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A Role of Antioxidants in Cervical Cancer

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ABSTRACT

Non-communicable diseases (NCDs) will account for 70% of the mortality globally in future.Cancer is the second most common cause of morbidity and mortality in both developed and developing countries. Substantial epidemiological data on populations indicate an association between many human cancers and lifestyle/diet.Cervical cancer remains a great problem for woman health, as it is the second deadly cancer of females worldwide. Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, supposed oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants.ROS are products of a normal cellular metabolism and play vital roles in the stimulation of signaling pathways in plant and animal cells in response to changes in intra- and extracellular environmental conditions. Imperfections in the antioxidant enzyme systems are stated to play chiefpart behind this antioxidant deficiency, which is responsible for the production of reactive oxygen species and ultimately, DNA damage in cervical cells. The key of the presentstudy was evaluating the enzymatic and non-enzymatic antioxidants in cervical cancer patients in serum and cervical tissue.

Keywords:
Non-communicable diseases, Cervical cancer, Oxidative stress,Reactive Oxygen Species,Antioxidant.

1. Introduction

Cancer is one of the most feared and outrageous disease, afflicting the human race. It is a complex disease, where there is a high degree of genetic variability not only among different types of cancer, but also among different patients and even to the extent, that different cells in the same tumor show variability.

Oxidative stress has been recognized as an important factor affecting the pathogenesis of degenerative and inflammatory diseases, cancer and aging. Indeed, reactive oxygen species (ROS) is believed to play a pivotal role in the etiology of cervical cancer, and ROS overproduction and subsequent oxidative DNA damage have been implicated to enhance the development of cervical cancer that are caused by carcinogenic agents.

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Durackova., 2010). ROS are products of a normal cellular metabolism and play vital roles in the stimulation of signaling pathways in plant and animal cells in response to changes in intra- and extracellular environmental conditions (Jabs., 1999). Most ROS are generated in cells by the mitochondrial respiratory chain (Poyton et al., 2009). During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion (O2-), hydrogen peroxide (H2O2), hydroxyl radical (OH•) and organic peroxides as normal products of the biological reduction of molecular oxygen (Fridovich., 1978).

The electron transfer to molecular oxygen occurs at the level of the respiratory chain and the electron transport chains are located in the membranes of the mitochondria (Goossens et al., 1995 and Goossens et al., 1999). Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which can generate reactive nitrogen species (RNS) (Poyton et al.,

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2009). RNS can further generate other reactive species, e.g., reactive aldehydes-malondialdehyde and 4-hydroxynonenal-by inducing excessive lipid peroxidation (Hussain et al., 2003).DNA, Proteins and lipids are also significant targets for oxidative attack and modification of these molecules can increase the risk of mutagenesis (Schraufstatter et al., 1988).Under a sustained environmental stress, ROS are produced over a long time and thus significant damage may occur to cell structure and functions and may induce somatic mutations and neoplastic transformation (Fang et al., 2009 and Khandrika et al ., 2009). Indeed, cancer initiation and progression have been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation (Visconti and Grieco., 2009).

Furthermore, cancer, which is considered an impairment of body functions over time, caused by the accumulation of molecular damage in DNA, proteins and lipids is also characterized by an increase in intracellular oxidative stress due to the progressive decrease in intracellular ROS scavenging (Minelli et al., 2009). Acting to protect the organism against these harmful pro-oxidants is a complex system of enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase and catalase) and nonenzymatic antioxidants (e.g., glutathione (GSH), vitamins C and D).The keyof the presentstudy was evaluating the enzymatic and non enzymatic antioxidants in cervical cancer patients in serum and cervical tissue.

2. Material And Methods

The study was conducted during the period January 2020 to march 2020 in the Department of Clinical Biochemistry, BHARATH INSTITUTE OF HIGHER EDUCATION AND RESEARCH, Chennai, Tamil Nadu.Two groups were maintained and forty four Samples were used. Ethical clearance was obtained from Institutional Ethical Committee conducted at the Meenakshi Medical College and Hospital.

