Determining oxidant and antioxidant status in patients with genital warts

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Objectives: Warts are abnormal skin growths caused by human papilloma virus (HPV) infections within the skin of patients. Genital warts usually appear in the perianal and perigenital regions. Asymptomatic warts may be activated after years and may damage natural immunity. The inflammation that occurs during this process may lead to an imbalance between the prooxidant and the antioxidant systems. The aim of this study was to investigate erythrocyte glutathione peroxidase (GSH-Px) activity, serum paraoxonase enzyme levels, and oxidative stress levels in patients with genital warts.

Patients and Methods: In total, 32 patients with genital warts and 35 healthy subjects were included in this study. Erythrocyte GSH-Px activity, serum catalase activity, and paraoxonase enzyme, and malondialdehyde (MDA) levels were determined.

Results: Erythrocyte GSH-Px activity, serum MDA levels, and catalase activity were significantly higher in patients with genital warts than in controls ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively). However, serum paraoxonase enzyme levels were not significantly different between groups ($P > 0.05$). Serum triglyceride levels were significantly lower in patients with genital warts than in controls ($P < 0.01$). However, there were no statistically significant differences between groups with respect to total cholesterol, high-density lipoprotein cholesterol, or low-density lipoprotein cholesterol levels (all $P > 0.05$).

Conclusions: Our data suggest that oxidative stress is increased in genital warts. Increased oxidative stress levels may contribute to the pathogenesis of genital warts, and prolonged HPV infection due to chronic inflammation could also affect oxidative stress.

Keywords: Genital warts, Malondialdehyde, Glutathione peroxidase activity, Catalase activity, Paraoxonase-1, Lipid parameters

Introduction

Viral warts are benign proliferations of the skin and mucosa that are caused by human papilloma virus (HPV) infection. HPV is a double-stranded DNA virus that has over 120 subtypes.[1] Genital warts usually appear in the perianal and perigenital regions and are usually asymptomatic. Asymptomatic genital warts may activate after years and may damage natural immunity. The inflammation that occurs during this process may lead to an imbalance between the prooxidant and the antioxidant systems.[2]

Oxidative stress appears as a result of destroyed balance between oxidants and antioxidants in the body. Oxidative stress constitutes the basis of many inflammatory skin diseases.[3] Oxidative stress may have an important role in the pathogenesis of genital warts. Several studies have suggested that imbalances in the oxidant/antioxidant systems may play an important role in HPV infection.[2,4] Moreover, oxidative stress may be a possible link between HPV infections and skin cancers.[5]

Catalase and superoxide dismutase are the most important antioxidant enzymes in cells.[6] When hydrogen peroxide (H_2O_2) levels increase, glutathione peroxidase (GSH-Px) becomes the most effective antioxidant.[7] A reduction in glutathione peroxide leads to an increase in H_2O_2 that causes severe cellular damage.[8] Malondialdehyde (MDA) is a well-known indicator of oxidative stress that is formed from unsaturated phospholipids, glycolipids, and cholesterol by peroxidative reactions.[8]

Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme with three activities, or paraoxonase, arylesterase, and dyazoxonase activities, which protect against such peroxidation.[9] PON1 has antioxidant activity and anti-inflammatory properties and plays an important role in protection against

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atherosclerosis by preventing the oxidative modification of serum lipoproteins and by hydrolyzing accumulated lipid peroxide.[10,11] PON1 has been associated with diseases characterized by high oxidative stress, such as cardiovascular disease and diabetes.[12] Oxidative stress down-regulates serum PON1 expression due to changes in the redox status.[13]

Several studies have suggested that serum PON1 activity may play an important role in the pathogenesis of various dermatological diseases.[3,14,15] However, to the best of our knowledge, there are no data concerning serum PON1 enzyme levels in patients with genital warts. Therefore, the aim of this study was to investigate erythrocyte GSH-Px activity, serum paraoxonase enzyme levels, and oxidative stress levels in patients with genital warts.

