



HEALTH SCIENCES

Plasma antioxidant capacity in cervical cancer patients

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Abstract: The oxidative pathway and the alteration of the antioxidant defense have drawn attention to the pathophysiology of cervical cancer (CC). In our preliminary study, it was possible to corroborate the findings regarding the antioxidants of patients affected by CC. The total antioxidant activity and lipid peroxidation (LP) were evaluated in 14 people diagnosed with CC and 14 volunteers without CC (control group). Results showed low antioxidant activity in CC group. LP was not significant when analyzing CC group and control group. Our results indicate that changes in antioxidant defense may contribute to the pathophysiology of CC.

Key words: uterine cervical neoplasms, antioxidant effects, lipid peroxidation, oxidative stress.

INTRODUCTION

According to the 2020 estimate, cervical cancer (CC) will have an incidence of 24.74/100.000 habitants in Maranhão, thus becoming the most frequent neoplasm in this state (INCA 2020). CC is curable when diagnosed early; however, it is responsible for a high mortality rate (Borges et al. 2018). Studies show the Human Papilloma Virus (HPV) as the main etiology, but the carcinogenesis of CC is still not well understood (Fontham et al. 2020).

Reactive Oxygen Species (ROS) can cause irreversible damage to biomolecules, such as proteins, lipids, and DNA, contributing to cell damage and death. Regarding the endogenous antioxidants, the excess of ROS is called Oxidative Stress (OS), a known pathophysiological component of the cancer cells resulting from increased metabolic activity (DeBernardinis & Chandel 2016).

There are three cooperative mechanisms between HPV and OS that contribute to carcinogenesis: genotoxicity, genomic instability

generated by the virus, and the direct action of the E6 protein increasing ROS in the oncogenic HPV subtypes (Zhen & Li 2017).

Thus, the present work aims to test the antioxidant defense and lipid peroxidation in plasma samples from patients diagnosed with CC.

MATERIALS AND METHODS

Study participants and ethical issues

A case-control study was carried out with 14 patients diagnosed with CC, who were allocated in CC group and 14 volunteers without CC, who, in turn, were allocated in control group. The research took place in Imperatriz, Maranhão – Brazil, with patients from the public health system. Inclusion criteria were: patients diagnosed with CC. On the other hand, presence of other disease, use of drugs and nutraceuticals, including ascorbic acid, vitamin E and tocopherol; smoking, alcohol consumption and pregnancy were considered as exclusion criteria. Peripheral blood samples

were collected, respecting biosafety and sample preservation. The blood was centrifuged at 4000 rpm for 10 minutes, and the plasma was removed and stored in a freezer (at -20°C).

The Study was approved by the Research Ethics Committee of the Federal University of Maranhão (CAAE: 94200518.7.0000.5087), and all participants were previously informed about the research and signed the Free and Informed Consent Form (FICF).

TBARS assay

Lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS) assay performed according to Esterbauer & Cheeseman (1990). Malondialdehyde (MDA) from plasma reacts with thiobarbituric acid (TBA 0.38 %) at 95°C for 30 minutes in $\text{pH} = 2.5$. Measurements were performed at 532 nm.

ABTS assay

Antioxidant activity was determined using the Trolox equivalent antioxidant capacity (TEAC/ABTS) assay by Miller et al. (1993) modified by Ferrante et al. (2019) based on the absorbance of ABTS^{\bullet} radical. The compound has a maximum absorbance of 734 nm, allowing the antioxidant activity of the sample to be evaluated based on the discoloration of the solution, compared to the Trolox standard antioxidant (6-hydroxy-2 acid, 5,7,8-tetramethyl chroman-2-carboxylic acid).

Anthropometric parameters, questionnaires and medical interviews

The questionnaire items were: age, education, history of preventive examinations, gynecological-obstetric history, family and personal pathological history, CC staging, symptoms, and life habits.

Statistical analysis

The data was analyzed by Student's t-test, Pearson's coefficient (r) and linear regression, with the aid of BioEstat program (Graphpad Software, version 5.3). All data were expressed as average \pm standard deviation (SD), and $p < 0.05$ was considered statistically significant.

RESULTS

The ABTS method showed low antioxidant activity rate in individuals with CC when compared to those without CC (Fig. 1). The final average for CC group was 1.7615 ± 0.0839 mM, while for control group it was 1.8544 ± 0.0778 mM. The final p for the analyzes was 0.00543 (bilateral), degrees of freedom (DF) = 26 and $t = -3.0357$.

In CC group and control group, the average TBARS was 9.9607 ± 1.9221 μM and 10.3448 ± 2.7849 , respectively. Both groups had no significant difference ($p_{\text{unilateral}} = 0.3373$, $p_{\text{bilateral}} = 0.6747$, DF = 26 and $t = -0.4245$).

There was no significant correlation between TEAC and TBARS ($p = 0.5028$ and $r = 0.1956$).

