

RESEARCH ARTICLE

Arsenic Exposure and Haematological Derangement in Cervical Cancer Cases in India

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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. CaCx is said to be mediated by HPV but recent data published reveal the role of Oxidative Stress in different Cancers. Arsenic is also one of the agents for causing Oxidative Stress. Arsenic has been linked with different types of cancer. Arsenic is considered responsible for generation of free radicals and eventually for apoptosis. Early diagnosis of CaCx is presently a matter of concern and clinical presentation in advanced stages become difficult for complete clinical response. For determination of oxidative stress, Malondialdehyde (MDA) was taken as an identifier and arsenic estimation was performed with the help of Atomic Absorption Spectrometer (AAS). RBC count and Haemoglobin levels were performed according to standard protocol. MDA was in direct proportion with arsenic concentration and inversely proportional to RBC and Haemoglobin in CaCx patients. Arsenic is one of the major causative agents for oxidative stress and hence may be a risk factor leading to cancer including CaCx.

Keywords: Arsenic - LPO - RBC - WBC - cervical cancer

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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 (Bray et al., 2005). 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 (Barrionuevo - Rosas et al., 2014). Chronic inflammation and infection over a long period of time have been recognized as a major risk factor for the initiation of Cervical Cancer (Moss et al., 2005).

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 (Beevis et al., 2007). Evidences has indicated that Reactive Oxygen Species (ROS) are involved in the initiation and progression of carcinogenesis (Cork et al., 2005). ROS might be affecting the tumor suppressor genes or immunological defence mechanism in our body. ROS can also initiate the lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death if the antioxidant potential is insufficient (Sharma et al., 2007). Oxidative damage to proteins lead to formation of malondialdehyde (MDA) which may lead to carcinogenesis (Kolanjipanna et al., 2004; Bartsch et al., 2007; Dalle - Donne et al., 2008; Panayiotidis et al., 2008).

During first clinical presentation, MDA level is elevated than the normal subjects in Cervical Cancer patients (Kumar et al., 2015). Knowledge about the disease is very less in common people; awareness about the disease and risk factors at the ground level is an important concern. Arsenic has been reported several times to be an important factor, might be responsible for causing cancers (Pawin et al., 2015). Different risk factors has been identified as a potential risk factor like multiple sex partner, unhygienicity and there is need for identification of more risk factors. (Wei-Dong et al., 2015).

Lipid Peroxidation is a normal cellular metabolism. LPO is one of the most extensively studied consequences of Reactive Oxygen Species (ROS), perturbing the

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integrity of the plasma membrane and the function of the cell. Polyunsaturated Fatty Acids (PUFAs) are extremely susceptible to Peroxidation. LPO mediated oxidative stress can disrupt the major cellular components and function like DNA strand breakage, rise in intracellular free calcium ions, damage to membrane ion transporters and specific proteins. MDA, an aldehyde product of peroxidation has been established to possess mutagenic and carcinogenic effect. It has been reported for its association with bases like dG, dC and dA (Belda et al., 2004)

Arsenic occupies 33rd location on the periodicity of elements as heavy metal as in context of toxicology (Mandal et al., 2002). A number of reports provide evidences about toxic and carcinogenic metals like arsenic having capabilities to bind with different nuclear proteins and DNA causing oxidative deterioration of biological macromolecules. Arsenic exposure has been established to be one of the factors for generation of ROS (Bergmann et al., 2002). Accumulation of arsenic over a long period of time in the body has annihilating impact on overall human health and has also been implicated in cancer.

In our current investigation, we have tried to establish correlation between Arsenic and MDA, Haemoglobin levels and RBC count.

Materials and Methods

The blood samples were collected intravenously from the 51 cervical cancer patients who came for the treatment in Mahavir Cancer Institute and Research Center, Patna and 48 healthy women of comparable age group, with their consent. Part of blood was used for RBC count, estimation of Haemoglobin level and arsenic concentration and serum was prepared from rest of blood for MDA estimation.

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.

1 Haematological study RBC 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).

MDA Assessment

Serum of CaCx patients was prepared by centrifuging blood sample at 3000g for 10 minutes. LPO assay was performed by a standard protocol with slight modification (Okhawa et al, 1995).

Estimation of Arsenic

1ml of blood was transferred to EDTA vials of randomly selected 21 out of 51 cervical cancer patients for estimation of arsenic concentration. Further, sample preparation and estimation of arsenic was done by standard protocol of Atomic Absorption Spectrometer graphite flame (Perkin Elmer) model number 900T.

Statistical analysis

Statistical analysis was done using Stastical Package for Social Sciences (SPSS) (version 16.0).

