A study of oxidative stress in Post Menopausal cervical cancer patients in Udaipur, Rajasthan, India - an institutional study

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ABSTRACT

Background: Cervical cancer is a preventable disease because it has a premalignant stage which may last up to 10-20 years before its progression to invasive carcinoma. The potential role of ROS in DNA damage as well as the nutritional etiology of cervical neoplasia (which include low dietary intake of vitamin C, carotenoids, vitamin E, and folate) has been suggested.
Objectives: The objective of this study is to measure the oxidant status**.** To estimate and compare serum levels of MDA (malondialdehyde) in postmenopausal cervical cancer patients and healthy age matched control.
Methodology: This case control study consist of 90 patients of cervical cancer along with 60 healthy subjects of précised matched age within the institution. blood samples was collected from all participants and were analysed for oxidant MDA (malondialdehyde) .Results obtained were analyzed statistically to see the significance of differences.
Results: The Mean concentration of S.MDA *(*nmol/L) in case group was 5.77 ±2.40 while that of control group 2.03±0.70 and the difference among them found to be highly significant. The mean age of cervical cancer group(case) is 51.26 ±5.43 yr while that of control group is 55.60±4.50 year. According to demographic data .Smoking history present in 16% and 11% in case and control group respectively.History of alcohol present in 9.9% and 4.2% in case and control group respectively.Among total 90 patients of cervical cancer 86(95.55%) females were married.
Conclusion: In conclusion, this study has shown that the patients with cervical cancer have high lipid peroxidation (MDA), which is a marker of oxidative stress; and this worsened as the disease progressed. There is a need for antioxidant supplementation in these patients to reduce oxidative stress.
Keywords: cervical cancer, MDA, postmenopausal, Oxidative Stress

INTRODUCTION

According to the National Institute of Cancer Prevention and Research (NICPR) India, Cervical Cancer is the second most common cancer-causing death in women in the country. In India, around 96,922 women are newly diagnosed, and 60,078 women die of cervical cancer every year as per

GLOBOCAN 2018 [1,2]. Considering the current incidence rates, the annual load of new cases is expected to increase to 225,000 by 2025 in India. [3]

Cellular metabolism and energy production require oxygen. This can lead to formation of potentially damaging compounds known as free radicals or reactive oxygen species, which creates a condition known as oxidative stress. This can cause cell damage and play fundamental role in various diseases, aging and neoplastic transformation. [4,5] Malondialdehyde (MDA), the principal end-product of polyunsaturated fatty acid peroxidation is a biomarker of free radical-mediated damage and oxidative stress. [6] Malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, the primary one being M1G. [7] This aldehyde is a highly toxic molecule and its interaction with DNA and proteins has often been referred to as potentially mutagenic.

Many population studies have suggested that diet rich in vitamin C, vitamin A, vitamin E, beta-carotene, selenium and folate may protect from cervical cancer and low level in red blood cells and tissues may be associated with cervical cancer. [8] In addition, accumulating research evidence suggests that many dietary antioxidant agents may be used alone or in combination with traditional chemotherapeutic agents to prevent the occurrence of cancer, their metastatic spread, or even to treat cancer, without causing significant levels of toxicity.

METHODS

This case control study has been conducted on 90 postmenopausal patients of cervicalcancer who are visiting Radiotherapy Department, Super Specialty Block, MB Hospital, Udaipur. The control group consisting of 60 healthy subjects of précised matched age within the institution.

Exclusion criteria- include late pregnancy, any other carcinoma of female reproductive system, DM, supplementations of vitamins.

Inclusive criteria- as mentioned above all consecutive patients with positive Pap smear are to be enrolled.

The same inclusive and exclusive criteria are to be applied for control subjects as well. All the above exclusion factors will be confirmed from thepatient reports and history.

Patients that were coming to Gynecology OPD and patients with positive Pap smear were enrolled. Selection of cases was based on detailed inclusion & exclusion criteria and clinical history.10 ml blood sample were collected through venipuncture under aseptic precautions in sterile plain and EDTA vial. Samples were incubated & centrifuged at 3000 rpm for 15 minutes. Precautions were taken to avoid hemolysis & other contamination & separated serum were analyzed for test MDA (malondialdehyde). Results obtained were analyzed statistically to see the significance of differences by calculating p value by using online student t test calculator.

RESULTS

This case control study consist of 90 patients of cervical cancer of age Group from 45-60 year along with 60 healthy subjects.

The mean age of cervical cancer group(case) is 51.26 ±5.43 yr while that of control group is 55.60±4.50 year.

Parameters	**Case (n=150)**	**Control(n=150)**	**P value**
Age	51.26 ±5.40	55.60±4.50	0.08

Table 1: Age wise distribution of the participants

P value <0.001 significant

Parameters	**Case(n=90)**	**Control(n=60)**
Hindu	90(100%)	57(95%)
Muslim	0 (0%)	3(1.8%)
Smoking	16(14.4%)	11(6.6%)
Alcohol	11(9.9%)	7(4.2%)
Married	86(95.55%)	58(95%)

Table 2: Demographic characteristic of the participants

(P value <0.001 significant)

According to demographic data , Religion wise 100% of cervical cancer patients were hindu and 96% 95%controls were hindu. Smoking history present in 16% and 11% in case and control group respectively.

History of alcohol present in 9.9% and 4.2% in case and control group respectively. (Table 2)

Among total 90 patients of cervical cancer 86(95.55%) females were married.

