

## Evaluation of Serum Protein Carbonyl Levels and Total Antioxidant Capacity in Patients With Basal Cell Carcinoma

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**Abstract-** Basal cell carcinoma (BCC) is one of the most common types of human cancer. Since oxidative stress is believed to be one of the pathogenic mechanisms involved in BCC formation, understanding the probable relation of inflammatory, oxidative factors, such as serum protein carbonyl and total antioxidant capacity, to BCC can help identify other pathogenic aspects of this tumor. Forty cases of BCC and forty cases of healthy controls without BCC or any other systemic disease were included in this study. After 5-ml venous blood samples were obtained from each patient, the separated serum fractions were stored at -70° C until analysis. Serum protein carbonyl level in the BCC group was significantly higher than that in the healthy controls ((5.86±3.25 μM vs. 0.86±0.20 μM,  $P<0.001$ ). The average serum total antioxidant capacity was 3.41±0.05 mM in the BCC group and 1.15±0.03 mM in the control group (statistically significant at  $P<0.001$ ). Oxidative stress might play a role in the pathogenesis of BCC.

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### Introduction

Photocarcinogenesis is one of the long-term effects of overexposure to ultraviolet radiation (UVR) from the sun. The resulting DNA photo-damage, formation of mutations, malignant transformation, and eventually, clonal expansion are the chain of events that occur in the development of UV-induced skin cancers, including melanoma, squamous cell carcinoma and basal cell carcinoma (BCC). Many of the biological properties of UVA (range, 320-400 nm) are strictly dependent on the presence of free radicals, which are predominantly represented as reactive oxygen species (ROS) intermediates, whereas UVB (range, 290-320 nm) often damages DNA directly (1). 8-Hydroxyguanine is one of the products of ROS-induced DNA damage that is inserted into DNA strands and initiates the process of tumor formation. Single-stranded breaks and oxidized pyrimidine bases are the other ROS-related effects on DNA (2-4). Signal transduction genes such as activation protein 1 and nuclear factor-KB pathways are also involved in photocarcinogenesis, which results in an imbalance between cell proliferation and apoptosis (3).

Reactive nitrogen species, the other group of free radicals, can nitrate and deaminate DNA and thereby cause strand breakage and mutation (4). The reaction of these radicals with superoxide anion produces a toxic substance with a potency to play a role in the aging process and age-related diseases (5). Proteins and cellular phospholipid membranes might be the other targets of oxidative reactions mediated by UVR and ROS. An unstable OH is the major cause of lipid peroxidation, which leads to cell membrane disintegration (6,7). Protein carbonyl derivatives are the products of cellular protein oxidation that predominantly accumulate and persist in the dermis (8). Irreversible changes in amino acids such as lysine, arginine, proline, and tyrosine occur during carbonylation (9). Protein carbonyl derivatives (ketones and aldehydes) are produced by direct metal-catalyzed oxidation of amino acids, oxidative cleavage of the peptide backbone via the amidation and glutamic acid oxidation pathways, and interaction of protein amino acid side chains with lipid peroxidation products or with reactive carbonyl derivatives (ketoamines, ketoaldehyde, and deoxyosones) (10,11). As carbonylation results in the introduction of reactive aldehyde or ketone groups in

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proteins, these groups are considered as reliable and quantifiable markers of oxidative stress. High levels of protein carbonyl are observed in several human diseases such as diabetes mellitus, arthritis, neurodegenerative disorders and pemphigus vulgaris (12-17). Moreover, this oxidative destruction may also affect the functions of enzymes, receptors and transport proteins. Consequently, inactivation of repair enzymes and loss of fidelity of DNA polymerases result in secondary DNA damage. In order to cope with these deleterious effects of oxidation, living organisms have developed complex antioxidant systems that include enzymes such as superoxide dismutase, catalase, the thioredoxin system, and glutathione peroxidase as well as non-enzymatic compounds such as albumin, ceruloplasmin, ferritin, ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, ubiquinol-10, reduced glutathione, methionine, uric acid, and bilirubin (18-20). Due to the difficulty involved in separating and measuring each antioxidant component and also the interactions between them, several methods have been developed to assess the total antioxidant capacity of human serum or plasma. Cao's research demonstrated that the antioxidant capacity of human serum is stable and is part of a tightly regulated homeostatic mechanism (21).

The purpose of this case-control study was to evaluate the oxidant and antioxidant status in patients with cutaneous BCC through the measurement of serum protein carbonyl concentration and total antioxidant capacity, in order to further understand the molecular pathogenic mechanism of BCC.

## Materials and Methods

After ethical approval for the study was obtained from the Ethical committee of Mashhad University of Medical Sciences, 40 patients with BCC who provided their written informed consent and fulfilled the inclusion criteria were included in this study. The case group had clinically and pathologically confirmed BCC and had not undergone any treatment for it. Patients who had taken antioxidant supplements or anti-inflammatory or immunosuppressive drugs in the recent 2 months prior to the study were excluded. Forty people without any type of cutaneous or systemic disease who were age- and sex-

matched were selected as the control group.

**Sample preparation:** From each person, 5 ml of blood was taken from the brachial vein and allowed to clot for 30 min at room temperature. After centrifugation at 3000 rpm for 10 min at 4° C, serum was separated and stored at -70° C until analysis.

