Antioxidant capacity total in non-melanoma skin cancer and its relationship with food consumption of antioxidant nutrients

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Abstract

The non-melanoma skin cancer is the most common cancer and accounts for more than half of the diagnoses of cancer, and basal cell carcinoma (BCC), the most frequent cutaneous neoplasm, corresponding to 70-80% of cutaneous tumors. Oxidative stress is an important trigger for skin carcinogenesis. Thus, it is important to evaluate oxidative stress, in order to discern effective therapeutic strategies able to stop it or attenuate it, thereby prevent the installation of non-melanoma skin cancer. Cross-sectional study with controls, involving 84 individuals of both sexes aged between 38-84 years, divided into two groups: control group of healthy people (n = 24) and the case group included individuals who presented non-melanoma skin and they have undergone surgery (n = 60). The blood samples of the individuals were obtained for evaluation of biomarkers of oxidative stress (F2-isoprostane, nitrite, thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity). The usual dietary intake and nutritional status of the subjects were evaluated. The significance level for this study was 5%. Patients in the case group had higher serum concentrations of biomarkers of oxidative stress, F2-isoprostane concentrations were significantly higher compared to controls. The results showed high rates of overweight and obesity in the case and control groups. The dietary concentrations of antioxidant minerals zinc, copper and selenium in the case group were significant lower compared to controls. The correlation between markers of oxidative stress and dietary concentrations of antioxidant nutrients showed the influence of food intake of vitamins A and E in reducing oxidative stress, since these nutrients behave as important antioxidants, acting as sweepers of RL, by removing of the body the negative effect. The dietary concentrations of antioxidant minerals zinc, copper and selenium in the case group were significant lower compared to controls. The correlation between markers of oxidative stress and dietary concentrations of antioxidant nutrients showed the influence of food intake of vitamins A and E in reducing oxidative stress, since these nutrients behave as important antioxidants, acting as sweepers of RL, by removing of the body the negative effect.
effects on the redox balance of the skin. We emphasize the importance of adopting healthy eating habits that optimize the consumption of antioxidant nutrients as a strategy to prevent oxidative damage to the skin.

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Key words: Skin Neoplasms. Oxidative stress. F2-isoprostanes. Antioxidants. Biomarkers.

Introduction

The National Cancer Institute (NCI) estimates about 580,000 new cancer cases for 2014 and the most incident cancers in the Brazilian population will be: non-melanoma skin cancer (182,000), prostate (69,000); breast (57,000); colon and rectum (33,000), lung (27,000) and stomach (20,000).

The non-melanoma skin cancer (NMSC) is the most common cancer and accounts for more than half of the diagnoses of cancer. Risk factors include: clear skin sensitive to ultraviolet radiation, excessive exposure to sun, weather conditions (tropical climate and climate at very high altitudes), the personal and family history of the disease. Basal cell carcinoma (BCC) is the most common skin cancer, accounting for 70-80% of skin tumors. Environmental factors have a strong influence on the tumorigenic process, and this is the reason of the occurrence of the variations in their incidence in different geographical locations and ethnicities.

The skin is the largest body organ and it is an important link between the environment and the body whose function is to protect it from physical, chemical and microbiological damaging agents which can alter the skin structure and function, by inducing the formation of reactive oxygen species (ROS) and nitrogen (RNS).

These species are essential to maintain cellular homeostasis and survival, when at equilibrium between formation and removal. However, when there are marked changes in the balance, a pro-oxidant state is generated, thus leading to oxidative stress.

Ultraviolet radiation (UVR) from sunlight is a potent environmental risk factor in the pathogenesis of skin cancer. Reactive oxygen species and nitrogen produced by UV radiation are associated with both the initiation, promotion, and with the progression of carcinogenesis. They are capable of inducing the production of transcription factors such as activator protein 1 (AP-1) and nuclear factor kappa B (NF-KB). The NFκB activated, in turn, stimulates the production of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF α) and interleukin-1β (IL-1 β), and it may play an important role in redox regulation.

