BLOOD BIOCHEMICAL PARAMETERS IN CARCINOMA BREAST

Dissertation submitted to

THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY

in partial fulfillment of the regulations
for the award of the degree of

M.D. BIOCHEMISTRY BRANCH - XIII

DEGREE EXAMINATION

THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY

CHENNAI - 600 032, TAMIL NADU

MARCH - 2008

CERTIFICATE

This is to certify that the dissertation titled " **BLOOD BIOCHEMICAL PARAMETERS IN CARCINOMA BREAST**" is the bonafide original work of **Dr. G.BHUVANESWARI,** in partial fulfillment of the requirements for M.D., (Biochemistry), Branch - XIII, Examination of the Tamil Nadu Dr.M.G.R. Medical University to be held in March 2008.

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Acknowledgement

The author finds it a pleasure to offer her special thanks to Dr. A.Renuka M.D.,Ph.D., D.N.B., Director & Professor, Institute of Biochemistry, Madras Medical College, for her patience, sense of perfection and an indepth understanding of the subject, who with her experience and expertise guided the author throughout the study and without whose help, this work could not have been brought into a good shape.

The author is grateful to **Dr. A. Manamalli M.D.,** Former director for her constant encouragement and timely guidance.

The author expresses her sincere thanks to Dr. Pragna B. Dolia M.D., Additional Professor, Institute of Biochemistry, Madras Medical College, who with her keen observation and expert guidance always pointed out the author's mistakes and constantly encouraged her during the study.

The author is very much indebted to **Dr. N. Durairaj**, M.S., Professor and Director Institute of Surgery Government General Hospital, Chennai, who constantly guided her during the course of the study.

The author wishes to express her gratitude to Dr. Ramadevi M.D., Additional Professor, Institute of Biochemistry, Madras Medical College for her encouragement and guidance during the study.

The author expresses her gratitude to Dr. G. Chandrasekhar, Additional Professor, Dr. M. Shyamraj, Dr. V.K. Ramadesikan, Dr. Sumathi, Dr. Sivasubramaniyan Assistant Professors, Institute of Biochemistry, Madras Medical College, Chennai, who constantly encouraged and guided her to adopt the right approach for this study.

The author is very thankful to all her colleagues and other staff of the Biochemistry department who were of immense help during every part of this study.

The author owes a special thanks to Mrs. Basilia, statistician, for her help in doing the statistical analysis for the study.

The author is indebted to those patients and persons from whom blood samples were collected for conducting the study.

Special Acknowledgement

The author gratefully acknowledges and sincerely thanks Professor, Dr.T.P.Kalanithi M.D., Dean, Madras Medical College, Government General Hospital, Chennai-600003, for granting permission to utilize the facilities of this institute for the study.

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A STUDY ON BLOOD BIOCHEMICAL MARKERS IN CARCINOMA BREAST

INTRODUCTION

Tumors or ulcers of breast have been reported as early as 1600 BC as reported in ancient Egyptian chronicles, the Papyrus. Currently carcinoma breast is the most common malignancy and the most common cause of cancer deaths in women worldwide. In India, carcinoma breast is the second most common malignancy in women.

Mammography, ultrasonography are being used for screening of breast cancer while tissue diagnosis is the gold standard for diagnosis of breast cancer. Cancer that is detected early can potentially be cured when the tumor is small enough to be completely removed surgically. Unfortunately, most cancers do not produce any symptoms until the tumors are either too large to be removed surgically or cancerous cells have metastasized. Hence there is a need to detect cancer at an early stage.

Tumor markers are used for the detection of risk, population screening, diagnosis, staging and prognosis. It can also predict the response to therapy, monitor treatment, detect the presence of occult metastatic disease, and monitor the course of the disease. Several tumor markers are in use nowadays which include alpha fetoprotein, carcinoembryonic antigen, human chorionic gonadotropin, prostate specific antigen, and relatively less specific markers like lactate dehydrogenase, alkaline phosphatase, ferritin, and gamma glutamyl transpeptidase. However the analytical method of many of these are unapproachable for general population as the facilities for these are available only at

sophisticated and well equipped centers with latest technology and are expensive. There is therefore a need for simple biochemical investigation which can be assayed, are less expensive and can detect metastasis.