3. Sources Of Chemicals

Reduced glutathione, bovine serum albumin, ribonuclease and antibodies were obtained from Sigma Chemical Company, St. Louis, MO, USA. Ascorbic acid, adenosine triphosphate, 1-amino 2-naphthol 4-sulphonic acid (ANSA) and glutathione were obtained from Sisco Research Laboratories, Mumbai, India. 1-choloro-2,4-dinitrobenzene (CDNB) and 5,5'-dithionitrobenzoic acid (DTNB) were obtained from S.D. Fine Chemicals, Mumbai, India.DNA markers, normal melting and low melting point agarose, acrylamide and N,N'-methylene bisacrylamide were purchased from Amersham Pharmacia, Germany. All other chemicals used were of analytical grade obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Glaxo Laboratories, CDH division, Mumbai, India. All other chemicals, reagents and solvents used were analytical grade.

4. Experimental Design

The patients were divided into TWO groups and each group consists of thirty patients.
Group I:Control (Normal Patients).
Group II:Cervical cancer patients.

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5. Enzymatic And Non-Enzymatic Antioxidants

The activity of superoxide dismutase was determined by the method of Marklund and Marklund (1974).The activity of catalase was assayed by the method of Sinha (1972).The activity of glutathione peroxidase was assayed by the method of Rotruck et al. (1973).The activity of glutathione reductase was measured by the method of Staal et al. (1969).The level of reduced glutathione was measured by the method of Moron et al. (1979).The level of ascorbic acid was estimated by the method of Omaye et al. (1979).The level of vitamin E was estimated by the method of Desai (1984).Measurement of serum 25 (OH) VITAMIN Dwas measured by immunodiagnostic direct Elisa kit method.

Statistical analysis

Data are expressed has Mean ± SD, and independent 't' test was used to compare the various parameter between normal healthy control and patient with breast cancer. P value <0.05 is considered statistically significant. The data was analyzed using SPSS (Statistical package for Social Science) software V.16.0.

6. Results And Discussion

Enzymatic antioxidants:-

Table: 1 shows the level of SOD, CAT and Gpx in control and experimental group was found to be significantly ($p < 0.05$) decreased in Cervical cancer (Group II) patients when compared to control (Group I) patients. The concentrations of SOD, CAT and Gpx were expressed as mean ± SD.

Particulars	Group-I (Control Patients)	Group – II (Cervical Cancer Patients)
SOD	3.81 ± 0.40	2.31 ± 0.2 [a*]
CAT	228 ± 23.1	102 ± 10.5 [a*]
Gpx	38.1 ± 3.9	18.2 ± 1.5 [a*]

Each value is expressed as mean + SD for Thirty patients in each group
Units : SOD - Units/min/mg protein,,CAT - μmoles of H2O2 liberated/min/mg protein,GPx - μmoles of GSH oxidised/min/mg protein, a: as compared with Group IStatistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

The event of free radicals attacking bio a membrane, leading to oxidative destruction of the polyunsaturated fatty acid (PUFA) in the membrane is well documented in the process termed as "Lipid Peroxidation" (Slater, 1984). Changes in the rate of LPO seem to be a general feature of

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cancerous cells and may be a prerequisite for cell division (Bartoli and Galeotti 1979; Chessman et al., 1986).

There has been a strong association between free radical reactions and a variety of pathological events such as cancer, diabetes, atherosclerosis, aging etc. (Vasavi et al., 1994). In biological systems, the steady state level of LPO is often assessed by the measurement of LPO breakdown products such as Malondialdehyde (MDA) (Knutson et al., 2000) Malondialdehyde (MDA) is one such reliable marker for estimating tissue injury (Ando et al., 1995).

