Short Comunications

Oxidative Imbalance and Risk Factors Involved in Premalignant and Malignant Lesions of the Uterine Cervix

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ABSTRACT

Objetive: To determine the indicators of oxidative stress and risk factors in patients with premalignant and malignant lesions of the cervix.

Methods: A total of 150 women were studied: 40 with low-grade squamous intraepithelial lesion, 40 with high-grade squamous intraepithelial lesion, 40 with stage IIB squamous cell carcinoma and 30 with negative cytology taken as a control, from the Gynecology Service of the "Chiqui Gómez" Polyclinic, and Oncology Service "Celestino Hernández" Hospital of Santa Clara, aged between 19 and 70. The information on the risk factors was obtained through an individualized interview, and oxidative stress indicators were determined by spectrophotometric techniques. The comparison and association between groups was carried out with the support of the statistical program SPSS.

Results: The levels of antioxidant enzymes showed a significant decrease in both groups of squamous intraepithelial lesion, glutathione decreased significantly in the three groups of lesions, while malonildialdehyde significantly increased in the same groups with respect to the control. The risk factors with the highest incidence in the affected women were early sexual contact, the number of sexual partners, the use of oral contraceptives, exposure to environmental toxins and the habit of smoking.

Conclusions: There is an oxidative imbalance in the cases due to the decrease of the antioxidant enzymatic system, where the reduced levels of glutathione contribute to the increase of the lipid peroxidation. Risk factors may influence exposure to the papilloma virus and lead to the development of premalignant and malignant lesions in the cervix.

Key word: oxidative stress, premalignant and malignant lesions, risk factors.

INTRODUCTION

Premalignant and malignant lesions of the uterine cervix occur in most cases in the squamo-columnar junction of the cervical epithelium, considered the area of greatest susceptibility to infection by human papillomavirus (HPV). This infection is transmitted sexually, is present in 99% of cervical cancer cases and can also cause carcinomas of the penis, vagina, vulva and anus.[1]

More than 200 types of HPV have been identified and classified according to their oncogenic risk. The lowest-risk HPVs (LR-HPV) reported most frequently are types 6, 11, 40, 42, 43, 44, 54, 57, 61, 70, 72 and 81, which tend to develop warty skin lesions and the mucous membranes; while the high risk (HR-HPV) includes types 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82, the Types 16 and 18 of greater oncogenicity closely related to precancerous and cancerous lesions of the cervix.[2]

The risk of contracting a genital HPV is influenced in the first place by sexual activity, although risk co-factors are added that promote the progression of the lesions. Among the multiple events that may explain this fact, are sexual activity at an early age, promiscuity, multiparity, co-infection with other viruses (herpes simplex (HSV) type 2, cytomegalovirus (CMV), human herpesvirus types 6 and 7 (HHV-6)), intra-type viral variants, persistence or viral load, genetic predisposition, prolonged use of oral contraceptives, toxins exposure, among others. It that could contribute substantially to the occurrence and persistence of infection.[3]

During cervical carcinogenesis the epithelial tissues are exposed to numerous pro-oxidant sources and are the targets by choice of HPV. The disturbance of the redox balance in favor of the reactive oxygen and nitrogen species (RONS) can cause functional alteration in the cell membranes of lipids, proteins and nucleic acids. This alteration could induce activation of transcription factors that modify the expression response of the genes and modulate the function of the proteins sensitive to the redox state.[4]

The release of ROS can occur during the inflammatory response to viral infection, where neutrophils and macrophages at the oxidative onset, give rise to pro-oxidant cytokines from activated phagocytes. It can also occur as a result of the immune response to the virus's proteins or as a consequence of the viral expression of the genes, which could damage the DNA and facilitate the integration of the virus.[4]

Oxidative stress (OS) studies can offer information about the imbalance between the production of reactive oxygen metabolites and the activation of the antioxidant system, which could be an interesting co-factor in cervical carcinogenesis. Therefore, the objective of the study was to determine oxidative stress indicators and risk factors in patients with premalignant and malignant lesions of the cervix.

Methodology

A cross-sectional analytical research was carried out, with case-control design in the Blood Chemistry Laboratory of the Biomedical Research Unit, belonging to the University of Medical Sciences of Villa Clara, with the collaboration of the gynecology clinic of the "Chiqui Gómez Lubián" polyclinic of Santa Clara and the multidisciplinary consultation of the Oncology Service of the "Celestino Hernández Robau" Hospital, during the year 2018.

Once the sample was selected taking into account inclusion / rejection criteria and obtaining the consent of the participants, a total of 150 women aged between 25 and 70 years, taken at random, were included. Four groups were formed: 40 women with low-grade squamous intraepithelial lesion (LSIL), 40 women with high-grade squamous intraepithelial lesion (HSIL), 40 women with stage IIB squamous cell carcinoma and 30 women with negative cytology taken as control. In all the groups the diagnosis was made by cervico-vaginal cytology (Papanicolau smear), the groups of cases were corroborated by colposcopy and / or biopsy.

Individualized interview was conducted for each patient, that included: personal, sexual, reproductive, lifestyle and personal and family pathological data; by which the risk factors with the highest incidence were determined.

The OS indicators included the study of the antioxidant enzymatic system (superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) as a cofactor of the enzyme glutathione peroxidase), as well as the levels of malonildialdehyde (MDA) as an indicator of damage to lipids.

Determinations were made in blood plasma and the analytical techniques used spectrophotometric methods (Genesys 10 UV ®), with reagents supplied by the firms Merck and HELFA.

