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Evaluation of Oxidative Stress in Patients with Non-Melanoma Skin Tumours

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Abstract

Background: Since oxidative stress induced by ultraviolet exposure has been shown to cause development of cutaneous cancers, solar ultraviolet radiation (UVR) is considered to be a major etiological factor for non-melanoma skin tumors (NMSTs). We aimed to compare the oxidative stress parameters between patients with NMST and healthy individuals.

Material and Methods: A total of 28 patients with clinically and histologically proven NMSTs (24 basal cell carcinoma, 4 squamous cell carcinoma) and 33 control subjects who were matched for age and gender were included in the study. Serum total oxidant status (TOS), total antioxidant status (TAS), paraoxonase (PON), arylesterase (ARE), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG) and total cholesterol levels of the participants were measured. The oxidative stress index (OSI) was determined.

Results: The TAS and ARE levels of patients with NMSTs were statistically lower than control subjects (P=0.005 and P<0.032, respectively). The TOS levels and OSI values were statistically higher in patients than healthy controls (P=0.005 and P<0.001, respectively). Patient and control groups were similar with regard to PON, HDL, LDL, TG, and total cholesterol levels (all P>0.05).

Conclusion: An increased systemic OS is determined in patients with NMSTs. Altered oxidant/antioxidant balance may play a role in the etiopathogenesis of NMSTs.

Introduction

Non-melanoma skin tumors (NMSTs) are the most common cancer in human beings. The two most frequent NMST types are basal cell carcinomas (BCC) and squamous cell carcinomas (SCC). The risk of NMST development depends on phenotypic, genotypic, and environmental factors. Exposure to ionized radiation, arsenic or organic chemicals, human papillomavirus infections, immunosupression and genetic predisposition are accused in the etiopathogenesis of NMSTs. Although ultraviolet radiation (UVR) is a well-known major etiologic factor for NMSTs, the details of pathogenesis of tumor growth still remains to be explained [1]. Skin cancer formation is a complex process composed of three stages, including initiation, promotion and progression, driven by some cellular, biochemical, and molecular changes [2]. Since the incidence and morbidity of NMSTs has gradually increased, many studies which investigate

the pathogenesis of these tumors have arisen [**3**].

The overproduction of reactive oxygen species (ROS) during oxidative stress (OS) was shown to play a key role in the cell damage and cancer growth [**4**]. In normal aerobic metabolism, ROS are continuously produced at low concentrations and are not harmful for the organism since they are detoxified by the antioxidant metabolism [**2**]. However, endogenous or exogenous factors such as age and UVR have important effects that impair the balance between formation of oxidative molecules and antioxidant defence systems of the cells. UVR induces increased production of ROS . Eventually, OS emerges as ROS accumulates in the tissue [**3**].The overproduced ROS has effects on all stages of cancer formation [**2**].

In humans, there are three separate paraoxonase (PON) genes including PON1, PON2, and PON3, which are located on the same chromosome adjacent to each other. Among PON family, PON1 enzyme is the most investigated one. PON1 has paraoxonase (PON) and arylesterase (ARE) activities, both of which carry antioxidant and anti-inflammatory properties. PON1 is synthesized in the liver and then released into the circulation where it is found in the high-density lipoprotein (HDL) structure [**5**]. PON1 activity has been shown to be reduced in some internal cancers such as overian and endometrial cancer [**6, 7**]. Cell membrane is one of the major targets of OS. The overproduced ROS cause lipid peroxidation. Low density lipoprotein (LDL) is one of the end products of lipid peroxidation, which has an important role in carcinogenesis. On the other hand, high density lipoprotein (HDL) prevents enzymatic and non-enzymatic ROS formation, by acting as a powerful antioxidant.

In recent studies, various oxidant and antioxidant molecules and enzymes were suggested to involve in the etiopathogenesis of cutaneous and internal cancers [**3, 4, 8, 9**]. In this study, we aimed to determine the systemic oxidant and antioxidant status of patients with NMSTs by measuring total oxidant status (TOS), total antioxidant status (TAS), the oxidative stress index (OSI), and PON and ARE enzyme activities.

Materials and Methods

Subjects: This was a single centre, cross sectional study. The study included a total of 28 patients with clinically and histologically proven NMSTs and 33 control subjects who were matched for age and gender. Patients or control subjects who were pregnant, nursing, smoking, who have any systemic or other dermatological diseases, positive HIV test, allergic story, and active infectious diseases were not included in the study. The study was performed in accordance with Clinical Practice Guidelines and Helsinki Declaration. Permission was taken from the Commission of Non-invasive Clinical Research and Assessment of the hospital. All patients participating in the study were informed and signed written inform consents.

