

Early Diagnosis of Cervical Carcinoma through Serum Biochemistry

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Abstract:

The potential causes of deranged serum biochemistry in cervical cancer are discussed with emphasis on changes in antioxidants level. In the present study, the levels of Reduced Glutathione (GSH), Glutathione peroxidase (GPx), Lipid peroxidation (LPO), Superoxide dismutase (SOD), Catalase, Glutathione-S-transferase (GST), Vitamin E (Vit.E) were evaluated. The increased lipid peroxidation with concomitant decrease in antioxidants was notable in cervical cancer patients. Results showed significant decrease in levels of GSH, GST, GPx, catalase and Vit. E in patients as compared to normal control subjects. The selected serum parameters are very sensitive and useful biomarkers for the study of cervical cancer and suggest cost effective tests in detecting cervical carcinoma

which can be assayed in any general purpose laboratories.

Key words: cervical carcinoma, serum parameters, antioxidants, biomarkers

Introduction

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. Cancers are caused by abnormalities in the genetic material of the normal cells. This abnormality may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals or infectious agents. The heritability of cancers is usually affected by complex interactions between carcinogens and the host genome.

Definitive diagnosis requires the histological examination of a biopsy specimen, although the initial indication of malignancy can be symptomatic or radiographic imaging abnormalities. Most cancers can be treated and some forced into re-emission, depending on the specific type, location and stage. Once diagnosed, cancer is usually treated with a combination of surgery, chemotherapy and radiotherapy.

Cervical cancer, the second most common gynaecological malignancy worldwide, has been reported to occur in abundance in different populations. According to World Health Organization, in India approximately 1, 34, 420 women are diagnosed with the disease every year, and of them 72, 825 die (WHO 2010).

The etiology of cervix cancer is multifactorial. Hormonal, genetic and environmental factors appear to interplay in the pathogenesis of cervical cancer. The risk factors associated with cervix cancer, may exert their effects via generation of reactive oxygen species (ROSs) such as super oxide (O_2^-), hydroxyl radical ($OH\cdot$), peroxy radical, hydrogen peroxide (H_2O_2) and

hydro peroxide (LOOH), which induce oxidative damage of DNA, lipid per oxidation (LPO) and neoplastic transformation. The lipid peroxide formation is normally prevented by a host of antioxidants. Experimental evidences revealed that reactive oxygen metabolites (ROMs) are involved in initiation, promotion and progression of carcinogenesis, where inactivation or loss of certain tumour suppressor genes occurs. The extent of ROS induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defence mechanism. Therefore, it is important to pay more attention to antioxidants defense (Agner *et al.* 1998).

Study group

After obtaining institutional ethical clearance, blood samples were collected from women visiting the out -patient department (OPD) of Cancer Hospital & Research Institute, Gwalior for family planning, medical termination of pregnancy or for routine gynaecological examination. All the women were physically examined by the resident doctors of cancer Hospital and samples were collected and stored for testing.

The women selected were asymptomatic with no previous history of HPV infection or any cervical neoplasia and were clinically normal. Unmarried women, women in their second or third trimester of pregnancy or with past history of hysterectomy were not included in the study. Since HPV infection is a sexually transmitted disease, there were less chances of infection in unmarried women and in the Indian scenario, it is not possible to get the correct information regarding premarital sexuality. Consent was taken from each of the participants included in the study. A questionnaire form consisting of questions related to different socio-demographic factors including age of women, marital status, parity, age of conception, diet, socio-economic status, place of residence, history of any disease or HPV infection, pre and post –

menopause status and pregnancy were filled and recorded for each participant.

The standard protocols were used for studying following parameters:

1. Blood GSH was performed with method described by Ellman (1959).
2. Lipid peroxidation in haemolyasate was measured by method of Ohkawa *et al.* (1979).
3. Haemolysate SOD (E.C. 1.15.1.1) activity in hemolysate was assayed by the method of Winterbourn *et al.* (1975).
4. Catalase (E.C. 1.11.1.6) activity in hemolyasate was assayed following the procedure of Sinha (1972).
5. Glutathione peroxidase (E.C. 1.11.1.9) activity was measured by the procedure of Rotruck *et al.* (1973).
6. Glutathione transferase (E.C. 2.5.1.18) activity in hemolysate was measured by method of Beutlar (1984).
7. Vitamin E activity was measured by the method of Desai (1984).

Result and Discussion

Cancer is characterized by excessive proliferation of cells. Cervical cancer is one of the major public health problems among women worldwide and is the most common malignant neoplasms among females in many developing countries.

The present study was planned to understand the biochemical changes in blood of CaCx patients. The study was undertaken to evaluate the correlation between the cervix cancer and oxidative stress as well as status of anti-oxidant enzymes in cervix cancer.