Results

As per calculations, mean± S.D of MDA level (nMol/ml) in cervical cancer patients and normal persons was found to be 48.87±11.97 and 24.39±13.39, respectively with p-value <0.0001. It demonstrates the comparative hike in MDA level in cervical cancer patients than normal persons.. On the other hand, mean±S.D of haemoglobin level (g/dl) in cervical cancer patients (9.17±3.75) was significantly lower than healthy women (13.10±1.98) with p-value <0.001 (Table. 1). Whereas red blood cells count in cervical cancer patients and healthy women yielded noticeable difference as seen in text. Mean±S.D of RBC count in cervical cancer and normal women was calculated to be 3.6±0.82 and 6.1±1.7, respectively which has significance value <0.001. The Cervical cancer patient who had higher level of arsenic was being confirmed by peak Atomic Absorption Spectroscopy (AAS) as shown above in Table 1.

Table 1. Mean±S.D value of MDA level, Haemoglobin level, RBC count, patients

Parameters	Healthy Women Mean±S.D	Cervical Cancer Cases Mean±S.D	p-value
MDA Level (nMol/ml)	24.39±13.39	48.87±11.97	<0.0001
Haemoglobin Level (g/dl)	13.10±1.98	9.17±3.75	<0.001

Accumulation of arsenic in the body of cervical cancer ranged from 0 to 1000 ppb from the data obtained from AAS. The mean±SD was calculated to be 100.73±65.97 for arsenic concentration in cervical cancer patients. 14 patients out of 21 had extremely higher value and 7 showed negligible amount of arsenic.

MDA level was found to be increased (51.76±7.32) in high concentration as compared to patients with low or no arsenic concentration (38.98±9.62) with p-value < 0.001. On the other hand, mean±SD of RBC count (2.4±0.58), Hb (6.9±3.34), in patients with high arsenic level and mean±SD of RBC count (3.9±0.76 thousand/dl), Hb (7.54±3.06 g/dl) in those without arsenic in blood.

Discussion

Arsenic is an established causative agent for oxidative stress. Inorganic arsenic includes arsenite [As (III)] and arsenate [As (V)] and can be in methylated form, either as monomethylarsenic acid [MMA(V)] or dimethylarsinic acid [DMA(V)]. *In vitro* and *in vivo* studies of arsenic exposure have suggested involvement of higher production of peroxy radicals (ROO), superoxide anion radicals (O₂⁻), hydroxyl radical (OH[•]), hydrogen peroxide (H₂O₂), dimethylarsenic radical [(CH₃)₂As[•]] and non-protein sulfhydryl and oxidant induced DNA damage (Bhadoria et al., 2007). Oxidative stress has been found to be elevated in almost all metabolic disorders and related as one of the causative agent for cancer etiology as well its progression. Increased concentration of self-generated ROS in the cells undermines the endurance of the plasma membrane resulting into apoptosis. Such an endogenous stimulus is intensified not only in several diseases and cancer but also during the toxicity caused by accumulation of pesticides and arsenic. MDA level almost doubles in cervical cancer patients when compared with healthy persons as apparent in Table 1. MDA is generated as the by-product of the lipid peroxidation process, acts as mutagen and possesses the capacity of activating proto-oncogenes or tumour suppressor genes leading to cancer.

Anaemia in cancer is quite usual which can be treated with re-oxygenation or erythropoietin doses which are indicated to provide improved survival rate in cancer. But still it is not under practice in cervical cancer patients and is under clinical trials. Kidney begins manufacturing more erythropoietin on signal received at the time of hypoxic condition. Low level of haemoglobin and RBC count in blood signifies that erythropoietin is hypoactive which means oxygen supply throughout the body is sufficient (Bookemeyer, 2002). Higher the oxygen-supply to the organ, greater the production of superoxides in the cell, mainly due to incomplete electron transfer or reduction of oxygen by damaged mitochondria. Oxidative stress is observed to be higher whereas RBC count and Hb level to be lower in CaCx patients, indicating increased oxidative stress results in lower RBC count. (Kumar et al., 2007). Furthermore, patients with higher arsenic level show higher MDA level and lower RBC count as compared to patients with no arsenic level, which signifies arsenic and oxidative stress together have impact of RBC count and haemoglobin level. It is possible that arsenic and oxidative

stress induce RBC death. In addition, arsenic has binding affinity with haemoglobin at its cyst-rich area which might affect Hb level.

However oxidative stress is directly related to necrosis and apoptosis which manifests several malfunctions in the body and may be attributed to high level of arsenic but more study is needed to establish a vital relationship for considering it as a cause for cervical cancer.

Arsenic induced oxidative stress in Carcinoma of cervix patients has strong association with RBC count and Haemoglobin level. It was found that high level of arsenic increases oxidative stress in the patients which in turn increases MDA levels in Cervix Cancer patients. However, this needs further confirmation with large number of sample size with high statistical power to establish arsenic as one of the factors for cervical cancer.

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