A study to evaluate the anti-oxidant concentration in carcinoma of the cervix in south Indian women

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Abstract

Aim and background: In the presence study was investigated that the anti-oxidant concentration in carcinoma of the cervix in south Indian women. Cervical cancer has a major impact on women's lives worldwide, particularly in developing countries where it is the leading cause of cancer deaths among women. Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species and their elimination by protective mechanisms, referred to as antioxidants. **Materials and Methods:** This is a prospective study. All the patients were divided into two groups and each group consists of thirty patients. All the patients were selected based on the inclusion and exclusion criteria. **Results:** Enzymatic and Non enzymatic antioxidants levels 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. **Conclusion:** In the present study we conclude that 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.

Key words: Cervical Cancer, Enzymatic and Non enzymatic antioxidants.

DOI: 10.48047/ecb/2023.12.9.239

I. Introduction

One fourth of the global burden of cervical cancer is shared by India. Because of our vast population, we are unable to implement primary prevention by HPV vaccination and secondary prevention by universal screening programmes to reduce the burden of cervical cancer in India. It is well established that persistent infection with high risk HPV is the necessary cause of cervical carcinoma. More than 80% of sexually active women acquire the infection, but most often the infection is transient and cleared from the body by the immune mechanism. Antioxidants are important to maintain and protect the immune status of the individuals from oxidative damage. The persistence of HPV in the body was modified and negated by improving the immune status of the individual. It had been shown that antioxidant vitamins, such as α-carotene, β-carotene, vitamin E, and vitamin C could act as efficient scavengers of free radicals and oxidants to prevent free-radical damage to DNA and mutations and are considered to have antineoplastic effects on the cervix [1]. At present, very few Indian studies are available related to this area. Evidence based knowledge generated through studies was help in creating awareness among the public about the importance of healthy diet and by simple dietary measures, risk of cervical carcinoma reduced considerably. At the National level besides the initiatives and programmes to prevent cervical cancers, the possibility of antioxidant reach nutrition supplements was also explored to the under privileged women in the community.

Reactive oxygen species (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. Most ROS are generated in cells by the mitochondrial respiratory chain [2]. During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion ($O2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$) and organic peroxides as normal products of the biological reduction of molecular oxygen. 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. Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which can generate reactive nitrogen species (RNS) [3]. RNS can further generate other reactive species, e.g., reactive aldehydes-malondialdehyde and 4-hydroxynonenal-by inducing excessive lipid peroxidation. DNA, Proteins and lipids are also significant targets for oxidative attack and modification of these molecules can increase the risk of mutagenesis. 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 [4].

Vitamin C, vitamin E, beta-carotene and lycopene, are potent antioxidants that can prevent reactive oxygen species from oxidizing cellular proteins and DNA, and are considered to have anti-neoplastic effects in the cervix. Studies like ours will throw light into the importance of vitamins and nutrients in curtailing and modifying disease progression in cervical carcinoma. Evidence based knowledge generated through such studies will help in creating awareness among the public about the importance of healthy diet and by simple dietary measures risk of pre-invasive and invasive carcinoma of the cervix can be reduced considerably.

II. Materials and Methods

The study was conducted during the period June 2021 to Dec 2021 in the Department of OBG, Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu. Ethical clearance was obtained from Institutional Ethical Committee conducted by institutional ethical committee. Samples received from the patients with consent.

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.

Experimental Design

The patients were divided into **TWO** groups and each group consists of thirty patients.

Group I : Control (Normal subjects).

Group II : Cervical cancer patients.

Biochemical Methods

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).

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 cervical cancer. P value <0.05 is considered statistically significant. The data was analyzed using SPSS (Statistical package for Social Science) software V.16.0.

III. Results

Enzymatic Antioxidants

Table: 1 shows the levels 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.

Table: 1. Levels of SOD, CAT and Gpx in control and experimental group

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 H_2O_2 liberated/min/mg protein

GPx - µmoles of GSH oxidised/min/mg protein

a: as compared with Group I

Statistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

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.

