25 patients with subtype II and 3 patients with subtype III. Additionally, 12 patients had ocular complaints due to rosacea. None of the patients were smokers and had any treatments (drugs or sunblock) for 6 months.

The age, sex and skin phototype matched 40 healthy control subjects recruited from patients who were referred to the outpatient clinic for cosmetic complaints or nevus, and had no systemic or dermatological diseases. No control subjects had received supplementation with antioxidant vitamins, such as E and C, and all control subjects were non-smokers.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000. The study protocol was approved by the local ethics committee, and an informed consent was obtained from each subject.

Blood samples

Blood samples were obtained following an overnight fast, collected into empty tubes and were immediately stored on ice at 4 °C. The serum was then separated from the cells by centrifugation at 2400 ***g*** for 10 min, and the lipid parameters and enzyme activities were measured immediately. The remaining serum samples were stored at −80 °C and used for the analysis of the PON1 activity and LOOH levels.

Measurement of serum paraoxonase and arylesterase activities

Paraoxonase and arylesterase activities were measured using paraoxon and phenylacetate substrates. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase inabsorbance at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17 000 M^{-1} cm^{-1}.[17] Paraoxonase activity was expressed as the U/L of the serum. Phenylacetate was used as a substrate to measure the arylesterase activity. The enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol: 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 μmol phenol generated/min under the above conditions and expressed as the U L^{-1} of the serum.[18]

Measurement of serum LOOH levels

Serum LOOH levels were measured with the ferrous ion oxidation-xylenol orange assay as previously described.[19] This particular assay depends on the oxidation of the ferrous ion to the ferric ion via the effect of various oxidants. Consequently, the ferric ion produced is measured with xylenol orange. The levels of LOOH are reduced by the application of triphenyl phosphine (TPP), which is a specific reductant for lipids. The LOOH levels can be estimated through the difference in the values that appears due to the absence or presence of TPP.

Other parameters

The levels of the triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C) and low-density lipoprotein cholesterol-(LDL-C) were determined using commercially available assay kits (Roche®, Mannheim, Germany) with an autoanalyzer (Roche®/Hitachi Modular P-800®).

Statistical analysis

The results were expressed as the mean ± standard deviation. The non-parametric continuous variables were compared using the Mann–Whitney *U*-test, and the parametric variables were compared using the Student's *t*-test. The qualitative variables were assessed using the chi-squared test. Comparisons among multiple groups were compared using the one-way analysis of variance with a post-hoc analysis using the Tukey's and Dunnett's tests. The results were considered to be statistically

significant when the P value was less than 0.05. The data were analyzed using the SPSS® for Windows computing programme (version 11; SPSS Inc., Chicago, IL, USA).

Results

The demographic characteristics of the subjects with rosacea and the controls are presented in Table 1. There were no statistically significant differences between the rosacea patients and control subjects with respect to age and gender ($P > 0.05$) (Table 1).

There were no statistically significant differences between patients with rosacea and the controls with respect to the serum TG, TC, LDL-C and HDL-C levels (all; $P > 0.05$) (Table 1).

Serum paraoxonase and arylesterase activities were significantly lower in patients with rosacea than controls subjects ($P = 0.010$ and 0.005 respectively), whereas the serum LOOH levels were significantly higher ($P = 0.009$) (Table 2).

There were no statistically significant differences between the clinical subtypes of the disease, menopause situation or ocular involvement with respect to the serum paraoxonase and arylesterase activities and LOOH levels (all; $P > 0.05$).

Discussion

In this study study, we tested the hypothesis that increased oxidative stress and decreased PON1 activity may play a role in the pathogenesis of rosacea. We observed that patients with rosacea had decreased serum paraoxonase and arylesterase activities and increased serum LOOH levels when compared with the healthy subjects.

Table 1 Demographic characteristics and laboratory findings of the rosacea and control subjects

Parameters	Rosacea (n = 39)	Controls (n = 40)	P
Age (years)	47 ± 12	46 ± 13	0.611
Gender (male/female)	17/22	20/20	0.654
Weight (kg)	66.9 ± 11.5	65.2 ± 8.9	0.23
TG (mg dL^{-1})	113.33 ± 92.94	123.80 ± 64.56	0.562
TC (mg dL^{-1})	174.10 ± 31.63	180.83 ± 33.42	0.362
HDL-C (mg dL^{-1})	47.49 ± 7.98	49.40 ± 16.51	0.516
LDL-C (mg dL^{-1})	103.95 ± 30.76	106.67 ± 27.15	0.678

Values are mean ± SD.
TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2 Serum lipid hydroperoxide levels and PON1 enzyme activities in rosacea and control subjects

Parameters	Rosacea (n = 39)	Controls (n = 40)	P
Paraoxonase (U L^{-1})	74.54 ± 38.30	116.32 ± 80.79	0.010
Arylesterase (kU L^{-1})	141.29 ± 22.27	155.84 ± 31.69	0.005
Lipid hydroperoxide (μmol L^{-1})	8.17 ± 1.91	7.12 ± 1.55	0.009

Values are mean ± SD.