ROS induce lipid peroxidation (LPO) which is responsible for: changes in membrane permeability, destroying the cell membrane structure, failure of the mechanisms of exchange of metabolites, and in an extreme condition, cell death. During the LPO, the enzyme cyclooxygenase catalyzes the production of prostaglandins, which in combination with nitric oxide causes: inflammation, benign tumors, solar keratosis (SK), which can progress to squamous cell carcinoma (SCC), probably because the cells inflammatory produce reactive oxygen species, thereby increasing oxidative damage to DNA and the immune system.

The objective of this article is to evaluate the markers of oxidative stress in people who had non-melanoma skin cancer in healthy people and in this regard and its association with habitual food intake relative to antioxidant nutrients, in order to contribute to consolidate new therapeutic formats and to program an effective preventive approach that aims at adopting healthy eating habits that ensure a satisfactory intake of antioxidant nutrients.

Material and methods

Cross-sectional study with controls, involving 84 individuals between 38-84 years. The individuals were divided into two groups: cases (previously diagnosed with non-melanoma skin cancer, n = 60) and control (people who have never had a non-melanoma skin cancer, n = 24). People with non-melanoma skin cancer were recruited among patients who were attended at the Dermatology Clinic of the HGV, in 2011, whose results of the biopsy revealed a diagnosis of basal cell carcinoma (BCC) or squamous cell (SCC).

The eligibility criteria for the case and control groups were: more than 20 years old; no pre-existing disease, including: melanoma, insulin dependent diabetes mellitus, severe heart disease (with hemodynamic repercussions or cianosantes), liver dysfunction (jaundice or cirrhosis), renal failure (requiring dialysis), HIV +; no use of chemotherapy or radiation in the previous six months; no severe psychiatric illness that would limit the ability of understanding and acceptance to participate by donating the material, no consume of supplementation of vitamins and minerals. The specific inclusion criterion for control group was: no diagnosis of BCC / SCC and for the case group was the previously diagnosed BCC / SCC whose skin lesion has already undergone excision. The exclusion criterion was not accepting participate. The study was approved by the Ethics Committee of the Federal University of Piauí,

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Assessment of nutritional status

Weight, height, triceps skinfold (TSF), arm circumference (AC), arm muscle circumference (MAMC) were the nutritional assessment parameters used. The simplified normal range according Jellife (1966) was used to interpret the results. For the classification of EN compared to the percentage of AC, TSF and MAMC values of Blackburn, GL and Bistrian, BR were used (1977) and for the elderly, the values of AC, TSF and MAMC were compared to the 50th percentile according to sex and age recommended in National Health and Nutrition Examination Survey - NHANES III (Kuczmarski et al, 2000).

For classification of nutritional status according to body mass index (BMI) to less than 60 years age group, were adopted benchmarks set by the World Health Organization (2000) and for the age group over 60 years, the benchmark established by the Pan American Health Organization (PAHO, 2001) was adopted.

Assessment of usual dietary intake of antioxidant nutrients

The individuals filled a FFQ (Food Frequency Questionnaire) validated by Crispin and Ribeiro and Panato. The estimate of habitual food intake was made from the methodology developed by Sichieri and Everhardt (1998), in a validation study. Diet composition was assessed by NutWin 2.5 software. The amounts of dietary intake of the antioxidant nutrients were adjusted by energy, according to the method residual nutrient by Stampfer and Willett (1986). It was used as a reference the estimated average requirement (EAR) defined for antioxidant nutrients.