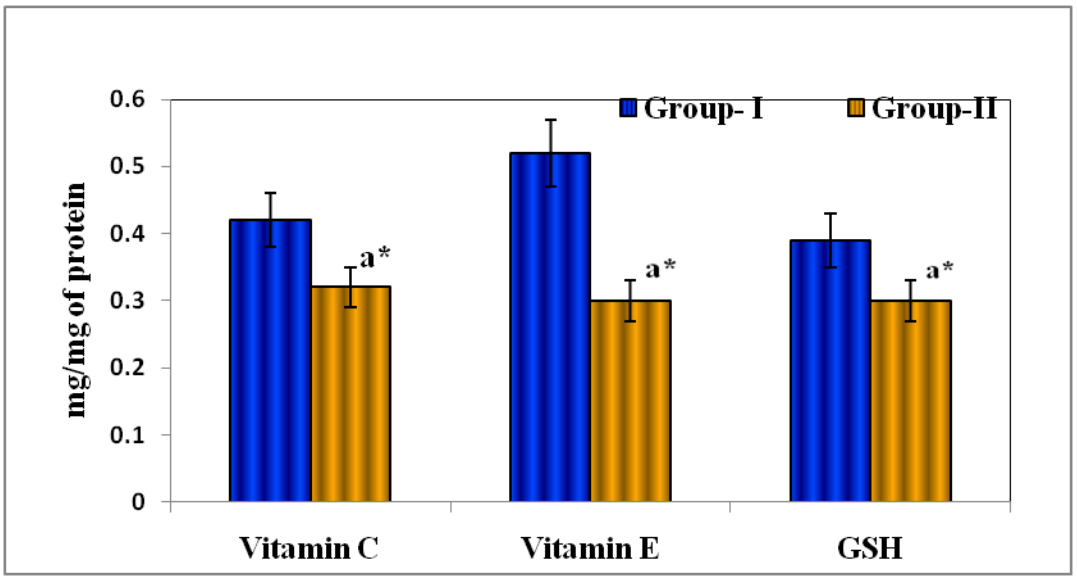

Fig. 2. Levels of Vitamin E and Vitamin C in control and Experimental animals

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

IV. Discussion

The event of free radicals attacking biomembranes, leading to oxidative destruction of the polyunsaturated fatty acid (PUFA) in the membrane is well documented in the process termed as "Lipid Peroxidation" [5]. Changes in the rate of LPO seem to be a general feature of cancerous cells and may be a prerequisite for cell division [6].

There has been a strong association between free radical reactions and a variety of pathological events such as cancer, diabetes, atherosclerosis, aging etc. [7]. In biological systems, the steady state level of LPO is often assessed by the measurement of LPO breakdown products such as Malondialdehyde (MDA). Malondialdehyde (MDA) is one such reliable marker for estimating tissue injury [8].

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) [9]. This kind of DNA damage and peroxidative pathways would result in initiation of cancer through mutagenesis.

Oxygen radicals play an important role in the complex course of multistep carcinogenesis [9], 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[3]. Oxygen radicals that are implicated in the genotoxic agents can initiate and promote cancer development . 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 cervical cancer. 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 [10].

The elevated levels of LPO in cervical cancer patients 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.

ROS are involved in the cell growth, differentiation, progression and death. 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 [11].

SOD may play an important role in protecting cervical cancer cells against ROS. 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. Decreased activity of SOD has been reported in cancerous conditions. GPx scavenges the highly reactive lipid hydro peroxide in the aqueous phase of cell membrane. Catalase the enzyme which catalyses the disproportionation of H_2O_2 and GPx is the first line of defence against oxidative damage[12].

The activities of SOD, CAT and GPx were found to be decreased in cervical cancer patients, 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 H_2O_2 which in turn causes the inhibition of these enzymes. 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 cervical cancerous condition.

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 [13].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.

α-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. The enormous production of lipid peroxides formed may be the reason for the decreased levels of vitamin E in cervical cancerous condition.

In biomembranes, 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. 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 [14].

Glutathione is an important intracellular antioxidant. This tripeptide helps to detoxify free radicals, peroxides and electrophilic compounds of endogenous and exogenous origin. 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 radicals, translocation of amino acids across cell membranes, detoxification of foreign compounds and the biotransformation of the drugs [15]. The elevated level of lipid peroxides and its utilization of GSH may be the reason for the decreased levels of GSH in cancerous condition.

Conclusion

In the present study we conclude that 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.

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