Blood samples: Venous blood samples were collected from all participants in the early morning after an overnight fast of at least 8 hours. None of the collected samples was icteric or haemolysed. The samples for measuring PON and ARE activities, TAS and TOS levels were separated by centrifugation at 2500g for 10 min, and sera were stored at – 80 °C until use. All samples were assayed at the same time.

Measurements of total oxidant status (TAS), total antioxidant status (TAS), and oxidative stress index (OSI): Serum TAS level was measured using a recent automated colorimetric measurement method based on the bleaching of characteristic colour of a more stable ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) radical cation by antioxidants. The results were expressed as mmol Trolox equivalent/L [**6**].

Serum TOS level was measured using a recent automated colorimetric measurement method, calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H2O2 Eq/L) [**7**].

The ratio of TOS to TAS represents the OSI, an indicator of the degree of OS. The OSI value is calculated according to the formula: OSI (arbitrary unit) = TOS (mmol H2O2 Eq/L) / TAS (mmol Trolox Eq/L) X 100 [**5**].

Measurements of paraoxanase (PON) and arylesterase (ARE) activities: Serum PON and ARES activities were determined by using commercially available kits (Rel Assay Diagnostics). Serum PON activity toward paraoxon was measured following hydrolysis of paraoxon to yield p-nitrophenol and diethyl phosphate in the absence of NaCl. The molar extinction coefficient of p-nitrophenol was 17,000 M-1 cm-1 at pH 8; the results were expressed as U/l. Serum ARES activity was determined by the presence of phenol following the reaction of phenylacetate. The molar extinction coefficient of phenol was 4,000M-1 cm-1; the results were exp-

Table 1. Demographic Characteristics of Patients with NMST and Healthy Controls

	Patients with NMSTs (n=28)	Control subjects (n=33)	P
Age (years)	65 (45-78)	60 (50-73)	0.082
Females (n/%)	13 / 46.4	18 / 54.5	0.527
Males (n/%)	15 / 53.6	15 / 45.5	

NMSTs : Non-melanoma skin tumors

**Continuous variables were expressed as median and ranges and compared with Mann Whitney U test. Categorical variables were presented as percentages and numbers and compared with chi-square test.

Table 2. Total Antioxidant Status (Tas), Total Oxidative Status (Tos), Oxidative Stress Index (OSI), Arylesterase (ARE) And Paraoxonase (Pon) Activities of Patients with NMSTs and Control Subjects*

	Patients with NMSTs (n=28)	Control subjects (n=33)	P
TAS (mmol Trolox Eq/L)	3.29 (2.30-4.30)	3.50 (2.50-4.60)	0.005
TOS (µmol H2O2 Eq/L)	20.11 (6.38-64.91)	14.61 (3.35-68.64)	0.005
OSI (arbitrary unit)	0.67 (0.28-1.63)	0.43 (0.10-1.69)	<0.001
ARE activity(kU/L)	199.92 (182.62-220.16)	211.24 (149.38-302.36)	0.032
PON activity (U/L)	83.70 (27.05-281.58)	143.36 (6.63-296.96)	*

NMSTs: Non-melanoma skin tumors

*Continuous variables were expressed as median and ranges and compared with *Mann Whitney* U test.

Table 3. Serum Total Cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein-Cholesterol (HDL) and the Triglyceride (TG) Levels in Patients with NMSTs and Healthy Controls*

	Patients with NMSTs (n=28)	Control subjects (n=33)	P
Total cholesterol (mg/dL)	193.96±4.19	207.39±37.73	0.171
LDL (mg/dL)	118.23±28.93	131.71±32.44	0.115
HDL (mg/dL)	45.85±13.65	46.41±11.04	0.868
TG (mg/dL)	135.80±71.46	142.61±71.16	0.724

*Normally distributed data are reported as mean±SD and compared with t test.

ressed as kU/l.

Measurements of other biochemical parameters: The levels of triglycerides (TG) total cholesterol, HDL, and LDL were determined using commercially available assay kits of Siemens Advia 2400 (USA).

Statistical analysis: All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 11.5 for Windows; SPSS Inc., Chicago, IL, USA). Distribution of the variables was checked using the Kolmogorov–Smirnov test. Categorical variables were presented as percentages and numbers and compared with the chi-square test. Normally distributed continuous variables were reported as mean±SD and t test was used for comparisions. Continuous variables with unequal distribution were expressed as medians and ranges and compared with Mann Whitney U test. A P value < 0.05 was considered statistically significant.

Results

The study included a total of 28 patients (15 males and 13 females) with NMSTs. Twenty-four patients had BCC and 4 of them had SCC. The control group consisted of 33 he-

althy individuals (15 males and 18 females) (**Table 1**).
The levels of TAS and ARE activities of patients with NMSTs were significantly higher than healthy subjects (P=0.005 and P=0.032, respectively). Patients had significantly higher levels of TOS and higher OSI values when compared with control subjects (P=0.005 and P<0.001, respectively) (**Table 1**). The PON1 enzyme activities and concentrations of HDL, LDL, TG, and total cholesterol of patients and controls were similar (all P>0.05) (**Tables 2 and 3**).

