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A Study of Vitamin-D and Biochemical Changes in Breast Cancer

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Abstarct: Breast cancer is the most common lethal cancer in women and the second most common among women worldwide. chemical carcinogen which interacts with membrane lipids, proteins, DNA and consequently induces free radical production. Cancer cell show various degree of differentiation, and there, normally is an inverse relation between the degree of cell differentiation and clinical aggressiveness of a cancer. To evaluate the antioxidants like both enzymatic and non-enzymatic levels in control and breast cancer patients, the Vitamin D in control and Breast cancer patients. Enzymic and nonenzymic antioxidants were examined. The activities of marker enzymes to confirm Breast cancer and the activity of Vitamin D were analyzed**.** The level of Vitamin D was found to be decreased in breast cancer patients. A decrease in the levels of enzymic antioxidants such as SOD, CAT, GPx, GR and nonenzymic antioxidants such as glutathione, vitamin C and vitamin E were observed in breast cancer patients. Carcino embryonic antigen is a marker of breast cancer were elevated in breast cancer patients.

.**Keywords**: Antioxidant, vitamin, glutathione and carcinogen.

INTRODUCTION

Cancer is a group of more than hundred diseases that develop across time characterized by varying degree of morphological disorientation, reduced control over growth and function through invasive growth and metastasis. This uncontrolled division can compromise the function of the host and ultimately may cause death. The description of cancer as a hundred diseases arises from the observation that cancer can appear as a result of different causes which can be hereditary or environmental factors in a variety of sites within the body and that each type of cancer displays its own growth rate, prognosis and treatability.

An essential feature of malignancy is that the malignant phenotype is passed on from the parent to the daughter cell. This suggests that the fundamental lesion of malignancy resides in the DNA. Cancer is caused by multiple irreversible mutations in genes critical for cell growth (Evan and Vousden 2001). Since the DNA repair network plays a key role in faithful maintenance of the genome, inherited or acquired abrogation of its function is detrimental and potentiates increased genome instability leading to cancer (Schar, 2001). Although mammalian cells are richly endowed with both efficient DNA repair and free radical scavenging system it is probable that some DNA lesion generated via intrinsic and extrinsic mutagenesis would escape these DNA "surveillance" processes and constitute an early step in human carcinogenesis (Ahuja and Rajeshwari, 2003).

Breast cancer is a malignant tumor, which is a group of cancerous cells that grow into other areas around it. Breast cancer starts in the cells of the breast, but can spread to other areas of the body, which is called metastasizing. Tumors are made when there is a buildup of unneeded, old, or damaged cells and they form a mass, which then becomes a tumor. The most common type of breast cancer starts in cells in the breast ducts. Breast ducts are the tubes that carry breast milk.

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2. MATERIAL AND METHODS

SETTING:

The study was conducted during the period Jan 2020 to March 2020 in the Department of Biochemistry, University of Madras, Guindy Campus, Chennai, Tamil Nadu and Department of Biochemistry, Bharath Institute of Higher Education and Research, Selaiyur, Chennai, Tamil Nadu Source of Sample and Data: Department of Biochemistry and Pathology, Bharath Institute of Higher Education and Research.

Number of study groups : 2

Sample size : 50

Study design : Biochemical and molecular changes in Breast cancer

ETHICAL CONCERN

Ethical clearance was obtained from Institutional Ethical Committee conducted at the Bharath Institute of Higher Education and Research.

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.

EXPERIMENTAL DESIGN

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

Group I : Control (Normal Persons).

Group II : Breast cancer patients.

BIOCHEMICAL PARAMETERS

Serum 25 OH vitamin D, marker enzymes and enzymic and non enzymic Antioxidants were the biochemical parameters estimated in the standard methods.

COLLECTION OF BLOOD SAMPLE

The blood samples of the respondents were collected after an overnight fast, 1ml in sodium fluoride coated sugar tubes and 3ml in plain tubes between 8 am to 9 am. 1 ml of blood for postprandial blood sugar was collected 2 hrs after breakfast. The blood drawn was allowed to coagulate and the serum was separated by centrifuging and stored at -20°C until assayed.

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MEASUREMENT OF SERUM 25 OH VITAMIN D

Method : 25(OH)D was measured by immunodiagnostic direct Elisa kit method

Quantification of carcinoembryonic antigen (CEA)

Quantitative estimation of CEA was based on the solid phase enzyme linked immunosorbent assay. The values were expressed as ng/ml serum.

Estimation of Protein

Protein was estimated by the method of Lowry *et al.* (1951).

Assay of superoxide dismutase (SOD): The activity of superoxide dismutase was determined by the method of Marklund and Marklund (1974).

Assay of catalase

The activity of catalase was assayed by the method of Sinha (1972).

Assay of glutathione peroxidase (GPx)

The activity of glutathione peroxidase was assayed by the method of Rotruck *et al.* (1973).

