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OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN CERVICAL CANCER PATIENTS

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ABSTRACT

Cervical cancer (CaCx) is a global public health problem as it is the second most common cancer leading to the death of women worldwide. Many references revealed that the low levels of antioxidants induce the generation of free radicals leading to DNA damage and further mutations. In the present study attempt have been made to evaluate the levels of serum Lipid peroxide, Nitric Oxide ($NO^{\bullet}$), Erythrocytic - Superoxide Dismutase (RBC -SOD), Vitamin-C, serum Copper (Cu) and serum Zinc (Zn). 120 patients were divided in 4 groups according to the increasing CaCx stages i.e. stage I, II, III & IV respectively. All the patients were around the age group of 25-65 years. 30 healthy women between the same age group were treated as controls. Highly significant increased values of MDA, $NO^{\bullet}$ and Cu were observed ($p<0.001$) whereas the activity of RBC-SOD, levels of Vitamin-C and Zn were significantly decreased in CaCx patients as compared with healthy controls ($p<0.001$). Cu/Zn ratio was found to be altered in CaCx patients. From our findings it can be concluded that the oxidative stress is induced among CaCx patients, which inturn increases the risk of CaCx.

KEY WORDS

Lipid peroxide, Cervical cancer, RBC-SOD, Nitric Oxide, Vitamin-C, Copper, Zinc.

INTRODUCTION

When we think of cancer in general terms, we are apt to conjure up a process characterized by a steady, remorseless and inexorable progress in which the disease is all conquering and none of the immunological and other defensive forces will help leading to faltering footsteps to the grave.

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 (1).

CaCx is said to be mediated by Human Papilloma Virus (HPV) but recent data published also revealed role of oxidative stress in CaCx (2). The imbalance between the pro-oxidants and antioxidants in favour of pro-oxidants is called oxidative stress. To assess the status of oxidative stress in CaCx patients following parameters were studied.

MDA is being extensively used in assessing the process of lipid peroxidation. Reactive Oxygen Species (ROS) generated causes peroxidation of Polyunsaturated Fatty Acids (PUFA) of the membrane called lipid peroxidation. This interrupts the membrane integrity which may be one of the possible reason of CaCx progression (3).

$NO^{\bullet}$ acts as an intracellular secondary messenger and provides an efficient system for cellular regulation, interaction and defence. Its role is strictly dependent upon its chemical reactivity with oxygen and metal. Recent references revealed the involvement of altered $NO^{\bullet}$ level in pathogenesis of CaCx (4). Some of the references showed that $NO^{\bullet}$ in high or low concentration than the basal levels has tumorogenic effect in CaCx (5).

SOD mainly functions to provide a defensive action against the potentially damaging reactivities of the superoxide radical generated by all aerobic metabolic reactions. As SOD is a free radical metabolizing enzyme, it catalyzes the dismutation of superoxide radical to H_2O_2. This protects the cell membrane

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from damage by ROS. But the decreased SOD levels may lead to increased lipid peroxidation resulting in the cellular rigidity and deformability (6). Altered activity of SOD in CaCx patients have been revealed recently (7).

Vitamin -C is a water-soluble antioxidant vitamin. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing agent and antioxidant agent it directly reacts with $O_2^{\bullet}$ and $OH^{\bullet}$ and various lipid hydroperoxides. It plays important role in sparing Vitamin-E as well, which is another lipid soluble antioxidant. So, it may be said that the role of Vitamin-C is very beneficial in CaCx treatment (8,9).

Strong oxidizing agent, interacts with organic substances only sluggishly, they are supported by transition metal like copper which creates more reactive species such as $OH^{\bullet}$(10).

Zinc is an integral part of biomembrane, it may be involved in the control of membrane integrity, in membrane stability and in lipid peroxidation- related injuries. The role of zinc in RNA and DNA polymerase is its inhibitory effects on phosphodiesterase and its activating effect on membrane bound adenylcyclase. These all suggest a role for zinc in carcinogenesis (11).