Table I

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 24)</th>
<th>Case (n = 60)</th>
<th>Value of p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.7 ± 11.6</td>
<td>62.7 ± 14.2</td>
<td>0.0492</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.4 ± 13.1</td>
<td>63.9 ± 10.1</td>
<td>0.6300</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.9</td>
<td>0.4750</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.3 ± 3.5</td>
<td>26.3 ± 3.7</td>
<td>0.2710</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>20.2 ± 6.8</td>
<td>18.4 ± 7.8</td>
<td>0.1750</td>
</tr>
<tr>
<td>AC (cm)</td>
<td>30.9 ± 3.1</td>
<td>30.0 ± 4.5</td>
<td>0.1910</td>
</tr>
<tr>
<td>MAMC (cm)</td>
<td>24.6 ± 3.0</td>
<td>24.5 ± 4.0</td>
<td>0.7100</td>
</tr>
</tbody>
</table>

Legend: BMI - Body Mass Index; TSF - Triceps Skin Fold; AC - Arm Circumference; MAMC - Circumference Muscular Arm. Mann-Whitney test, p > 0.05 difference is not significant.

Results

The mean and standard deviation for age and anthropometric parameters used to assess nutritional status of the subjects in the case group and control values are shown in table I. There was a statistically significant difference in age between the groups.

The mean values of BMI and TSF suggest overweight and accumulation of fat mass respectively in both the control group and a study group, and the mean values of nutritional indices and AC and MAMC suggest normal the concentration of lean and fat mass in the

Determination of the concentrations of total antioxidant capacity

The total antioxidant capacity of plasma collected with citrate was determined using the commercial Antioxidant Assay Kit (Cayman, USA). The assay is based on the ability of the sample antioxidant to inhibit the oxidation of ABTS® (2,2-azino-di [3-ethylbenz-thiazoline sulfonate]) to the ABTS® • + metmyoglobin. The amount of produced ABTS® • + can be monitored by absorbance at 750 or 405 nm.

Statistical Analysis

For statistical analysis a database was constructed, by using the Statistical Package for the Social Sciences - SPPS version 15.0 (2007). The results were displayed as mean and standard deviation. Statistical analysis initially involved the application of the Kolmogorov-Smirnov test to verify normal distribution of the quantitative variables. For comparison of means, ANOVA Student’s t test or Mann-Whitney test was used as appropriate; the correlation between variables was obtained by Spearman correlation. In all tests, it was adopted p <0.05 considered statistically significant.
two groups. The values of anthropometric data did not differ between groups.

Table II shows the estimated dietary concentrations of antioxidant nutrients from case and control groups. It was possible to detect a statistically significant difference in the habitual dietary intake of vitamin A, E and the minerals zinc, copper and selenium between groups.

Figure 1 shows the comparison of serum concentrations of total antioxidant capacity of the control group (1.8 ± 0.4 mMol/L) and case group (2.3 ± 1.1 mMol/L).

The association between CAOT and dietary concentrations of antioxidant nutrients in the control group showed negative weak (r = -0.488) and significant correlation (p = 0.015) between CAOT and Vitamin A.

Discussion

The main environmental risk factor for NMSC is exposure to ultraviolet radiation, which can cause excessive amounts of reactive oxygen species and nitrogen species, contributing to the installation of oxidative stress6,7, which has a major effect on cutaneous immune system, inducing a state of location6,7,8 immunosuppression, compromising the antitumor defense system.

![Antioxidant Capacity Total control groups (n = 24) and case group (n = 60). Legend: ANOVA test for repeated measures. There was no statistically significant difference (p = 0.8767).](image)

Fig. 1.—Antioxidant Capacity Total control groups (n = 24) and case group (n = 60). Legend: ANOVA test for repeated measures. There was no statistically significant difference (p = 0.8767).

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**Table II**

Dietary concentrations averages and standard deviations of the consumption of antioxidant nutrients in the diet to the case and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 24) Average ± SD</th>
<th>Case (n = 60) Average ± SD</th>
<th>EAR(^{#})</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (μg)</td>
<td>380.9 ± 197.1</td>
<td>558.0 ± 570.0</td>
<td>625</td>
<td>0.0010</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>162.4 ± 19.3</td>
<td>57.9 ± 50.6</td>
<td>75</td>
<td>0.2220</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>6.1 ± 14.2</td>
<td>9.6 ± 7.02</td>
<td>12</td>
<td>0.0010</td>
</tr>
<tr>
<td>Copper (μg)</td>
<td>1040.0 ± 1009.0</td>
<td>800.0 ± 400.0</td>
<td>700</td>
<td>0.0330</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>10.1 ± 12.6</td>
<td>6.5 ± 3.6</td>
<td>9.4</td>
<td>0.0310</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>52.2 ± 44.5</td>
<td>76.2 ± 38.8</td>
<td>45</td>
<td>0.0280</td>
</tr>
</tbody>
</table>