The determination of the extracellular SOD enzymatic activity was carried out using the kinetic method described by Marklund (1990), the CAT by the kinetic method of Aebi (1974) and the concentration of total proteins by the method of Lowry (1951), used for refer the units of specific enzymatic activities (UAEE = UAE / mg of proteins). On the other hand, the concentration of GSH was carried out through the method described by Beutler (1986) and MDA by the method of Esterbauer (1990).

Statistical analysis was made from a database using the SPSS program, version 18.0 of Windows® XP Titan Ultimate. It was verified by applying the Chapiro Wills goodness of fit test that the data did not fulfill a normal distribution ($p < 0.05$). Therefore, to compare the mean ranges, non-parametric tests were used, specifically the Mann-Whitney test, with a confidence interval of 95%.

The research was approved by both the Scientific Committee and the Ethics Center and part of a national project hired by CITMA, which took into account the ethical regulations for human

work. The team of researchers gave the objective explanations of the study to the participating women, who issued their authorization in writing (consent), respecting the principle of autonomy.

RESULTS

Average age of the women participating in the research was 52 years.

When performing the comparisons of the oxidative stress indicators in the LSIL group, a significant decrease was observed in SOD (p=0,028), CAT (p=0,012) and GSH (p=0,045) with respect to the control, as well as an increase in significant level of MDA (p=0,040).

Patients with HSIL behaved similarly; decreased levels of SOD (p=0.014), CAT (p=0.030), GSH (p=0.043), while MDA increased significantly (p=0.012) with respect to the control group.

In the group with diagnosis of squamous cell carcinoma, there were no significant differences in SOD and CAT, GSH decreased significantly (p=0.031), and MDA showed a significant increase (p=0.027).

Risk factors in all groups of women with cervical lesions had a similar behavior, so they were analyzed as a single group of cases (n=120)

The early onset of sexual intercourse (84%), the number of sexual partners (64%), the use of oral contraceptives / IUD (57%), exposure to environmental toxins (43%) and smoking habit (30%) had higher incidence.

DISCUSSION

Enzymatic antioxidant system is considered the first line of defense against the excessive production of RONS. These species come from endogenous and exogenous sources, whose toxicity can affect important biomolecules, which leads to a sustained state of OS.[5]

Several of the mechanisms that generate ROS stimulate carcinogenic events, inducing genetic mutations and activating signaling pathways that promote cell proliferation and survival. These events promote activation of oncogenes, loss of p53 functionality, mitochondrial dysfunction and the appearance of growth factors and cytokines, especially in sites of chronic irritation, inflammation and infection. This is so, that HR-HPV infection in cervical cancer is able to modulate signaling pathways associated with cell cycle control, apoptosis and DNA damage response, as well as influencing the expression of the E6 and E7 oncoproteins especially during viral integration to the host genome.[6]

Alteration of the antioxidant enzyme system found in the study, given by the decrease in SOD and CAT enzymes, and GSH levels, agrees with several authors.[7] This could be due to the

variability of the individual response due to differences in antioxidant status, exposure period and genetic factors. The affectation of the defense system in the neutralization or sweep of ROS could lead to an aberrant regulation of redox homeostasis and consequently adaptation of cancer cells to stress.

GSH is an intracellular thiol whose essential function is to protect cellular constituents from oxidative damage, especially during lipid peroxidation. Function that would be affected due to the decrease in its levels that lead to the overproduction of free radicals, which could explain the increase in lipid peroxidation and consequently the neoplastic transformation.[8]

The breakdown of lipids due to the formation of reactive compounds during the peroxidation process can alter the cellular integrity due to changes in the permeability and fluids of the lipid membrane.[8] These results agree with several studies carried out in cervical cancer, although it has also been described in diseases related to aging, atherosclerosis and others cancers.

Although the E6 and E7 oncoproteins of HR-HPV are able to immortalize the cells for the neoplastic transformation to occur, other conditions are required. There are genetic and environmental co-factors that are closely related to the persistence of the virus in the tissue and that increase the risk of developing cancer.

The early onset of sexual intercourse and promiscuity are co-factors of marked incidence in studies of cervical lesions. This occurs because the cervical epithelium, specifically the transformation zone, presents physiological immaturity during adolescence, which increases the vulnerability of the tissue to any type of sexually transmitted infection, particularly HPV.[9]

Contraceptive methods can also influence the development of cervical lesions, so that the consumption of contraceptive pills presents an increased relative risk as the time of consumption increases. Has been mentioned that there are mechanisms by which estrogens and progestogens can favor the cellular effects of HPV infection. On the other hand, IUDs could produce traumas in the transformation zone during their placement, which would induce a cellular immune response that could eventually allow the persistence of HPV and pre-invasive development.[9,10]

Exposure to pollutants derived from chemicals, tobacco smoke and / or environmental pollution has been associated with the development of cervical lesions. It has been described that the accumulation of carcinogens, such as nicotine and its degradation product cotinine in cells of immune surveillance of the cervical mucus producing glands, interfere in the normal functioning of this cell, promoting the entry of HPV. Similarly, exposure to any polluting product can negatively influence the immune system, thus affecting the endocervical and exocervical epithelium that is constantly changing. It could also cause genetic mutations at the cellular level.[10]

FINAL CONSIDERATIONS

Oxidative imbalance was evidenced in the groups of premalignant and malignant lesions of the uterine cervix, given by the decrease of the antioxidant enzymatic system and increase of lipid peroxidation. Likewise, the presence of genetic and environmental co-factors related to HPV infection was corroborated, which favors the persistence of the virus and the progression of the lesions.

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