Researchers have shown that the HPV is involved in cervical cancer. The mechanism by which HPV acts is by damaging the DNA. Many researchers have shown the potential of ROS to damage the DNA causing mutations. It is evident that both HPV and ROS have same effect, therefore, the role of ROS in CaCx has to be studied. The present study invites attention on the possible role of ROS in the progression of cervical cancer.

MATERIALS AND METHODS

The present study was carried out in Department of Biochemistry, Dr.V.M.Govt Medical College, Shree Chatrapati Shivaji Maharaj General Hospital and Shree Sidheshwar Cancer Hospital, Solapur (Maharashtra).

The present study included 150 subjects. Out of 150 subjects 30 were controls and 120 were patients with CaCx. Further, CaCx patients were divided in 4 different groups according to their stages (I, II, III & IV). The female subjects within the age group of 25-65 years were selected. They were clinically and histopathologically diagnosed for CaCx. 30 healthy female subjects from the same socio-economic status, having no history of smoking, alcoholism, any type of carcinoma etc. were treated as controls. The subjects having history of smoking, alcoholism and other diseases which induce oxidative stress such as diabetes mellitus, pulmonary diseases, respiratory diseases etc. having no such concurrent or past history of diseases were excluded from the study.

After obtaining prior consent, venous blood was collected from the subjects under aseptic condition by venipuncture using 10ml sterile disposable syringe and needle. About 8ml of blood was collected, out of which 4ml was poured in heparinised bulb and 4 ml was allowed to clot. Serum and plasma were separated by centrifugation at 3000 rpm for 10 min. at room temperature. The samples were stored at 4ºC before analysis and all the samples were analyzed on the same day of collection.

All the methods were standardized first and standard graphs were obtained. Serum Lipid peroxide was measured by precipitating lipoporoteins with trichloroacetic acid and boiled with thiobarbituric acid which reacts with Malondialdehyde to get pink colour as per Kei Satoh's Method (12). For Nitric oxide serum was deproteinized first and then Nitrate, the stable product of nitric oxide, present in filtrate is reduced to nitrite, which is measured by diazotization of sulphanilamide and coupling with Naphthylethylene Diamine as in Najawa & Cortas method (13). RBC-SOD by Winterbourn's Method which is based on ability of SOD to inhibit the reduction of Nitroblue tetrazolium by superoxide, which is generated by the reaction of photoreduced riboflavin and oxygen (14) . Plasma Vitamin-C was estimated by Aye Kyaw's Method where phosphotungstic acid first deprotienize the plasma and then react with ascorbic acid to produce blue colour (15). Serum Copper and Zinc were estimated by Atomic Absorption Spectrophotometer (AAS).

All the results were expressed in mean ±SD.Statistical analysis was done by using student's 't'-Test. Correlation coefficient was determined between lipid peroxide and Vitamin-C. $p<0.05$ was considered as significant whereas $p<0.001$ was considered as highly significant.

RESULTS

Significantly higher levels of serum lipid peroxide in the form of MDA ($p<0.001$) and serum $NO^{\bullet}$ ($p<0.001$) were observed in patients with CaCx when compared with healthy controls. Maximum rise in MDA and $NO^{\bullet}$ were observed in stage IV when compared with healthy controls (Table 1). Significantly lowered activity of RBC-SOD ($p<0.001$) and decreased levels of plasma Vitamin-C ($p<0.001$) was observed in all the stages of CaCx patients when compared with healthy controls. It was

Table 1 : Showing comparative status of oxidants and antioxidants in controls and different stages of CaCx patients