Measurement of serum protein carbonyl was done using the Protein Carbonyl Colorimetric Assay Kit (Item No 10005020) from Cayman Chemical, USA. The assay is based on a reaction between dinitrophenylhydrazine and protein carbonyls that leads to the production of hydrazone, which can be analyzed spectrophotometrically at 360 nm.

Cayman's Antioxidant Assay kit (Item No. 709001) was used to measure the total antioxidant activity of the samples that prevents the oxidation of ABTS<sup>R</sup> (2, 2'-azino-di-3 ethylbenzthiazoline sulfonate) to ABTS<sup>R+</sup> by metmyoglobin. The product was measured by reading the absorbance at 405 nm.

After the assays were performed according to Cayman's protocols, the concentration of protein carbonyls and total antioxidant capacity were determined based on the standard curves. The SPSS 15 statistical package was used for data analysis, and a *P* of  $\leq 0.05$  was considered to indicate statistical significance. Data are represented as the mean $\pm$ SD values.

## Results

There were 28 males (70%) and 12 females (30%) in the BCC group (age, 64.4 $\pm$ 8.8 years). The control group included 26 males (65%) and 14 females (35%) (age, 63.2 $\pm$ 6.1 years). The differences were not statistically significant.

The serum protein carbonyl level in the patients (5.86 $\pm$ 3.25  $\mu$ M) was significantly higher than that in the healthy controls (0.86 $\pm$ 0.20  $\mu$ M). According to the *t*-test, the difference in the serum protein carbonyl level between the two groups was statistically significant (*P*<0.001). The maximum and minimum amounts of protein carbonyl were 10.52  $\mu$ M and 0.62  $\mu$ M, respectively, in the BCC group. In the healthy controls, the maximum and minimum values were 1.14  $\mu$ M and 0.42  $\mu$ M, respectively (Table 1).

**Table 1. Comparison of serum protein carbonyl between patients and control groups (*P*<0.001)**

Index	Patients	Controls
Number	40	40
Average	5.86 $\mu$ M	0.86 $\mu$ M
Standard deviation	3.25 $\mu$ M	0.20 $\mu$ M
Minimum	0.62 $\mu$ M	0.42 $\mu$ M
Maximum	10.59 $\mu$ M	1.14 $\mu$ M

The average level of total antioxidant capacity was significantly higher in the BCC group than in the control group (3.41 mM vs. 1.15 mM,  $P < 0.001$ ). In the BCC group, the maximum TAC value was 3.52 mM, and the

minimum value was 2.52 mM. The maximum and minimum levels of TAC in the control group were 1.20 mM and 1.10 mM, respectively (Table 2).

**Table 2. Comparison of total antioxidant capacity in patients and control groups ( $P < 0.001$ )**

Index	Patients	Controls
Number	40	40
Average	3.41 mM	1.15 mM
Standard deviation	0.05 mM	0.03 mM
Minimum	2.52 mM	1.10 mM
Maximum	3.92 mM	1.20 mM

## Discussion

Oxidative stress has been associated with photo-aging as well as photocarcinogenesis. ROS lead to oxidation of the amino acids arginine, proline and lysine, which leads to the formation of protein carbonyl groups. Increased levels of oxidized proteins have been found in keratinocytes from aged donors, and its stability makes it a good index of oxidation (22). An increased level of protein carbonyls can also indicate the probable role of oxidative stress in BCC. Based on the findings of this study, we concluded that there was a significant difference between patients with cutaneous BCC and the healthy control group with regard to oxidant-antioxidant balance, as the average level of protein carbonyl was higher in the former. However, there was also a compensatory rise in the total antioxidant capacity in the BCC group. This increase might be attributable to the response of the antioxidant system to the oxidative state. The fairly low average age of our patients may also explain the remedial rise in the antioxidant status. Bastiaens reported that the antioxidant level is increased in chronically photo-exposed skin due to the age-related compensatory mechanism against UVR (23). Moreover, Sander *et al.*, concluded that oxidative stress has different pathogenic mechanisms in melanomatous and non-melanomatous skin cancers. In non-melanomatous skin cancers such as BCC, a reduction in antioxidant defense occurs as a result of chronic UV exposure and leads to cancer formation, whereas melanomatous cells produce ROS and facilitate cancer progression (24). Additionally, the levels of antioxidant enzymes such as catalase and superoxide dismutase have been reported to decrease, according to an immunohistologic assay conducted on skin biopsy samples obtained from patients with BCC (25). The formation and subsequent accumulation of

protein carbonyls within the papillary dermis are noted in photo-aged skin and premature aging. However, protein oxidation was not as prominent in the epidermal layers as in the dermis, probably because of the more efficient antioxidant function of the epidermis (8). Of note,  $H_2O_2$  and other ROS agents contribute to protein oxidation after exposure to UVR in both fibroblasts and keratinocytes (8). It has also been demonstrated that ascorbic acid,  $\alpha$ -tocopherol, total thiol groups and ceruloplasmin are significantly depleted in patients with BCC (26). In a cohort study, people with a higher serum selenium level had a lower incidence of squamous cell carcinoma and BCC in the future (27). The effect of oxidant-antioxidant imbalance has been also found in inflammatory mucocutaneous diseases such as psoriasis, lichen planus and vitiligo (28-30). In conclusion, our study shows that oxidative stress might play a role in the pathogenesis of BCC and that therapeutic protocols based on augmentation of the antioxidant system might have the potential for the treatment of BCC in the future.

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