Research Article

MDA Elevation and Haematological Derangements at First Clinical Presentation in Cervical Cancer Patients

A. Nath[1], Rahul Kumar[2*], Vinita Trivedi[1], Krishna Murti[2], Shailendra Kumar[1], Chandan Kumar Singh[1], J.K. Singh[1], P. Das[3].

[1]Mahavir Cancer Sansthan & Research Centre, Patna, India.

[2]Department of Pharmacy Practice, National Institute of Pharmaceutical Education & Research, Hajipur, India.

[3]Department of Molecular Biology, Rajendra Memorial Research Institute of Medical Sciences, Patna, India.

***Corresponding author's E-mail:** rahulkumar5199@gmail.com

Accepted on: 12-12-2014; **Finalized on:** 31-01-2015.

ABSTRACT

Cervical cancer is the second most common gynecological malignancy worldwide. According to HPV Information Centre, Spain (Aug' 2014), in India approximately 1,22,844 women are diagnosed with the disease every year and of them 67,477 die due to the disease. The etiology is multifactorial. Malondialdehyde (MDA) is important peroxide which is a measurement of oxidative stress and can be a predictive in the early detection of cervix cancer or may help in developing a new therapeutic strategy. Cervical cancer has direct impact on the haematological parameters. This study has been undertaken to investigate the malondialdehyde (MDA) level and haematological changes at the first clinical presentation. Thiobarbituric Acid Reactive Substances (TBARS) assay was performed on the blood serum samples taken from the freshly diagnosed cervical cancer patients. For haematological changes, haematocytometer and Sahli's method was followed. Mean MDA level in the serum of freshly diagnosed cervix cancer patients was found to be elevated than the healthy volunteers and was found to be 37.90 ± 4.23 nmol/ml. Mean RBC count was found to be 3.30 ± 0.49 $x10^6/mm^3$ which was comparatively less than the normal and mean WBC was found to be 10.65 ± $1.85x10^3/mm^3$ which was comparatively higher than the normal. The mean hemoglobin content was found to be 10.425 g/dl. MDA may have a predictive role in treatment response. MDA levels are higher in patients of cervical cancer and suffer from haematological derangements. Hence, measures to control oxidative stress and to increase blood profile should be taken. Increased LPO may be taken as associated predictive markers, thus suggesting that CaCx cases should get nutritive supplements to control the blood LPO level and maintain a positive balance of antioxidants for a better outcome in terms of delayed recurrence and better Quality of Life (QoL).

Keywords: Cervical Cancer, MDA, Haematological changes, MDA, Oxidative stress.

INTRODUCTION

Cervical Cancer is one of the most common gynecological malignancy worldwide. Approximately, 5,00,000 new cases are diagnosed every year with a higher rate of incidence among women of lower socioeconomic status especially in developing countries[1]. India has a population of 432.20 million women aged 15 years and older who are at the risk of developing Cervical Cancer. 1,22,844 women are diagnosed every year with cervical cancer and out of them 67,477 die from the disease. It is a multifactorial disease and several risk factors include Human Papilloma Virus (HPV) infection, early age of intercourse, multiple sex partners, smoking, oral contraceptive use and low socioeconomic status[2]. Chronic inflammation and infection over a long period of time have been recognized as a major risk factor for the initiation of Cervical Cancer[3].

Carcinoma of Cervix (CaCx) tends to occur during midlife in women with most of the patients diagnosed between 25 to 65 years of age. CaCx rarely affects women under the age of 20. CaCx is said to be mediated by HPV but recent data published reveal the role of Oxidative Stress in CaCx. The imbalance between the pro-oxidants and antioxidants towards pro-oxidants is called Oxidative Stress[4]. Evidences have indicated that Reactive Oxygen Species (ROS) are involved in the initiation and progression of carcinogenesis[5]. ROS might be damaging the tumor suppressor genes or immunological defenses in our body. ROS can initiate the lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death if the antioxidant potential is insufficient[6]. Oxidative damage to proteins lead to formation of malondialdehyde (MDA) which may lead to carcinogenesis[7-10].

Increase in lipid peroxidation has been reported in patients with laryngeal and oral cancer[11,12]. Elevated lipid peroxidation and disturbed antioxidant activities have been reported in patients with malignant lymphoma[13]. Even in gastric cancer patients elevated erythrocyte lipid peroxidation with a concomitant decline in antioxidants has been published[14]. Increased MDA with haematological changes has also been reported in breast cancer[15]. These studies hint towards establishing a correlation between MDA and cervical cancer.