Carcinogenic compounds, mediate the high production of free radicals, which escape detoxification by the defense system and they attack cellular constituents such as Deoxy ribo nucleic acid (DNA) (Nakazarva et al., 1996). This kind of DNA damage and peroxidative pathways would result in initiation of cancer through mutagenesis (Dreher and Junod 1996)

Oxygen radicals play an important role in the complex course of multistep carcinogenesis (Cerutti, 1985; Copeland, 1983), which play a prior role and are mainly responsible for a variety of detrimental effects, biochemical changes such as cellular damage, tissue damage and DNA modification (Chance et al., 1979; Fridovich, 1983). Oxygen radicals that are implicated in the genotoxic agents can initiate and promote cancer development (Thurnham, 1991). Free radicals initiated auto-oxidation of cellular membrane can lead to cellular necrosis, and is now accepted to be important in a variety of pathological conditions, particularly cancer (Pryor, 1980). Unquenched free radicals can subsequently cause several toxic effects to the system, the major being LPO. Free radicals may be the most critical factors triggering plasma antioxidant depletion and lipid peroxidation and protein modification (JunMa et al., 2000; Eiserich et al., 1995; Sparrow et al., 1989).

The elevated levels of LPO in lung carcinoma bearing animals may be due to its poor antioxidative defense as well as either due to leakage of MDA from the tissue injury or due to the improper functioning of antioxidant system in cancerous condition. Wrang et al. (1996)

ROS are involved in the cell growth, differentiation, progression and death (Mates et al., 1999). They play a major role in cancer initiation and promotion (Ames, 1983) .The antioxidant defense enzymes namely SOD, GPx and CAT protect aerobic cells against oxygen toxicity and lipid peroxidation (Cerutti, 1985).

SOD may play an important role in protecting cancer cells against ROS (Yamaguchy, 1991). SOD activity and superoxide generation may be different from normal in in vivo tumor cells. Increased superoxide radical levels in tumor cells may explain the decreased activity of SOD in malignant tissues (Patwardhan et al., 1988a). Decreased activity of SOD has been reported in cancerous conditions (Oberley and Buettuer 1979).

GPx scavenges the highly reactive lipid hydro peroxide in the aqueous phase of cell membrane.

Catalase the enzyme which catalyses the disproportionation of H2O2 and GPx is the first line of defence against oxidative damage.The activities of SOD, CAT and GPx were found to be decreased in lung carcinoma bearing animals; this may be due to the enormous production of free radicals or weakening of antioxidant defense system in the cancerous condition or may be due to the higher production of lipid peroxides

The decreased activity of GPx and CAT may be due to the accumulation of H2O2 which in turn causes the inhibition of these enzymes (Rister and Banchner 1976). This may be due to the altered antioxidant defence system because of enormous production of free radical in the B(a)p induced carcinogenesis (Daniel and Joyce 1983).This decreased activities of enzymic antioxidants well correlates with the progression of the malignancy, indicating the impairment of free radicals and the weakened antioxidant defence system in cancerous condition.

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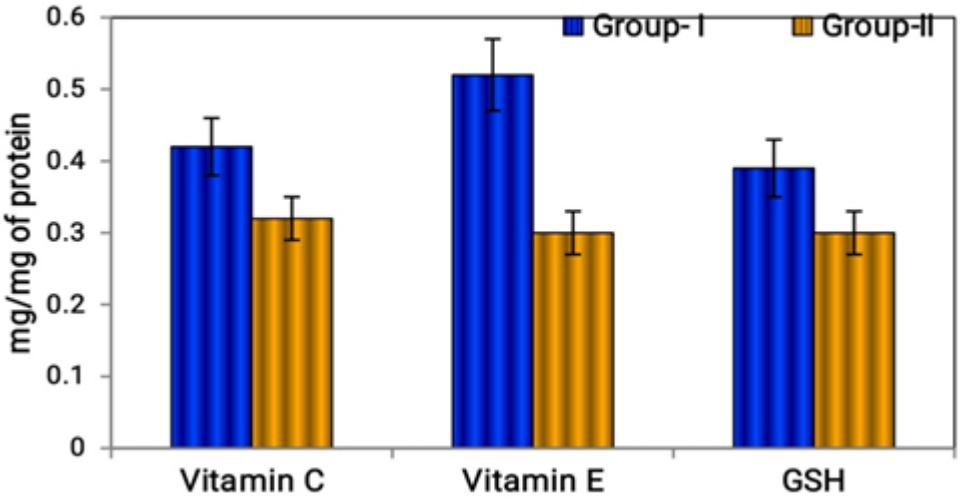
Non enzymatic antioxidants:-

Fig.2. represents the level of Vitamin E and Vitamin C in control and experimental animals ,was found to be significantly ($p < 0.05$) decreased in cervical cancer bearing (Group II) patients when compared to control (Group I) patients.