Groups	No. of Subjects	MDA (nmol/dl)	NO• (µmol/ L)	RBC-SOD (Unit/gm of Hb)	Vitamin-C (mg %)
Control	30	251±0.45	51.04±3.71	3265±249.5	1.32±0.08
CaCx Patients					
Stage-I	30	$292\pm0.44^{h}{}_{1}$	$54.30\pm3.69^{S}{}_{1}$	$3043\pm304.1^{S}{}_{1}$	$1.17\pm0.06^{h}{}_{1}$
Stage-II	30	$327\pm0.11^{h}{}_{2}$	$68.67\pm4.49^{h}{}_{2}$	$2820\pm326.0^{S}{}_{2}$	$1.06\pm0.08^{h}{}_{2}$
Stage-III	30	$338\pm0.10^{h}{}_{3}$	$77.72\pm3.88^{h}{}_{3}$	$2095\pm461.8^{h}{}_{3}$	$0.84\pm0.06^{h}{}_{3}$
Stage-IV	30	$376\pm0.12^{h}{}_{4}$	$84.27\pm6.61^{h}{}_{4}$	$1760\pm372.5^{S}{}_{4}$	$0.77\pm0.07^{h}{}_{4}$

observed that the concomitant decline in the activity of RBC-SOD and levels of Vitamin-C are associated with the progression of stages in CaCx (Table 1). Cu/Zn ratio was also found to be substantially higher in all the stages ($p<0.001$) when compared with healthy controls (Table 2).

DISCUSSION

Increased levels of MDA indicate upsurged lipid peroxidation which is a consequence of increased free radical generation. It causes profound alterations in the function of the cell membrane and also structural organization of DNA leading to mutations (16). Therefore, it can be stated that lipid peroxidation is one of the possible cause of CaCx progression.

ROS that are derived from NO• are released from inflammatory cells and can act on neighbouring dividing epithelial cells, leading to somatic mutations in crucial cancer-causing genes (17). NO• produced by inducible nitric oxide synthase (iNOS) in solid tumors has been implicated in enhanced vascular permeability and increased tumor blood flow and hence sustained tumor growth(18).

The probable reason for the decrease in SOD activity may be associated with free radical generation which cause damage (to enzyme) by cross linking or damaging the nuclear DNA leading to mutations. It may also be due to scarcity of trace elements like Zinc, Manganese etc. which acts as a cofactor for this enzyme.(7)

The decreased levels of Vitamin-C may be associated with its action as antioxidant where it gets utilized. Its synergism with Vitamin-E helps in sparing of Vitamin-E, during this process Vitamin-C gets utilized (8,9) which is seen as significant decline in plasma ascorbic acid. Negative correlation (r = -0.73) between Vitamin-C and MDA was noted leading to the

Table 2 : Showing comparative status of trace elements and its ratio in controls and different stages of CaCx patients

Groups	No. of Subjects	Copper (µg%)	Zinc (µg%)	Cu/Zn ratio
Control	30	109.7±10.85	97.6±8.71	1.13±0.13
CaCx Patients				
Stage-I	30	$117.4\pm12.26^{\mathbf{S_1}}$	$87.4\pm11.91^{\mathbf{S_1}}$	$1.37\pm0.34^{\mathbf{S_1}}$
Stage-II	30	$142.8\pm9.65^{\mathbf{h_2}}$	$76.5\pm10.62^{\mathbf{S_2}}$	$1.92\pm0.040^{\mathbf{h_2}}$
Stage-III	30	$159.2\pm8.68^{\mathbf{h_3}}$	$67.3\pm8.61^{\mathbf{h_3}}$	$2.42\pm0.45^{\mathbf{h_3}}$
Stage-IV	30	$172.9\pm9.17^{\mathbf{h_4}}$	$71.3\pm9.34^{\mathbf{n_4}}$	$2.48\pm0.45^{\mathbf{h_4}}$

Results were expressed as mean ± SD

Table 1 & 2: Statistical Analysis	p<0.001 (Highly significant)	P<0.05 (Significant)	P>0.01 (Non-significant)
Control V/s Stage I	$\mathbf{h_1}$	$\mathbf{s_1}$	--
Stage I V/s Stage II	$\mathbf{h_2}$	$\mathbf{s_2}$	--
Stage II V/s Stage III	$\mathbf{h_3}$	$\mathbf{s_3}$	--
Stage III V/s Stage IV	h_4	s_4	n_4