Materials and methods

This cross-sectional study was conducted in the Departments of Dermatology and Clinical Biochemistry at Yuzuncu Yil University School of Medicine between July 2012 and May 2013.

A total of 32 consecutive patients with genital warts (4 female, 28 male, mean age: 30 ± 8 years) and 35 control subjects (5 female, 30 male, mean age: 29 ± 7 years) were included. All patients had no systemic or other dermatological diseases. None of the patients was receiving supplementation with antioxidant vitamins such as vitamins E and C. All patients were nonsmokers.

Warts were diagnosed on the basis of history and physical findings by a dermatologist. Only patients with genital warts were invited to participate in the study. Patients with disease duration of more than 1 year and patients previously treated for genital warts were not included.

The age- and sex-matched healthy subjects were recruited from among patients who were referred to the out-patient clinic for cosmetic complaints and who had no systemic or dermatological diseases. No control subjects were receiving supplementation with antioxidant vitamins such as vitamins E and C. All control subjects were nonsmokers.

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 informed consent was obtained from each subject.

Blood samples

Blood samples were collected from the antecubital vein after an overnight fasting period, and the serum and erythrocytes were separated. The serum was separated from the cells by centrifugation at 1409*g* for 10 minutes, and lipid parameters and catalase enzyme activity were measured immediately. The remaining samples were stored at −40 °C and used for the analysis of GSH-Px activity and PON1 and MDA levels.

Measurement of serum paraoxonase enzyme levels

PON1 levels were measured using the sandwich enzyme immunoassay method via Eastbiopharm ELISA kits (Eastbiopharm®, Zhejiang, China). The intra-assay and inter-assay %CV values of the method were <10% and <12%, respectively. Paraoxonase levels are expressed as ng/ml.

Measurement of serum lipid peroxidation levels

Lipid peroxidation levels in the samples were determined based on MDA levels. Serum MDA levels were determined using high-pressure liquid chromatography via Chromsystems (Chromsystems®, Mannheim, Germany) kits and an Agilent 1200 series autoanalyzer (Agilent Technologies®, CA, USA). The results are expressed as μmol/l.

Measurement of serum catalase activity

Catalase activity was measured using H_2O_2 as the substrate.[16] The disappearance of H_2O_2 was tracked at 540 nm. The results are expressed as kU/l at 25 °C.

Measurement of erythrocyte GSH-Px activity

Measurement of GSH-Px enzyme activation was performed according to the method of Paglia and Valentina.[17] GSH-Px catalyzes oxidation of glutathione. When the oxide glutathione (GSH) is reduced, NADPH is oxidized and turned into NADP. This change was observed at a wavelength of 340 nm, and activation of GSH-Px was measured. The results are expressed as milliunits of GSH-Px activity per milligram of Hb. Hb was assayed using the commercial cyanmethemoglobin method (Sigma Diagnostics, St. Louis, MO, USA).

Other parameters

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

Statistical analysis

The results are 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 Student's *t*-test. The qualitative variables were assessed using the chi-squared test. 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 program (Version 13.0).

Results

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

Serum TG levels were significantly lower in patients with genital warts than in controls ($P < 0.01$). However, there were no statistically significant differences between patients with genital warts and controls with respect to TC, HDL-C, or LDL-C levels (all $P > 0.05$) (Table 1).

Erythrocyte GSH-Px activity, serum MDA levels, and catalase activity were significantly higher in patients with genital warts than in controls ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively). However, there was no statistically significant difference in serum PON1 levels ($P > 0.05$) (Table 1).

Erythrocyte GSH-Px activity was significantly correlated with TC levels ($r = -0.425$, $P < 0.01$) and LDL-C levels ($r = 0.451$, $P < 0.05$). However, serum MDA levels, catalase activity, and PON1 levels were not correlated with serum lipid parameters in patients with genital warts (all $P > 0.05$).

Discussion

In the present study, we investigated erythrocyte GSH-Px activity, serum PON1 enzyme levels, and oxidative stress levels in patients with genital warts. In addition, we measured the serum lipid profiles of patients with genital warts. We also examined the relationship between oxidant and antioxidant status and the serum lipid profiles of patients with genital warts.