Legend: Sd = Standard Deviation; Estimate average application EAR different for sexes (vitamin A 625 μg/d males and 500 μg/d female. Zinc 9.4 mg/d male 6, 8 mg/d female. Vitamin C 75 mg male and 60 mg/d female). Mann-Whitney test significant difference (p < 0.05). Dietary nutrient concentrations adjusted for energy.

**Table III**

Analysis of simple linear correlation between total antioxidant capacity and dietary concentrations of antioxidant nutrients of case and control groups

<table>
<thead>
<tr>
<th>CAOT</th>
<th>Dietary concentrations of antioxidant nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>G. Control</td>
<td>r = 0.064</td>
</tr>
<tr>
<td>p = 0.015</td>
<td>p = 0.226</td>
</tr>
<tr>
<td>G. Case</td>
<td>r = 0.064</td>
</tr>
<tr>
<td>p = 0.654</td>
<td>p = 0.888</td>
</tr>
</tbody>
</table>

Legend: CAOT - Total Antioxidant Capacity; Vitamin A; Vitamin C; Vitamin E. Spearman Correlation.
Researches directed to mechanisms for restoring the redox balance of the skin as a prevention or treatment for NMSC strategy have been encouraged and the use of antioxidants is emerging as a promising alternative\textsuperscript{20}. There is more and more evidence that antioxidants promote rapid repair of oxidative damage (through sequestration of reactive oxygen species in the lipid membrane, eg.), helping to reduce the production of free radicals, lipoperoxidation, promoting the repair DNA damage and protein and thus reducing oxidative damage to the skin\textsuperscript{31}.

This study showed a predominance of females in 19 (79.2\%) of the controls and 39 (65\%) of cases, similar to the results of Mantese et al.\textsuperscript{22}, Becher et al.\textsuperscript{23} Spindola et al.\textsuperscript{24}, which also performed studies in patients with NMSC. This result is due to an earlier search of health services by women, making them more aware of their own bodies and yet they constitute the target audience for several educational campaigns against cancer, alerting them about the problem, its risk factors and possible manifestations\textsuperscript{23}.

The average age was statistically higher in the case group compared to control (p = 0.0492). With advancing age there is an increase in ROS and RNS, depression of the immune system and reduction of DNA repair mechanisms, beyond the effects of cumulative sun exposure over a lifetime\textsuperscript{25}, thereby increasing vulnerability to NMSC.

The mean values of anthropometric indices AC, TSF and MAMC suggest accumulation of fat mass and lean mass. The classification of nutritional status showed 51.7\% in normal weight and overweight / obesity in 48.3\% of cases by BMI, signaling to the low nutritional impact caused by NMSC, since it has a high cure rate and effective treatment with the removal of skin lesion. The NMSC does not cause digestive and absorptive disorders that compromise the nutritional status of their bearers, which favorably affects the mental and emotional conditions, providing welfare, better appetite and greater freedom in the selection of food, which can lead to increased body weight.

These current results are aligned with Tartari et al.\textsuperscript{25}, Azevedo and Bosco\textsuperscript{26}, who assessed the nutritional status of patients with cancer by BMI and found high levels of eutrophication and / or overweight.

The mean BMI of the cases was not according to the results of Mantese et al.\textsuperscript{22}, Becher et al.\textsuperscript{23} Spindola et al.\textsuperscript{24}, which also performed studies in patients with NMSC. This result is due to an earlier search of health services by women, making them more aware of their own bodies and yet they constitute the target audience for several educational campaigns against cancer, alerting them about the problem, its risk factors and possible manifestations\textsuperscript{23}.