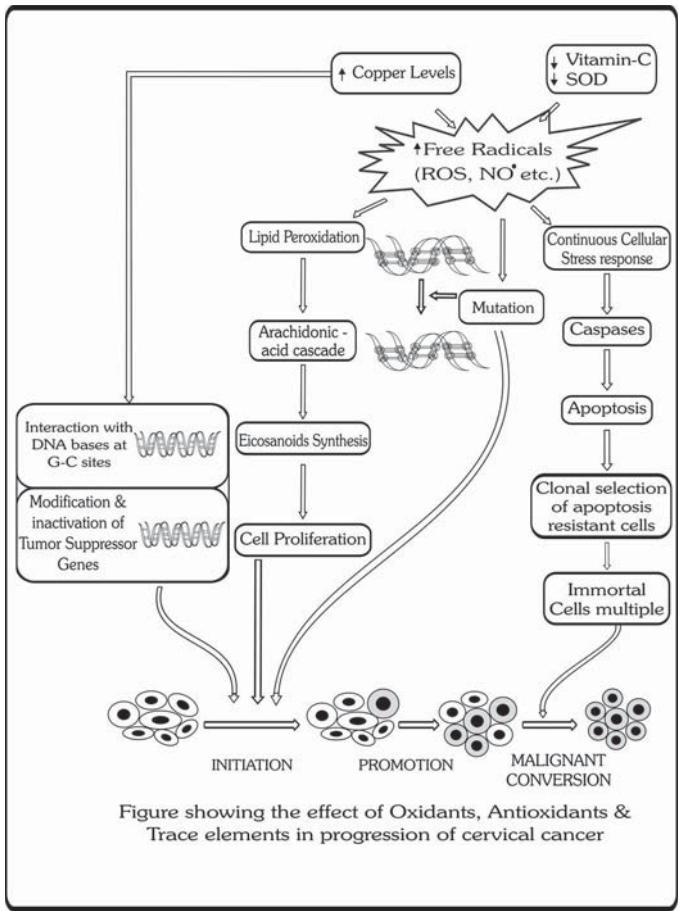

Figure showing the effect of Oxidants, Antioxidants & Trace elements in progression of cervical cancer

conclusion that free radicals are scavenged by ascorbic acid and thus it gets utilized.

Copper can interact directly with the bases of DNA at G-C sites(19) (See Fig.). The addition of copper to DNA *in vitro* mediates more extensive DNA base damage inducing more mutations(20). Copper may also elaborate other free radical species such as $OH^{\bullet}$, therefore, the inactivation/loss of certain tumor suppressor genes can lead to the initiation and/or progression of carcinogenesis. The elevation in copper levels may be due to mobilization of copper from tissue to serum(20).

Zinc is used for the growth of the cell and also it is useful in maintaining the integrity of the cell membrane. So, it may happen that the cancerous cell may consume the zinc which is present in the circulation for tumor growth and to maintain its membrane integrity (21). This might be the possible reason of depletion of zinc in CaCx patients.

Increased ratio of Cu/Zn is due to the significant decrease in Zn and concomitant increase in copper. As this ratio is altered, this could be considered as risk factor for tumor growth or carcinogenesis.

While there are several chronic diseases more destructive to life than cancer none is more feared. The present discussion focuses on the potential molecular mechanism of free radicals underlying the ability of oxidative stress in carcinogenesis. Our finding strongly supports that the oxidative stress is induced among CaCx patients which inturn increases the risk of CaCx. Further altered Cu/Zn ratio may be considered as a risk for sustained tumor growth. However, the role of other antioxidant factors and effect of antioxidant supplementation can be substantiated in future studies.

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