The patients with CC were 43.57 ± 14.74 years old and non-CC participants were 47.71 ± 11.68 years old. Regarding education, the result was homogeneous, showing that half of both groups did not complete the first stage of elementary school (until the 5th grade).

From CC group, 17.6% of the surveyed patients presented Stage I neoplasm, while Stage II and III accounted for 47% and 23.4%, respectively. Vaginal bleeding (46%) was the main complaint presented, followed by pelvic pain (42%), and asymptomatic patients accounted for 11% during the last examination.

In the context of preventive medicine, 42% of the diagnosed patients underwent biannual prevention, while 35% underwent uncertain intervals and 23% had only undergone one examination in their lifetime. In control group,

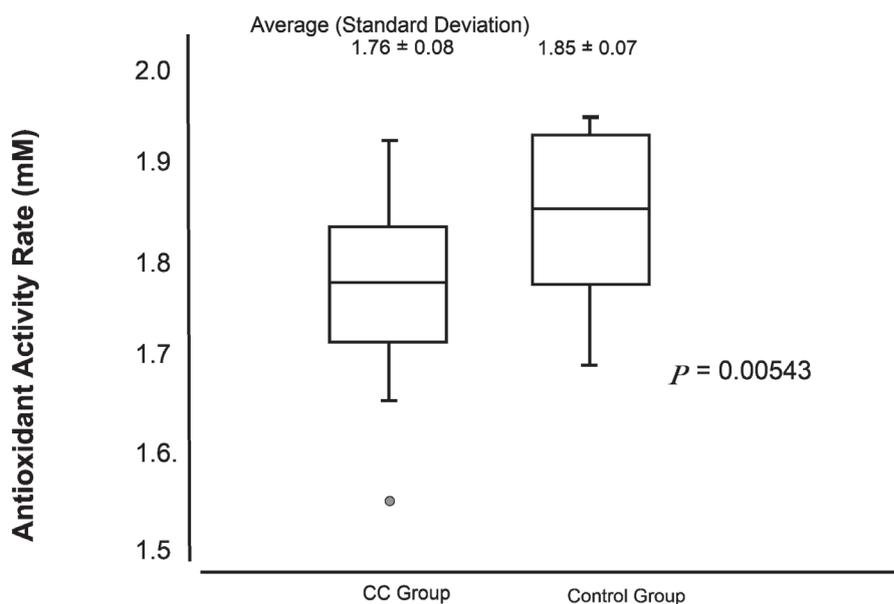


Figure 1. Result of the analysis of the Antioxidant Activity Rate. Student's t-test was used to compare the two groups. CC: cervical cancer.

79% biannual, 14% uncertain and 7% had a single examination.

In terms of sexual activity, 47% of CC patients started at 16 years of age or less, while 70% of control group were over 17 at the time of their first intercourse.

DISCUSSION

Significant changes were observed in the antioxidant activity of patients with CC, thus confirming that the existence of cancer alone can generate changes in the overall oxidation status in the patients' plasma. These results were in line with those of Kim et al. (2009) and Shah & Kalal (2019), who reported the altered antioxidant status of patients with CC compared with healthy controls, which was estimated using ABTS method and FRAP assay (ferric reducing antioxidant power), respectively. Srivastava et al. (2009) observed reduced glutathione (GSH) and decreased superoxide dismutase (SOD) in patients with cervical cancer.

The lipid peroxidation results diverge in CC patients' research. Gonçalves et al. (2005) identified increased lipid peroxidation in

patients with CC. Conversely, the most recent research shows that lipid oxidation is not significant in patients affected by CC (Borges et al. 2018). In the present study, no significant differences between MDA levels of affected and non-affected women. Difference in sample and method of lipid peroxidation analysis can change the results.

In light of the foregoing, this study has revealed that the preventive examination is an important tool, as there is a marked presence of this care in control group (79 % biannual).

Vaginal bleeding and pelvic pain were the commonest clinical features seen in CC patients. These results were similar to those revealed by studies conducted by Slimani et al. (2016).

Therefore, it is concluded that the decrease in antioxidant capacity seems to be an important factor in the genesis of cervical cancer, and that such change can be caused by radicals that act in biomolecules of a non-lipidic nature, such as DNA, sugar and proteins.

Acknowledgments

We thank all patients and volunteers who participated in the study and Laura C. Nery, source of inspiration.

This study was supported by Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA, Brazil).

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How to cite

NERY EC, NETO AMS, LYRA JS & FERREIRA MES. 2022. Plasma antioxidant capacity in cervical cancer patients. *An Acad Bras Cienc* 94: e20201733. DOI 10.1590/0001-376520220201733.

Manuscript received on November 3, 2020; accepted for publication on January 23, 2021

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Nery EC and Neto AMS performed sampling and interpretation of data, as well as the writing of the paper. Dr. Ferreira MES developed study design and monitoring, performed the statistical analysis, and corrected the final text. The physician Lyra JS analyzed clinical characteristics and helped finalize the paper.