Assay of glutathione reductase (GR)

The activity of glutathione reductase was measured by the method of Staal *et al.* (1969).

Estimation of reduced glutathione

The level of reduced glutathione was measured by the method of Moron *et al.* (1979).

The level of glutathione was expressed as μg/mg protein.

Estimation of ascorbic acid

The level of ascorbic acid was estimated by the method of Omaye *et al.* (1979).

Estimation of vitamin E

The level of vitamin E was estimated by the method of Desai (1984).

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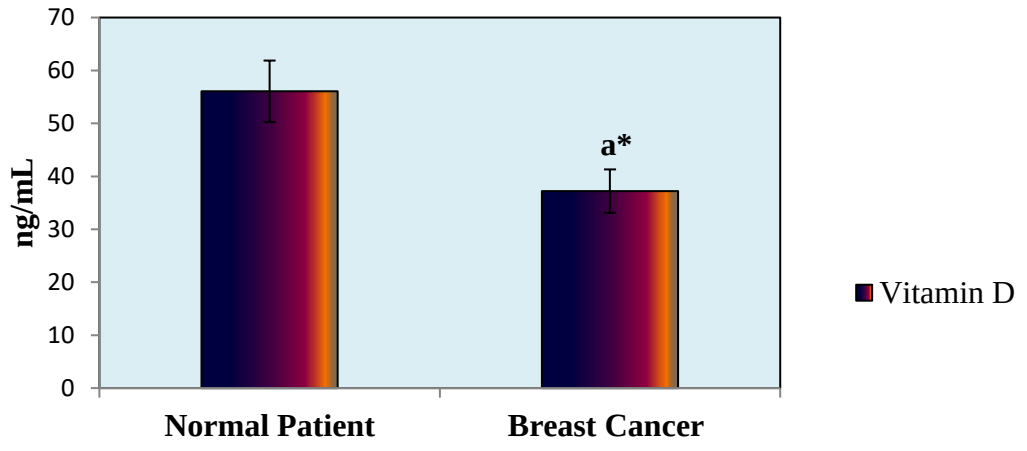
4. RESULTS

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

Fig. 1. Study of Vitamin D



Each value is expressed as mean ± SD for Twenty five patients in each group
a: as compared with Group I
Statistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

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 populations in south Asian countries are limited. The current study was conducted to assess the vitamin D status of 50 Tamil women of breast 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 breast cancer. Women who have breast cancer tend to have low levels of vitamin D in their body. Rose, et al 2013 have found how vitamin D might have a role in breast 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 breast 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 breast cancer, by making cells in the breast smarter.

Mammographic (breast) density reflects the epithelial and stromal components of the breast; fat appears dark and epithelium and stroma appear light or white. Women with very dense breast tissue, as seen on

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mammography, are at increased risk of subsequent breast cancer. Mammographic density can be modified, and such changes represent a potential biological marker for assessing the effects of dietary/supplemental factors on breast cancer risk.

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 breast (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 breast tissue and thereby increase risk of breast cancer.

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 Breast 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 (Breast Cancer Patients)**
SOD	4.71 ± 0.50	3.12 ± 0.3 ^{a*}
CAT	253 ± 25.1	122± 12.5 ^{a*}
Gpx	40.1 ± 4.1	20.2 ± 2.5 ^{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 oxidized/min/mg protein, a: as compared with Group I, Statistical significance: * $p<0.001$ $^{@}$ $p<0.01$ $^{\#}$ $p<0.05$, NS- Not significant

The event of free radicals attacking bio membranes, 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 cancerous cells and may be a prerequisite for cell division. There has been a strong association between free radical reactions and a variety of pathological events such as cancer, diabetes, atherosclerosis, aging etc. 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 (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 Deoxyribo nucleic acid (DNA). This kind of DNA damage and peroxidative pathways would result in initiation of cancer through mutagenesis (Dreher and Junod 1996).

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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. 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 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.

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. ROS are involved in the cell growth, differentiation, progression and death. They play a major role in cancer initiation and promotion. The antioxidant defense enzymes namely SOD, GPx and CAT protect aerobic cells against oxygen toxicity and lipid peroxidation. SOD may play an important role in protecting 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 (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 H_2O_2 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 H_2O_2 which in turn causes the inhibition of these enzymes. This may be due to the altered antioxidant defence system because of enormous production of free radical in the B(a)p induced carcinogenesis. This decreased activity of enzymic antioxidants well correlates with the progression of the malignancy, indicating the impairment of free radicals and the weakened antioxidant deface system in cancerous condition.

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 lung cancer bearing (Group II) patients when compared to control (Group I) patients.