Fig.2. Levels of Non Enzymatic Antioxidant



Each value is expressed as mean + SD for Thirty patients in each group. Units :Units/min/mg protein, a: as compared with Group I, Statistical significance: * $p<0.001$ @ $p<0.01$ # $p<0.05$, NS- Not significant

Vitamin C has multiplicity of antioxidant properties and has been proved to be the most important antioxidant in human plasma, because it disappears faster than other antioxidant when exposed to reactive oxygen species. Vitamin C is observed to have inverse relationship with incidence of cervical cancer. The ascorbate molecule must be involved in the feedback inhibition of lysosomal glycosidases responsible for malignant invasiveness (Cameron et al., 1979).The reduction of vitamin C in cancerous condition may be due to the stress, the requirement and utilization of these vitamins and other antioxidants increased progressively, since tumor cells utilize these antioxidants for cell proliferation.

□-tocopherol is a powerful chain breaking antioxidant and free radical scavenger that inhibits peroxidation of lipids. Vitamin E is one of the exclusive antioxidant that protect against carcinogenesis and tumor growth (Das, 1994). The enormous production of lipid peroxides formed may be the reason for the decreased levels of vitamin E in cancerous condition.

In bio membranes, vitamin E is an efficient antioxidant, the reason being its ability to penetrate, to a precise site into the membrane, which may be the important feature of protection against highly reactive radicals (Packer et al., 1979). Vitamin E levels were found to be significantly lower in cervical cancer patients when compared with their control subjects. The assessment of vitamin E provides further useful information in evaluation of cancerous condition (Torun et al., 1995).

Glutathione is an important intracellular antioxidant. This tripeptide helps to detoxify free radicals, peroxides and electrophilic compounds of endogenous and exogenous origin (Ley et al., 2000; Dolphin et al., 1989). It is endogenously synthesized in the liver and is the first line of defense against lipid peroxides. It plays a vital role in destruction of H_2O_2, lipid peroxides, free

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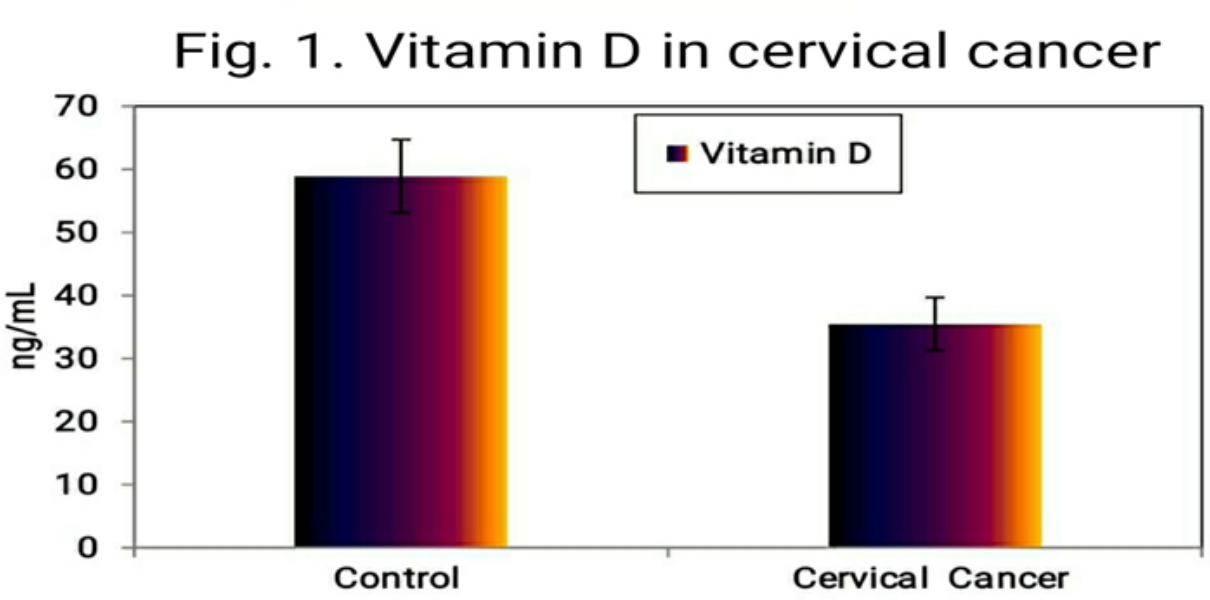
radicals, translocation of amino acids across cell membranes, detoxification of foreign compounds and the biotransformation of the drugs (James and Harbison 1982).