Changes in haematological parameters such as RBC, WBC, Haemoglobin has been of relevant consideration in context of cancer patients. Haematological parameters with progression of disease has a significant pattern with slight deviation[15] Changes in blood parameters might help us in establishing a better pharmacological management of the disease.

Increased MDA is responsible for Oxidative Stress. If the level of MDA goes on increasing, a critical level will come

above which the cells will have a permanent oxidative stress.

This could cause genomic instability and adaptation of cells to oxidative stress which in return can provoke malignancy.

The evaluation of Oxidative Stress and haematological derangements could significantly contribute in the planning of appropriate treatment and in increasing the patients Quality of Life[16].

MATERIALS AND METHODS

Subjects

Patients were enrolled from Department of Radiotherapy, Mahavir Cancer Sansthan & Research Centre, (MCSRC), Patna, India, after the Ethical Clearance from the Human Ethical Committee, MCSRC, Patna.

Patients were explained about the study and the consent was taken prior to the start of the study.

Patients were selected as per inclusion and exclusion criteria set before the initiation of work and strictly adhered.

Inclusion Criteria

Patients who were ready to sign the consent form and had confirmed histopathological investigation of Carcinoma of Cervix were included.

Exclusion Criteria

Patients who were not ready to sign the consent form and those of Stage IV were excluded initially. Patients with previous history of hysterectomy, existence of any comorbidity, with a history of prior treatment for cancer, any previous associations with a chronic debilitating disease like HIV, TB, or patients on other medications like insulin were excluded from the study.

2 ml of Venous blood was taken in EDTA vials from subjects on the very first day before the initiation of treatment.

Estimation of Lipid Peroxidation (LPO) Levels

Thiobarbituric Acid Reactive Substances (TBARS) Method

Serum of 31 Cervix Cancer patients and 10 healthy volunteers were assayed for lipid peroxidation by determining their malondialdehyde (MDA) levels. MDA level in each cervix cancer patients were estimated by standard procedure with slight modifications of Ohkawa.[17]

Blood samples were centrifuged at 3000 rpm for 10 min to obtain serum. 2.5 ml of 10% of TCA was added to 0.5ml of test serum, incubated at 95 °C for 15 min and the solution was centrifuged at 3000 rpm for 10 min. The supernatant was collected and 0.675% of TBA was added to the same and incubated again at 95 °C for 15 min. The color reaction was obtained to measure optical density using spectrophotometer at 532 nm and the amount of TBARs was calculated with the help of standard procedure.

Haematological Parameters

RBC and WBC Count

100 µl blood from cervical cancer patients were taken and dilution of 1:200 was made by using EDTA and saline. Single drop of blood solution prepared was put on haematocytometer chambers and counted under the microscope.

Haemoglobin Count

It was carried out using Sahli's method. 20 µl of blood was mixed with N/10 of HCl in the haematocytometric tube and few drops of distilled water was put in the solution upto the level marked (color matching).

RESULTS

Table 1: Characteristics of the CaCx patients at the time of first Clinical Presentation and Control

Characteristics	No. (%) Patients	Control
Median age, yr (range) Comorbidities	52 (29-65)	44 (32-60)
Diabetes Mellitus	3 (9.68)	0
CA 125	1 (3.22)	0
Histopathological features		
Squamous carcinoma	30 (96.77)	
Adenocarcinoma	1 (3.22)	
Grade		
I	7 (22.58)	
II	13 (41.94)	
III	11 (35.48)	

Patients and Control

The clinical and pathological characteristics of patients are summarized in Table 1. The age and comorbidity distribution of the patients and controls were similar. Patients were of median age 52 years and lies between the range of 29 to 65 years.

3 patients were suffering from diabetes mellitus and 1 had a positive CA 125. 30 patients had Squamous Cell Carcinoma and one of them had Adenocarcinoma. The patients were further categorized under different grades. 7 patients of Grade I, 13 patients of Grade II and 11 of Grade III were observed.