Parameters	**Case(n=90)**	**Control(n=60)**
Rural residence	82(91.6%)	28(46.66%)
urban residence	08(8.0%)	32(53.33%)
Family history present	24(26.66%)	4(6.6%)
Family history not present	66(73.33%)	56(93.33%)

Table 2A: **Demographic characteristic of the participants**

Parameters	**Case(n=90)**	**Control(n=60)**
illiterate	62(68.88%)	18(30%)
literate	19(21.11%)	18(30%)
semi literate	9(10%)	24(40%)

Table 3: Education wise distribution of case and control group

Parameters	**Case(n=150)**	**Control(n=150)**	**t-value**	**P value**
S.MDA ***(nmol/L)***	5.77 ± 2.40	2.03± 0.7	13.86	<0.001*

Table 4: Showing comparison of MDA Levels between case and control group

P value <0.001 significant

*significant

The Mean concentration of S.MDA *(*nmol/L) in case group was 5.77 ±2.40 while that of control group 2.03±0.70 and the difference among them found to be highly significant.(Table 4)

DISCUSSION

In this case-control study, the possible association between oxidative stress and progression of cervical cancer was investigated. All participants (60 controls and 90 cervical cancer cases) met the inclusion criteria for the study population.

Persistent infection with high-risk HPV types (HR-HPV) is the main etiological cause for the development of several epithelial tumors at different anatomic locations. The most strongly HPV-associated malignancy is cervical carcinoma, where almost all tumors are positive for HR-HPV DNA [9]. Cervical carcinoma is the second most common type of cancer in women worldwide, with approximately 530,000 new cases and 270,000 deaths per year, of which more than 85% occur in developing countries. HPV infection is implicated in a variable, although consistently high, proportion of vaginal, vulvar, anal, penile and head and neck carcinomas [10].

In addition to HPV infection, several cofactors contribute to cervical cancer development. These include low socioeconomic status, early sexual initiation, multiple sexual partners, smoking, Genital warts, multiparity, immunosuppression and use of oral contraceptives [11,12]. Cervical cancer is mostly a consequence of the continuous evolution of non-invasive precursor lesions called cervical intraepithelial neoplasia (CIN) that are characterized by different degrees of cellular atypia (dysplasia) [13]. CIN is divided into the following groups: CIN 1, characterized by mild dysplasia; CIN 2, which represents moderate dysplasia; and CIN 3 or carcinoma in situ, characterized by a severe dysplasia that may progress to an invasive squamous cell carcinoma (SCC). Moreover, changes in the glandular epithelium of the cervix, caused by HPV and other cofactors, are associated with the development of cervical adenocarcinoma . Furthermore, in persistent viral infection, HPV-induced carcinogenesis involves genetic and epigenetic changes that affect the expression of different cellular proto-oncogenes and tumor suppressor genes. Usually, this process requires an extensive period to accumulate sufficient alterations to trigger and sustain tumor development . Therefore, immune system evasion and HPV persistence are crucial factors for tumorigenesis [14]. This is highlighted by the fact that the great majority of HPV infections are self-limited and spontaneously resolved in few months with therapies and medications, and cancer development affects only a small proportion of infected individuals

The pathogenesis of cervical cancer is related to oxidative damage caused by persistent infection by one of the oncogenic types of human papillomavirus (HPV). [15] This damage comes from oxidative stress, which is the imbalance caused by the increase in reactive oxygen and nitrogen species and impaired antioxidant mechanisms, promoting tumor progression through metabolic processes. The incorporation of HPV into the cellular genome leads to the expression of oncoproteins, which are associated with chronic inflammation and increased production of reactive oxygen species, oxidizing proteins, lipids and DNA. The increase in these parameters is related, in general, to the reduction of circulating levels of enzymatic antioxidants—superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase; and non-enzymatic antioxidants—reduced glutathione, coenzyme Q10 and vitamins A, C and E, according to tumor staging. [16]

This study found marked elevation in serum malondialdehyde (MDA) among the cervical cancer patients compared with healthy controls. The level of serum MDA among the cases increased significantly at different stages of disease progression.

The mean MDA levels showed a significant progressive increase with advanced disease and a maximum rise in stage IV. This indicates excessive lipid oxidation and hence, oxidative stress with worsening disease. This was in support of the previous findings from studies by Naidu et al, [17] Nirmala et al [18] and Beevi et al[19]. The study by Naidu et al. [17] observed a maximum rise in MDA in stage IV when compared with healthy controls. Nirmala et al. [18] found increased levels of MDA in the circulation of cervical cancer patients attributed to increase in oxidative stress due to the deficiency of antioxidant mechanism. Also, Beevi et al. [19] found a link between oxidative stress and cervical cancer.

The present study has limitations that should be considered. Only one marker of oxidative stress was used: serum MDA through the TBARS method involving spectrophotometry. It is possible that more substantial results would have been obtained if other oxidant and even antioxidant markers had been used for a more accurate comparison of the REDOX equilibrium. Another limitation was not having better control over variables regarding the daily lives of the participants, as the analysis was limited to self-reported data on physical activity, eating habits, lifestyle habits, smoking, and alcohol intake. Finally, the sample size was small due to the detection of diagnosed cases during the study.

CONCLUSION

In conclusion, this study has shown that the patients with cervical cancer have high lipid peroxidation (MDA), which is a marker of oxidative stress; and this worsened as the disease stage progressed. There is a need for antioxidant supplementation in these patients to reduce oxidative stress.

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DECLARATIONS

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Ethical approval: The study was done after permission of institution Ethics committee.

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