Rosacea is a chronic inflammatory disorder with unknown aetiology.[1–3,20] Today, rosacea pathophysiology has been established with regard to two essential factors: vascular and inflammatory manifestations.[21,22] In the development of rosacea, inflammation has been largely accepted to be the central process, considering the growing understanding of immune system processes in the skin, as well as successful treatment with anti-inflammatory agents.[3,4,21–23]

The inflammation of rosacea may involve oxidative modulation of proteins and lipids, deactivation of natural defences by reactive oxygen species (ROS), altered lipid balance and the ROS-related stimulation of other inflammatory mediators such as cytokines.[3,4,23] Some studies suggest the implication of neutrophils, releasing potent inflammatory mediators and ROS in the inflammation of rosacea.[3,4,21–23] Damage to keratinocytes, fibroblasts and endothelial cells by ROS can result in the release of IL-1 and TNF-α, which contribute to the recruitment of leucocytesClick here to enter text. from the blood to the tissues.[4,23,24]

The skin is a biological barrier against environmental insults, and it is directly exposed to UV radiation.[25] Rosacea predominantly affects the convexities of the central aspect of the face, which are the major solar exposure sites. UV radiation has been proposed as a significant contributing factor for rosacea, which has been described as a photoaggravated disorder.[6,19,26] Sundaram *et al.*[27] have reported that both UVA and UVB radiation, even below the minimum erythema dose, generate a high concentration of oxygen radicals. These free radicals play a role in the damage seen with photoaging, an etiologic factor in the development of rosacea.[3,4,22,27,28]

It is known that activated inflammatory cells, especially neutrophils lead to ROS production, enhancing lipid and lipoprotein peroxidation, in rosacea.[4] LOOH and MDA are well-known indicators of oxidative stress formed from unsaturated phospholipids, glycolipids and cholesterol by peroxidative reactions.[29] An increase in MDA levels was revealed in patients with severe rosacea previously.[7] It has been reported that PON1 protects lipoproteins against oxidative modification.[11,12] On the other hand, increased oxidative stress including free radicals and peroxides oxidize sulphdyryl groups of paraoxonase and arylesterase enzymes and lead to a decrease in enzyme activity of PON1.[30]

The PON1 activity is determined genetically, however, various factors, such as diet, lifestyle and environmental factors can influence PON1 activity.[31] Smoking has also been shown to decrease the serum PON1 activity.[32] In contrast, the PON1 activity is positively correlated with the quantity of vitamins C and E in the diet.[33] In this study none of the patients or control subjects received supplementation with antioxidant vitamins, such as vitamin E and C, and also all of them were non-smokers. In our patient population, decreased serum PON1 activities may be responsible for the increased oxidative stress levels. Herein, the increase in oxidative stress markers such as LOOH and the decrease in antioxidant markers such as, paraoxonase and

arylesterase activities support the reports regarding the oxidative nature of rosacea.

Recent findings support the hypothesis that antioxidant imbalances could be involved in the pathogenesis of rosacea.[7,34,35] Skin naturally uses several antioxidant pathways to protect itself from the oxidative damage of UV-generated free radicals. SOD, catalase and glutathione peroxidase are examples of the enzymatic defence mechanisms against ROS toxicity.[26,27] In one study, severe rosacea was associated with high MDA levels and low SOD levels, which shows that in severe cases of rosacea, SOD can be overwhelmed.[7] Using topical or systemic antioxidants has been shown to be effective in the treatment of rosacea, suggesting that this disease process may be related to a deficiency in the antioxidant system or increased ROS activity.[4,23,36]

In conclusion, serum paraoxonase and arylesterase activities were found to be significantly lower in rosacea. Our results suggest that reduced PON1 activities may be related to increased oxidative stress levels. These findings support the hypothesis that decreased PON1 activities and increased oxidative stress levels may play a role in the pathogenesis of rosacea. Further studies are needed to elucidate the role of PON1 activity in the pathophysiology of rosacea.

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