Cancer of the cervix tends to occur during midlife in women, with half 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 Human Papilloma Virus (HPV) but recent data published also revealed role of oxidative stress

in CaCx (Beevis *et al.* 2007). The imbalance between the pro-oxidants and antioxidants in favour of pro-oxidants is called oxidative stress. Antioxidant species may act in vivo to decrease oxidative damage to DNA, protein and lipids thus reducing the risk of cancer (Sierans *et al.* 2001). Human body is equipped with various antioxidants like superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), glutathione peroxidase (EC 1.11.1.19) and glutathione transferase which can counteract the deleterious action of ROMs and protect from cellular and molecular damage (Abei 1980). These antioxidant enzymes defend body system against free radicals attacks.

In this study we tried to determine the association of different socio-demographic factors with HPV infection. We found that non-vegetarian diet and rural settings act as significant independent predictors for HPV infection (also reported earlier in North Indian population) (Aggarwal *et al.* 2006; Shrivastava *et al.* 2012). Many studies reported that vegetarians have lower risk of HPV infection because of the presence of fruits and vegetables in their diet which are rich source of antioxidants like vitamin C, E, Beta carotene and presence of folate (or in the form of folic acid) (Sedjo *et al.* 2002). Folate deficiency is known to cause reduced immunity (Dhur *et al.* 1990).

The antioxidants and lipid peroxidation products are being extensively studied because of their potential importance and pathogenic role in several diseases including cancer (Srinivas *et al.* 2000). The oxidation of lipids and cell membranes generate cytotoxic compounds implicated in the etiology of aging and cancer (Wayner *et al.* 1998). Oxidative stress has long been related to carcinogenesis in human cancers and animal cancer models (Chen *et al.* 2000). Reactive oxygen species can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death if the antioxidant potential is insufficient (Devi *et al.* 2000).

Biochemical evidence has demonstrated that reduced glutathione plays a central role in cellular defence against reactive oxygen species (McLellan and Wolf 1999). The tripeptide glutathione plays a very important role in protecting the cells from free radical-induced lipid peroxidation. Glutathione as a reductant is very important in maintaining stability of erythrocyte membranes, its sulfhydryl group reduces peroxides formed during O² transfer and thus it provides protection against free radical injury (Tarr and Samson 1993). Although GSH level was reported to be decreased in malignant region by many scientist (Weir *et al.* 1996). However contradictory reports are also available (Ray *et al.* 2000).

Taking above facts, reduced glutathione was taken as a parameter in normal and cancer patients to assess its level in cervix cancer patients of Gwalior and nearby region. Results present in table 1, clearly indicate that the levels of GSH were significantly decreased in patients suffering from cervix cancer. The normal blood level of GSH, which was 2.58 mg/ml, was decreased to 1.4 mg/ml, showing 45.7 % decrease when compared with control. Role of GSH in tumors are not clearly understood but results of lower GSH level seen in different stages of breast cancer patients support the hypothesis that the glutathione status inversely related to malignant transformation (Kumarguruparan *et al.* 2002). Therefore the data obtained in the present study will help the clinicians for giving judicious dosage of compounds for increasing the GSH concentration in future.

S. No.	Parameters	Control Group (n=97)	Patients (n=37)
1.	GSH	2.58±0.03	1.40±0.04
2.	LPO	7.24±0.07	7.50±0.11
3.	SOD	0.51±0.03	0.32±0.01
4.	Catalase	7.75±0.11	5.23±0.10
5.	GPx	3.95±0.06	2.90±0.10
6.	GST	48.21±30.09	13.30±3.89

7.	Vitamin E	2.14±0.18	1.86±0.09
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Table 1: Comparison of oxidative stress markers in cervical cancer subjects

Values are expressed as mean ± SE, Units: GSH- mg/ml; LPO: mg/ml; SOD: units/min/mg protein; Catalase: units/min/mg protein; GPx: units/min/mg protein; GST: units/min/mg protein, p< 0.05 values are significant vs control group

Lipid peroxidation products diffuse from the inflammatory site and can be measured in the blood. Erythrocytes and erythrocyte membrane from cervical cancer patients showed a higher release of lipid peroxides in vitro as compared to normal subjects. The TBARS concentrations in the untreated erythrocytes and erythrocyte membrane were also significantly higher in cervical cancer patients as compared to normal subjects. The above observation reflects insufficient antioxidant potential in the erythrocytes of cervical cancer patients (Kwiatkowska *et al.* 1999).

The normal blood level of LPO, which was 7.24 mg/ml, was increased to 7.50 mg/ml, showing 3.5% increase when compared with control. Oztruck *et al.* in 2000 found an increased erythrocyte lipid peroxidation in testis cancer and concluded that an increase in lipid peroxidation may play a role in the pathogenesis of testis carcinoma in addition to other causes. Increase in lipid peroxidation products has been reported in patients with laryngeal and oral cancer (Samir and Kholy 1999; Manoharan and Nagini 1994). Elevated erythrocyte lipid peroxidation and disturbed antioxidant activities have been reported in patients with malignant lymphoma (Abou-Seif *et al.* 2000). Arivazhagan *et al.* in 1997 reported an increase in erythrocyte lipid peroxidation with a concomitant decline in antioxidants in gastric cancer patients.