In this study, we observed that erythrocyte GSH-Px activity, serum MDA levels, and catalase activity were significantly increased in patients with genital warts. However, we observed no statistically significant difference in serum PON1 levels. We think that these findings show that increased oxidative stress levels may play an important role in the pathogenesis of genital warts.

Table 1 Demographic characteristics of the patients with genital warts and the control group

Parameters	Genital warts ($n = 32$)	Controls ($n = 35$)	*P*
Age (years)	30 ± 8	35 ± 8	0.487
Gender (male/female)	28/4	30/5	0.833
TG (mg/dl)	108.00 ± 7.92	154.10 ± 13.30	0.01
TC (mg/dl)	165.88 ± 6.67	180.31 ± 5.88	0.110
HDL-C (mg/dl)	43.16 ± 2.88	46.60 ± 1.71	0.310
LDL-C (mg/dl)	101.09 ± 5.91	102.86 ± 4.87	0.819

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

Viral warts are caused by HPV, which has several mechanisms for avoiding the immune response.[18] HPV are small double-stranded DNA viruses. HPV strains can be practically classified into low-risk (e.g., HPV-6 and HPV-11) and high-risk (e.g., HPV-16 and HPV-18) types, based on their risk of causing cervical cancer. HPV-6 and HPV-11 are associated with the majority of more benign cutaneous or mucosal proliferative lesions affecting the anogenital areas, such as genital warts or condylomas.[2,8] Moreover, there is epidemiologic and molecular evidence that a subset of HPV, referred to as high-risk HPV, is associated with human anogenital cancers, such as cervical cancers.[19] Warts are a common infectious disease caused by HPV, affecting the skin and mucosa.[20,21]

Reactive oxygen species (ROS) are toxic molecules that have important roles in many inflammatory skin diseases.[22] According to a view under dispute, T lymphocytes also produce ROS when faced with a certain stimulus. It has been proposed that the antioxidant system stimulates T cells; in contrast, in other studies, the opposite result was obtained, and it was reported that oxidative stress suppressed T-cell activation.[23] A T-cell defect is suggested to lead to disease in cases of infection with HPV.[24]

Oxidative stress is a condition arising when the overproduction of ROS is not matched by the antioxidant/repair pathways of the cell. ROS cause damage to cellular macromolecules, resulting in apoptosis or necrosis. The cellular damage caused by excessive ROS leads to the production of reactive biomolecules such as MDA. MDA is an end-product of lipid peroxidation and an important and specific indicator of oxidative stress.[25] Such reactive biomolecules exacerbate the harmful effects of ROS and may also evoke immune and inflammatory responses.[22,26] There could be a relationship between ROS production, antioxidant defense impairment, and inflammatory pathological processes.[22] It has been claimed that the balance of oxidants and antioxidants may play an important role in the spontaneous regression of HPV infections.[27] It is also thought that the antioxidant system has a connection with immunity.[22]

Limited studies are available regarding MDA levels and catalase activity in patients with non-genital or plantar warts,[2,25] and these studies are conflicting. Recently, Arican *et al.*[2] evaluated the role of oxidative stress in affected skin areas in a group of patients with plantar warts. The authors showed that MDA levels were significantly higher in the lesional area than in the non-lesional area. However, they could not detect a significant difference in catalase activity between the two areas. They concluded that cutaneous oxidative stress in patients with plantar warts may play

a role in the pathogenesis of the disease and that the use of topical drugs with antioxidative effects may be valuable in the treatment of warts. In another study, Sasmaz *et al.*[25] measured indicators of oxidative stress, such as catalase and MDA, in patients with non-genital warts. These authors reported statistically significant increases in catalase activity and MDA levels in the patients with warts compared with the control group. The authors suggested that non-genital warts may cause a systemic oxidative status. In the present study, we observed that serum MDA levels and catalase activity were significantly increased in patients with genital warts.