MDA Levels and different Grades

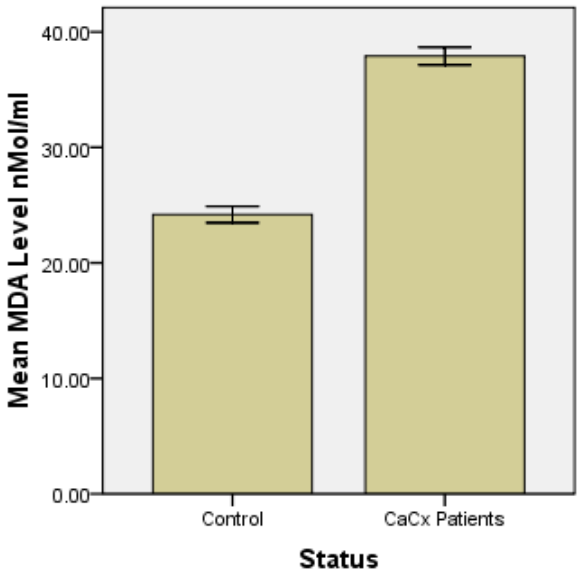

Figure 1. Bars (Mean ± S.E) comparing MDA levels in Healthy Volunteers (Control) and Cervical Cancer Patients.

Figure 1: Shows increased level of MDA in patients during their first clinical presentation than compared to that of healthy volunteers. Mean MDA in Cervical Cancer patient was found to be 37.90 ± 4.23 nMol/ml.

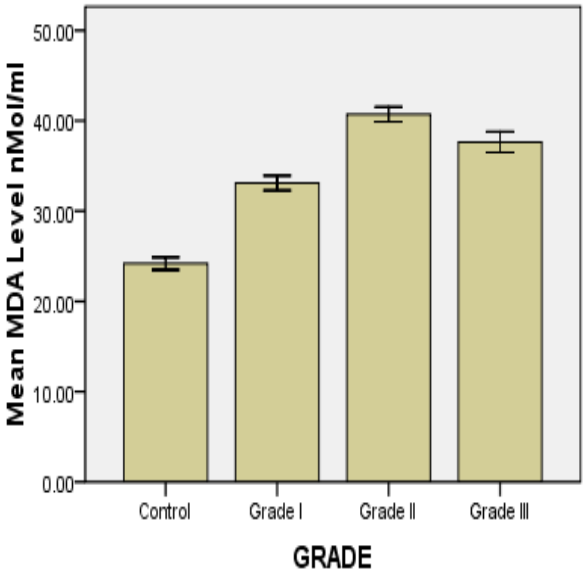

Figure 2 . Bars (Mean ± S.E) representing MDA levels in different GRADES.

Figure 2: Shows levels of MDA in different grades of Cervical Cancer. MDA level in grade I was found to be 33.12 ± 2.12 nMol/ml, in Grade II was 40.72 ± 2.91 nMol/l and in Grade III patients was found to be 37.47 ± 3.55 nMol/ml.

Haematological Derangements

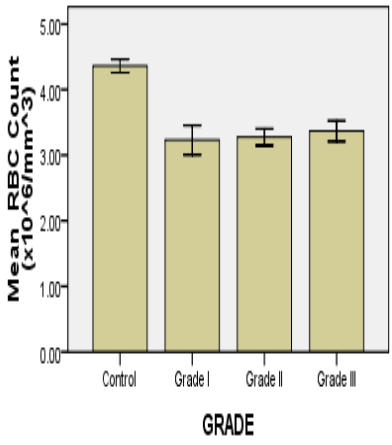

Figure 3. Bars (Mean ± S.E) representing RBC Count levels in different Grades.

Figure 3: Represents Mean RBC count was comparatively lower in the CaCx patients than compared to the normal. In healthy volunteers mean RBC Count was found to be 4.36 ± 0.32 × 10^6/mm^3 and in CaCx patients the count was 3.30 ± 0.49 × 10^6/mm^3 which was significantly lower. Decrease in the count showed a definite ascending pattern in different grades.

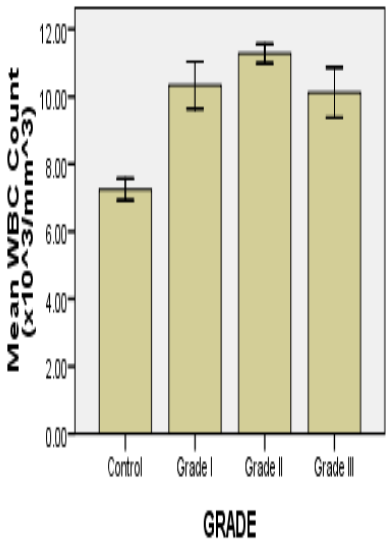

Figure 4 . Bars (Mean ± S.E) representing W.B.C. Count in different Grades.