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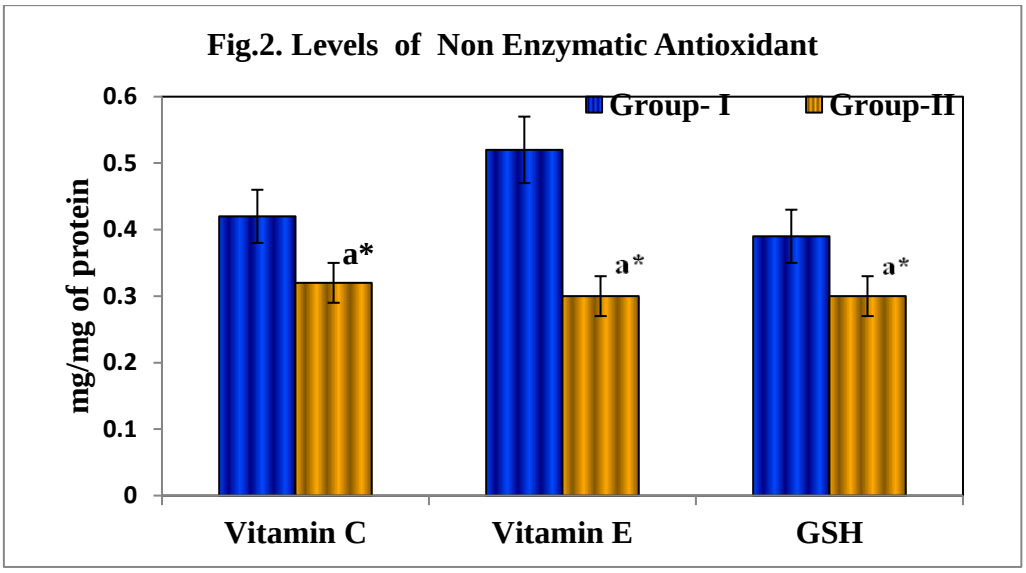

Each value is expressed as mean ± SD for Thirty patients in each group. Units: Units/min/mg protein. a: as compared with Group I, Statistical significance: * $p<0.001$ @ $p<0.01$ # $p<0.05$, NS- Not significant

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 lung cancer. The ascorbate molecule must be involved in the feedback inhibition of lysosomalglycosidases responsible for malignant invasiveness. 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 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 lung cancer patients when compared with their control subjects. The assessment of vitamin E provides further useful information in evaluation of cancerous condition. 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).

Marker Enzymes:

Fig.3. represents the level of CarcinoEmbronic Antigen (CEA) in control and experimental animals, was found to be significantly ($p < 0.001$) decreased in lung cancer bearing (Group II) patients when compared to control (Group I) patients.

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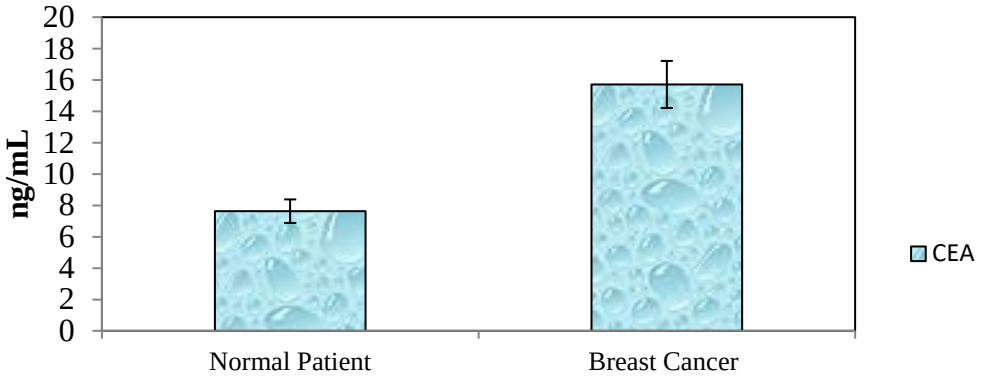
Fig.3. CEA



Each value is expressed as mean ± SD for Twenty five patients in each group
a: as compared with Group I
Statistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

Carcino embryonic antigen (CEA) is a cell surface glycoprotein expressed in fetal tissues and is transcriptionally silent in adults but is frequently over expressed in human carcinomas (Huang *et al.,* 1990). The elevated levels of CEA observed in lung, breast and other cancers suggest that CEA could play an important role in cancer progression and embryogenesis. Tumor CEA and normal CEA do not appear to have any differences at the genetic level. CEA is a reliable marker that is secreted by breast cancer cells into the serum in high levels. It is reported higher levels of CEA in breast cancer conditions and also in breast cancer measurement of CEA has appeared promising in assessing the progression of the disease and to the therapeutic response. The progression of the tumor growth directly depends on the increased levels of CEA. Hence breast cancer patients exhibited a significant incline in the levels of CEA.

CONCLUSION

From the above test results obtained in terms of vitamin D and biochemical studies, the present study proves that the early identification and marker of breast cancer to start treatment with either chemotherapy or radiotherapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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