The enhanced LPO may also be due to depletion of the activities of enzymes SOD, GST, GPx, and catalase which are the free radical scavenging enzymes or higher production of O₂ and H₂O₂. SOD and CAT are considered primary antioxidant

enzymes, since they are involved in direct elimination of ROMs. They also can act as anti-carcinogens and inhibitors at initiation and promotion/ transformation stage in carcinogenesis. There is a possibility of the accumulation of both the ROMs due to the reduced levels of antioxidants which may result in significantly higher LPO at cellular and molecular levels. However some contradictory reports indicating lower lipid peroxidation measured by plasma TBA has also been reported in breast cancer patients (Seven *et al.*, 1993). But Tas *et al.* in 2005 showed that lipid per oxidation in breast cancer tissue was enhanced compared to non-malignant tissues. They showed higher oxygen free radical production and decreased CAT activity supporting the oxidative stress hypothesis in breast carcinogenesis.

Superoxide dismutase (SOD) is a very important enzyme that functions as a cellular anti-oxidant. It is present in cell cytoplasm (copper-zinc enzyme) and in mitochondria (manganese enzyme) in order to maintain a low concentration of superoxide anion. It catalyzes the dismutation of super oxide anion into H_2O_2 . H_2O_2 is metabolized by catalase and glutathione peroxidase, which reduce it into water and molecular oxygen. The activity of SOD was decreased significantly in patients suffering from cervical cancer. The normal blood level of SOD, which was $0.51 \mu\text{g}/\text{min}/\text{mg}/\text{protein}$, was decreased to $0.32 \mu\text{g}/\text{min}/\text{mg}/\text{protein}$, showing 37.25% decrease when compared with control.

Enhanced lipid peroxidation observed in the erythrocytes of cervical cancer patients can be correlated with the decrease in catalase activity (Kaplan and Groves 1972). High activity of catalase in red blood cell has been reported to play a crucial role in protecting red blood cells against oxidative damage. Administration of catalase has been shown to have an important role in protection against H_2O_2 -mediated lipid peroxidation (Chow 1988).

The activity of catalase was decreased significantly in

patients suffering from cervical cancer. The normal blood level of catalase, which was 7.75 $\mu\text{g}/\text{min}/\text{mg}/\text{protein}$, was decreased to 5.23 $\mu\text{g}/\text{min}/\text{mg}/\text{protein}$ in patients of cervix cancer registering 32.52 % decrease when compared with healthy control.

Glutathione peroxidase is a cytoplasmic and mitochondrial enzyme that is important for detoxifying H_2O_2 in most cells. The activity of GPx was decreased significantly in the blood of patients suffering from cervix cancer. The normal blood level of GPx, which was 3.95 $\mu\text{g}/\text{min}/\text{mg}/\text{protein}$, was decreased to 2.9 $\mu\text{g}/\text{min}/\text{mg}/\text{protein}$ in patients of cervix cancer registering 26.58 % decrease.

The activity of GST was decreased significantly in patients suffering from cervix cancer. The normal blood level of GST, which was 48.21 $\mu\text{g}/\text{min}/\text{mg}/\text{protein}$, was decreased to 13.30 $\mu\text{g}/\text{min}/\text{mg}/\text{protein}$, showing 72.41 % decrease when compared with control.

The normal blood level of Vit. E, which was 2.14 mg/ml, was decreased to 1.86 mg/ml, showing 13.1 % decrease when compared with control. Recent clinical trials have demonstrated the significant cancer preventive potential of vitamin E in many different cancer sites, ranging from oral and pharyngeal cancer to prostate cancer (Shklar and Oh 2000). Evstigneeva *et. al.*, in 1998 reported that vitamin E was concluded to be a universal participant of antioxidant defense reactions in biological membranes, since it acts at all steps of membrane oxidative damage, and act as a first line of defense against peroxidation of PUFA (Singh *et al.*, 2000). Vitamin E present in high concentration in erythrocyte membrane may help to diminish lipid peroxidation. Vitamin E has been suggested to be one of the sensitive and functional markers of TBARS release from erythrocytes under in vitro conditions (Gutteridge 1978). A positive association between vitamin E deficiency and lipid peroxide formation has been reported (Chow 1991). Hence we feel that the increased lipid peroxidation in cervical cancer

patients, in the present study, can be ascribed to a large extent to the deficiency of vitamin E.

Conclusion

From the results of the present study, we suggest that increased lipid peroxidation and decrease host antioxidant defenses associated with the development of cervix cancer may offer a selective growth advantage to tumor cells over their surrounding normal counterparts.

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Both RST and SK have contributed equally to this paper.

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