Discussion

This is the first study that investigated the OS condition by TAS and TOS levels and PON1 enzyme activities in NMSTs. In this study, we detected higher total oxidant and lower total antioxidant status in patients with NMSTs when compared with healthy individuals. All these findings demonstrated an increased systemic OS in patients having NMSTs and suggested that OS may play a role in the etiopathogenesis of NMSTs.

It is well known that OS induced by UVR exposure may lead to the development of skin cancers. Solar UV radiation-induced skin cancer or photocarcinogenesis involves three distinct stages. Tumor initiation is the first step of carcinogenesis resulting in irreversible DNA mutation in normal cells. These mutations have been detected in tumor suppressor gene p53 in human NMSTs. Tumor promotion stage develops as the reversible clonal expansion of initiated cells giving rise to premalignant lesions, essentially by alterations in signal transduction pathways. In the tumor progression, the premalignant lesions converse into an invasive and potentially metastatic malignant tumor [**10**].

Most of the recent studies suggested that UVR involves in cutaneous carcinogenesis particularly by oxidative damage [**11**]. UVR exposure causes overproduction of ROS, and then results in inflammation. The emerging DNA damage, dysregulation of cellular signaling pathways and immunosuppression thereby give rise to the development of skin cancer [**10**]. Antioxidant defence systems protect the organism from the harmful effects of ROS. The antioxidant mechanisms include many enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase and non enzymatic molecules such as beta carotene, ascorbate, tocopherols, uric acid, glutathione, coenzyme Q10and proteins (metallothionein, ferritin) [**12**]. The changes in oxidant and antioxidant mechanisms of the organism have been shown to have important effects on carcinogenesis in in vivo and in vitro studies [**13**]. Overproduction of ROS and lipid peroxidation was found to be increased and antioxidant system was repressed in SCC, endometrial, laryngeal, and cervical cancers [**4, 9, 14, 15**]. In order to explain the pathogenesis of some internal cancers such as lung and endometrial cancer, oxidant and antioxidant molecules including malondialdehyde (MDA), SOD, and nitric oxide were measured separately and lower antioxidant enzyme activities were detected [**15, 16, 17**].

Recent studies have shown that antioxidant enzyme levels such as catalase, copper-zinc superoxide dismutase (CuZnSOD), and manganese superoxide dismutase are reduced and oxidative protein damage is increased in the presence of chronic UVR [**18, 19**]. As the ROS production is increased as a result of UV exposure, with the breakdown of antioxidant defence mechanism, ROS induced DNA damage and mutations are observed and the process of carcinogenesis is initiated. In the recent studies the role of antioxidant enzymes in plasma and serum samples in patients with NMST was investigated; however, there is not enough information about the antioxidant system in NMSTs. It is observed that plasma antioxidants such as ascorbic acid, alpha-tocopherol, and glutathione are reduced significantly in actinic keratosis and BCC [**20**]. In an immunohistochemical study, CuZnSOD, catalase and manganese SOD (MnSOD) were found to be reduced in the tissue samples of SCC and BCC, and the antioxidant capacity was suggested to be harmed in NMSTs [**3, 21**].

All these observations about OS in NMSTs determined the concentrations of oxidant and antioxidant molecules and enzymes separately; however, measurements are time consuming, expensive and individual values do not reflect global OS. To determine the TOS and TAS levels by recently established methods better reflects the global effects of various oxidants and provides the evaluation of efficiency of all antioxidants in the organism in a practical way [**8, 22, 23**]. The OSI value is

the most meaningful parameter showing the OS condition and is obtained by using the TOS/TAS ratio [**8, 9, 20**]. Our study is the first one that shows an increased OS in NMSTs, by measuring TOS, TAS, and OSI. In our study, PON and ARE enzyme activities were found to be decreased in the patient group, but the difference was not statistically significant. These results may suggest that PON activities may not involve in the OS in NMSTs. Since this is the first and the only study that investigated the activities of PON1 enzyme, our results need to be confirmed by other studies including larger patient groups.

Conclusion

The present study determines an increased systemic OS in patients with NMSTs. The cross sectional manner of this study is our limitation. Therefore, we cannot conclude a cause and effect relationship about OS in NMSTs. Although UVR induced ROS formation is a well-known entity, our findings suggest that a systemic OS may affect the whole organism. We believe that our findings will give rise in further investigations about OS in NMSTs. The levels of OS after excision of NMSTs, the alterations in OS in the follow-ups, and long term effects of OS on the organism should be investigated in the future studies.

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