Figure 4: Represents mean WBC to be 7.25 ± 1.0 × 10^6/mm^3 while in CaCx patients the WBC count was 10.65 ± 1.8 × 10^6/mm^3. WBC count is higher in patients but without any definite pattern.

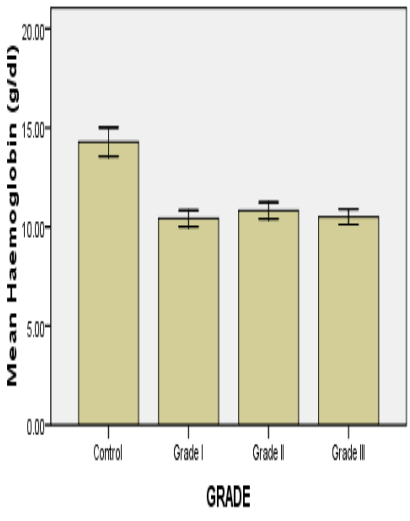

Figure 5. Bars (Mean ± S.E) depicting Haemoglobin concentration in different Grades.

Figure 5: Represents Mean haemoglobin level in CaCx was lower than that found in healthy volunteers. The normal mean level of Hb in healthy volunteers was 14.28 ± 2.29 g/dl and in the patients was found to be 10.61 ± 1.32 g/dl.

DISCUSSION

There are sufficient experimental evidences to justify the contribution of Free Radicals during process of Carcinogenesis which is caused by damaging DNA synthesis and repair mechanism[18,19]. The study on free radicals has been extensively studied in Oral, Layngeal, Malignant Lymphomas, Gastric and Breast Cancer[11-15]. Studies have also been performed in Cervical Cancer to study the same. The present study goes in conformity with the earlier studies. Elevated LPO in Cervical Cancer has been reported. But, whether elevated MDA is involved in pathogenesis of the disease or is the effect of the disease is still questionable. Several studies suggest that during cancer treatment, there is gradual increase in Oxidative stress[16]. Chemotherapy and Radiotherapy which is used for the treatment of carcinoma of cervix are responsible for increasing the MDA level. If it could be established as an intermediate of pathogenesis then we can use this concept as a new therapeutic approach. As increased ROS is detrimental to cancer cells and thus it contributes as an anti neoplastic agent. Oxidative Stress may also affects the normal cells. So, we should work to promote it as a Pharmacological insult. Oxidative Stress in future may be explored as a potent therapeutic breakthrough for carcinogenesis. Establishing the etiology of cancer has major scientific, clinical as well as public health concern.

There is gradual increase in MDA level in Grade I and Grade II but a sudden decline in the Grade III is remarkable change during cancer progression. The gradual increase in LPO is self explanatory but a sudden decline requires further study to establish the cause[18,19].

The study revealed that most of the patients has decreased level of haemoglobin. Most of the patients having Cervical Cancer comes from low socioeconomic status and poor hygiene. This may be correlated because majority of patients of carcinoma of cervix comes from low socioeconomic background are malnourished with poor hygiene level. It has been reported that malnutrition and folate deficiency leads to suppression of immunity[20]. Folate deficiency is responsible for decreasing immunity which might have given opportunity to HPV infection which is considered to be most important factor for causation of carcinoma of cervix. Infection will lead to increase in strengthening the defense mechanism which results in increased WBC[21].

In a nutshell, Cervical cancer is a multifactorial disease. MDA may be a new etiology in the pathogenesis of the disease. This may be exploited as a new therapeutic approach. As per current scenario, MDA level can be controlled by increasing the antioxidant level and folate supplementation. Future study is thus required at the pharmacological level to explore it as a new potential therapeutic approach[22]. Further study at a larger scale on different disease conditions are required to establish the fact.

To conclude, haematological derangements are significant at the first clinical presentation with elevated level of LPO. Elevated LPO can be used as a Predictive marker for disease progression.