The elevated level of lipid peroxides and its utilization of GSH may be the reason for the decreased levels of GSH in cancerous condition. Burk (1983) have reported that increased lipid peroxidation correlates with reduction of GSH and this leads to the alteration of polyunsaturated fatty acids (PUFA)

Vitamin D in Cervical cancer

Figure: 1 shows the level of Vitamin D level in control and experimental group was found to be significantly ($p < 0.001$) decreased in Cervical cancer patients (Group II) patients when compared to control (Group I) patients. The concentrations of vitamin D were expressed as mean ± SD.



Vitamin D deficiency is of particular concern among women in many south Asian countries due to low availability of vitamin D-rich foods, dark skin pigmentation, and cultural and religious practices that promote the wearing of concealing clothing. However, the information regarding the vitamin D status of many sub population in south Asian countries are limited. The current study was conducted to assess the vitamin D status of 50 Tamil men of cervical cancer and determine whether vitamin D status influences the susceptibility to promote better quality of life.

Many studies have shown that there is a link between vitamin D and cervical cancer cancer. Rosen, et al 2012 have found how vitamin D might have a role in cervical cancer. Vitamin D receptors are found on the surface of a cell where they receive chemical signals. By attaching themselves to a receptor, these chemical signals direct a cell to do something, for example to act in a certain way, or to divide or die.

Vitamin D has a number of anticancer effects, including the promotion of cancer cell death, known as apoptosis, and the inhibition of angiogenesis. There are vitamin D receptors in lung tissue, and vitamin D can bind to these receptors. This can cause cells like oncogenes to die or stop growing, and can stop the cancer cells from spreading to other parts of the body. Therefore, it is thought that vitamin D may help in protecting against cervical cancer, by making cells in the cervical smarter.

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Circulating levels of vitamin D are directly related to dietary vitamin D intake and cutaneous synthesis of vitamin D.15 The active form of vitamin D, 1,25-dihydroxyvitamin D, abbreviated 1,25(OH)2D, is produced by hydroxylation of the major circulating form of vitamin D, 25-hydroxy vitamin D, abbreviated 25(OH)D, a reaction catalyzed by the enzyme 25-hydroxy vitamin D-1_-hydroxylase.15 1,25(OH)2D is produced in the lung (amongst other anatomic sites, including the kidney, colon, and prostate), and the extent of its production there is probably dependent upon the availability of 25(OH)D for 1-hydroxylation. Therefore, it has been hypothesized that low circulating levels of 25(OH)D might impair local production of 1,25(OH)2D in lung and thereby increase risk of cervical cancer.

7. Conclusions

The present study is a Biochemical changes in cervical cancer in humans. The findings of the study have been summarized below. A decrease in the levels of enzymatic antioxidants such as SOD, CAT, GPx, GR and non-enzymatic antioxidants such as glutathione, vitamin C and vitamin E were observed in cervical cancer patients. From the above test results obtained in terms of biochemical studies, the present study proves that the early identification and marker of cervical cancer to start treatment with either chemotherapy or radiotherapy.

Conflict of interest
The authors declare no conflict of interest.

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