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The mean BMI of the cases was not according to official recommendations contained in the global perspective on food, nutrition and cancer prevention\textsuperscript{27} report, what could be a factor that would contribute to the generation of oxidative stress.

The average age of the group was 62.7 ± 14.2 years, an irregular and insufficient intake of protein sources, zinc intake in the study group, which is identified by EAR, therefore not reaching the recommended nutritional coverage, showing great probability of inadequacy of intake of these nutrients. In the case group, only the average intake of selenium and copper exceeded the EAR, the average vitamin A, C and E and the mineral zinc dietary concentrations were lower than the values of EAR, therefore not reaching the recommended nutritional coverage, showing great probability of inadequate intake of these nutrients.

The findings of this study suggest to inadequate zinc intake in the study group, which is identified by an irregular and insufficient intake of protein sources, represented by food: beef, fish and eggs and still no reports of intake of whole grains, cereals integrals, nuts walnuts, almonds, wheat germ, nuts, pistachios, liver and other foods rich in this nutrient.

The probable deficit of vitamins C, A and E can be identified by an irregular and even occasional consumption of foods such as orange juice, cashew, cherry, pumpkin, orange, papaya, melon, mango, strawbe-
It is noteworthy that the classification of food consumption patterns concerning the antioxidant nutrients allowed to establish the predominance of quantitative deficit of intake of all nutrients evaluated in the case group, revealing conducive to installation deficiencies tables, disabling the body to enjoy the effects played by these antioxidant nutrients.

The correlation found in this study between CAOT and dietary concentrations of antioxidant nutrients in the control group suggests that dietary intake of vitamins A could induce a compensatory mechanism to oxidative stress, slowing the production of free radicals and slowing the depletion of antioxidant defenses, contributing to lower serum concentrations of CAOT in this group. And the results of the same correlation in the case group, highlighted the influence of the consumption of food sources of vitamin A reducing oxidative stress, since the nutrient in focus behaves as an important antioxidant, acting as a scavenger RL, freeing the body of these negative effects on the redox balance of the skin.

A proper, balanced diet, rich in antioxidants and nutrients acts as a preventive mechanism in generating RL, hence it can be inferred that the individuals who had NMSC, included in this study, did not adopt positive strategies capable to protect the skin.

The probability of nutrients inadequacy in focus, even not acting alone in the propensity to skin cancer, may contribute to the depletion of endogenous antioxidant mechanisms, generating an imbalance oxidant / antioxidant, which can lead to oxidative stress. If people continue the current food consumption, this will cause serious nutritional compromise, thus disabling the body to meet the demands antioxidant, now increased, due to previously diagnosed NMSC.

In face of the action of these nutrients in the prevention of oxidative stress and inflammatory stress, it becomes imperative to adopt eating habits that ensure adequate intake of food sources of these nutrients, as a preventive strategy to oxidative damage in the population studied.

Conclusion

The assessment of nutritional status showed high prevalence of overweight and obesity and excess fat and lean mass, reflecting the low impact of the disease on nutritional status. The maintenance of BMI in excess of the levels recommended by the World Cancer Research Foundation proves to be able to explain the propensity for disease progression.

This fact suggests the need for nutritional counseling of these individuals, since adiposity may precipitate the pro-oxidant, increasing the chances of disease recurrence and cause other nutritional and metabolic disorders.

It was possible to confirm the previous diagnosis of NMSC led to the pro-oxidant status in the case group, probably due to the late effects of UVR, driven largely by the generation of a chronic oxidative stress, a chronic increase in free radicals, chronic inflammatory stress and depletion of antioxidant defenses. Factors such as being overweight, advanced age, striking sun exposure, alcohol consumption and smoking may also have contributed to the pro-oxidant status checked.

The mean dietary antioxidant nutrients concentrations of the case group pointed to high probability of inadequacy of vitamins A, C, E and the mineral zinc, not reaching the recommended nutritional coverage, favoring the pro-oxidant status verified in the cases, which greatly hinders the fight against free radicals produced.

References