Acknowledgement: The study was supported by Department of Pharmaceuticals, Ministry of Chemical & Fertilisers, Government of India. We are thankful to the clinicians, Nurses and Medical Physicists of Department of Radiotherapy, Mahavir Cancer Sansthan & Research Centre, Patna. Authors are also thankful to Department of Science & Technology, Government of India, for its support and cooperation.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P, Global Cancer Statistics, 2002, J CA Cancer Jclin (55), 2005, 74-108.
2. Bruni L, Barrionuevo – Rosas L, Serrano B, Brostons M, Albergo G, Osano R, Munoz J, Bosch FX, de Sanjose S, Castellsague X, Human Papilloma virus and Related Diseases in India. Information Centre on HPV and Cancer (HPV information cancer), Summary Report 2014.
3. Steven F Moss and Martin J Blaser, Mechanisms of disease inflammation and the origins of Cancer, Nature Clinical Practice Oncology, 2(2), Feb 2005, 90-97.
4. Beevis S.S, H. Rasheed, A Geeha, Evidence of Oxidative and Nitrosative Stress in patients with cervical Squamous cell Carcinoma, Clin Chem Acta, 375(1-2), 2007, 19-123.
5. Cork JA, Guis D, Wink DA, Krishna MG, Russo A, Mitchell JB, Oxidative Stress, redox and the tumor microenvironment, Semin Radiat Oncolo, 4, 2004, 59-266.
6. Manju V, ailaivani Sailaja J, alini N, Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case control study, Clin Biochem, 5, 2002, 621-625.
7. Dalle – Donne I, Ross R, Giustarini D, Milzani A, Colombo R: Protein Carbonyl groups as biomarkers of oxidative stress, Clin Chim Acta, 29, 2008, 23-38.
8. Manoharan S, Kolanjipanna K, Kayalvizhi M, Enhanced lipid peroxidation and impaired enzymatic antioxidant activities in the erythrocytes of patients with cervical carcinoma. Cell Mol Biol Lett, (9), 2004, 699-707.
9. Nair V, Bartsch H, Nair J, Lipid peroxidation-induced DNA damage in cancer prone inflammatory diseases – a review of published adduct types and levels in humans, Free Radic Bio Med, 43), 2007, 1109-1120.
10. Panayiotidis M, Reactive Oxygen Species (ROS) in multistage Carcinogenesis, Cancer Lett, 66, 2008, 5.
11. Samir M. and N.M. Kholy, Thiobarbituric acid reactive substances in patients with laryngeal cancer, Clin Otolaryngol, (24), 999, 232-234.
12. Manoharan S. and S. Nagini, Lipid peroxidation and antioxidant status in oral cancer patients, ed Sc. Research, (22), 1994, 91-292.
13. Abou – Seif, A., A. Rabia, Nasir, Antioxidants status, Erythrocyte membrane lipid peroxidation and osmotic fragility in malignant lymphoma patients, Clin Chem Lab Med, (38), 2000, 732-742.

14. Arivazhagan S, K. Kavitha, Nagini, Erythrocyte lipid peroxidation and antioxidants in gastric cancer patients, Cell Biochem Funct, (15), 997, 5-18.

15. A. Nath, Shweta Suman, Seem Kumar, Priyanka, Meena Sinha. TBARS Assay and haematological parameters in Relation to Breast Cancer, J. Ecophysiol. Occuo. Hith, 14(3), 2014, 10-116.

16. Domika Ivanova, Rumiana Bakalova, Dessisslava Lazarova, Veselina Gadjeva, Zhivko Zhelev, The Impact of Reactive Oxygen Species on Anticancer Therapeutic Strategies, Adv Clin Exp. Med, (22), 2013, 899-908.

17. Ohkawa H., Shishi N. and Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95, 979, 51-358.

18. Dix A. and Aikens J, Mechanisms and biological relevance lipid peroxidation initiations, Chem Res Toxicol, (6), 993, 2-18.

19. Marnett .J. Lipid peroxidation–DNA damage by Malondialdehyde, Mut Res Fund Mol Mech Mutagen, 24, 1999, 83-95.

20. Dunst, Kuhnt and Strauss G., Anemia in Cervical Cancers: Impact on survival, Pattern of relapse, and association with hypoxia and angiogenesis, Int. J. Radiat Oncol Biol Phys, (56), 003, 778-787.

21. Senem Demirci, Zeynep Ozsaran, and an AK Celik, The interaction between antioxidant status and cervical cancer: A case control study, Tumori (97), 011, 90-295.

22. Harry W Haverkos, Viruses, Chemicals and Co-carcinogenesis, Oncogene (23), 004, 492-6499.

Source of Support: Nil, **Conflict of Interest:** None.