PON1 is a calcium-dependent esterase that hydrolyzes a broad spectrum of oxidized lipids.[28] Due to an increase in lipid and protein oxidation products and a decrease in antioxidant enzymes and vitamins, oxidative stress is associated with decreased PON1 expression and activities.[29,30] Serum PON1 activity has been investigated in various dermatological diseases, such as rosacea.[3,14,15] However, to the best of our knowledge, serum PON1 enzyme levels have not been evaluated in patients with genital warts. In the present study, we observed no statistically significant difference between patients with genital warts and control subjects with respect to serum PON1 enzyme levels. As far as we know, this is the first study to investigate serum PON1 enzyme levels in patients with genital warts.

PON1 is an HDL-associated enzyme.[9] It has been suggested that reduced serum PON1 activity might be associated with decreased HDL-C levels and/or increased oxidative stress.[31] However, we found no correlation between PON1 levels and HDL-C levels.

In the current study, we observed that serum TG levels were significantly lower in patients with genital warts than in controls. However, there were no statistically significant differences between patients with genital warts and controls with respect to TC, HDL-C, or LDL-C levels. Additionally, we observed that erythrocyte GSH-Px activity was positively correlated with TC levels and negatively correlated with LDL-C levels. However, serum MDA levels, catalase activity, and PON1 levels were not correlated with serum lipid parameters in patients with genital warts.

Table 2 Antioxidant enzyme activities and oxidative and antioxidant levels in patients with genital warts and in controls

Parameters	Genital warts ($n = 32$)	Controls ($n = 35$)	*P*
Paraoxonase (ng/ml)	37.07 ± 2.16	38.17 ± 2.39	0.734
Glutathione peroxidase (IU/gHb)	90.37 ± 5.29	65.39 ± 3.86	0.01
Malondialdehyde (mmol/l)	0.06 ± 0.01	0.05 ± 0.01	0.05
Catalase (kU/l)	11.49 ± 1.21	7.63 ± 0.41	0.05

Values are mean ± SD.

GSH-Px is one of the body's most potent antioxidant defenses. GSH and GSH-Px salvage the cell from lipid peroxidation damage, and particularly from damage to the membranes.[32] GSH and GSH-Px also protect cells from damage by free radicals, and specifically lipid peroxides and H_2O_2. These enzymes act as members of the antioxidant system of the cell by degrading H_2O_2 before its conversion into a hydroxyl radical.[33] However, to the best of our knowledge, erythrocyte GSH-Px activity has not been evaluated in patients with genital warts. In the present study, we observed increased erythrocyte GSH-Px activity in patients with genital warts compared with control subjects. As far as we know, this is the first study to investigate erythrocyte GSH-Px activity in patients with genital warts. Therefore, the increased erythrocyte GSH-Px activity in patients with genital warts might be a peripheral response of the organism to increased oxidative stress.

There were several limitations of the present study. First, the investigation used a cross-sectional design. Second, the study sample was small, so these observations must be confirmed in a larger patient sample. Third, all patients had a disease duration of less than 12 months, and the HPV types remained unknown. Fourth, the activity of PON1 is under genetic and environmental regulation, but we were unable to perform PON1 genotyping analyses due to technical challenges.

Our data suggest that oxidative stress is increased in genital warts. Increased oxidative stress levels may contribute to the pathogenesis of genital warts, and prolonged HPV infection due to chronic inflammation could also affect oxidative stress. Finally, oxidative stress may still exist despite increased activities of antioxidant enzymes. We think that antioxidant drugs may reverse the increased oxidative stress levels in patients with genital warts, although more detailed studies are required to verify this opinion.

Disclaimer statements

Contributors EC, MRS, RB and SGB: Conception and design; **EC and MA**: Analysis and interpretation of the data; **EC and MA**: Critical revision of the article for important intellectual content; **EC, MRS and MA**: Final approval of the article; **EC, ZH and RB**: Collection and assembly of data.

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Ethics approval 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 informed consent was obtained from each subject.

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