This study was made to measure the levels of enzymes Lactate dehydrogenase (LDH), Gamma glutamyl transpeptidase (GGT), and superoxide dismutase (SOD) in patients with newly diagnosed carcinoma breast and study if there is any clinical correlation of the levels of these enzymes with the presence of early untreated breast carcinoma.

REVIEW OF LITERATURE

Epidemiology of breast cancer:

Breast cancer is the most common malignant neoplasm in women worldwide. This situation is also true in the United States. Based on data from National cancer institute's surveillance epidemiology and end results (SEER) program, 30% of all incident cancers among women are breast cancers, the most frequently diagnosed cancer.

International Geographic variation:

Globally, breast cancer is the leading cause of death among women, accounting for more than 3,00,000 deaths in 1990, of which 1, 74,000 occurred in developed countries and 1, 39, 500 occurred in developing countries. From the mid 1970s to the mid 1980s, mortality rates did not change greatly in many of the countries with high rates, whereas incidence occurred in many of the countries with low rates, resulting in a narrowing of international differences. Geographic variation was apparent with many countries but within country differences were considerably smaller than among countries. Rates in urban areas generally exceeded those in neighboring areas.

RISK FACTORS FOR BREAST CANCER:

Age: The most common breast cancer risk factor is age. Half of a woman's lifetime risk factor for breast cancer development occurs after age 65. Between ages 35 to 55, the risk for breast cancer development is only 2.5 %, and the risk for breast cancer death is only about one third of that.

Family History:

A family history of breast cancer in a first-degree relative is associated with approximately a doubling of risk. If both mother and sister have had breast cancer, the risk is even higher. Approximately 20-30% of women with breast cancer have a family history of the disease, but only 5-10 % have an inherited mutation in a breast cancer susceptibility gene. There are two distinct types of risk associated with a family history of breast cancer. Distinguishing between familial and true hereditary breast cancer is important because the associated level of risk varies widely. The majority of cases of genetic breast cancer are caused by mutations of BRCA-1 and BRCA-2 genes. Genetic testing for mutations of BRCA-1 and BRCA-2 is now a commercially available option for women with a family history suggestive of genetic mutation. If the affected person does not carry a genetic mutation, testing an unaffected relative is unlikely to be informative. For the majority of women with a family history of breast cancer that is not associated with an inherited mutation, the level of risk is much lower and rarely exceeds 30%.

Demographic factors:

Breast cancer is generally recognized as a disease that occurs more often among women of the upper social classes, as measured by either educational status or family income. Studies seem to indicate that these associations largely reflect the effect of correlated life-style factors such as late motherhood. Unmarried women over the age forty have been found to have a higher risk of breast cancer than women who have been marries, an

association attributed to a reduced risk associated with child bearing. Similarly, nuns have been found to have a higher than average risk for breast cancer.

Reproductive risk factors:

A late age at first birth is an important determinant of breast cancer risk. This was perhaps best demonstrated in Mc Mohan and colleagues' international study of breast cancer, in which women with a first birth after thirty years were shown to have approximately twice the risk of those with a first birth before age eighteen. Because nulliparous women have a risk similar to that of women with a first birth around 30 years, it is as hazardous to delay a first birth until after 30 years as to remain childless, in terms of risk of breast cancer.

Menstrual factors:

Numerous studies have shown that women with early onset menarche are at an increased risk of breast cancer, with those who begin menstruating before twelve years of age having approximately a 50% higher risk than those with menarche at age 15 years or later. Women who have an early age at menarche have an earlier onset of regular menstrual periods, however, whether menstrual irregularities have an independent influence on breast cancer risk remains unresolved. Women with late ages at menopause have been shown to be at an increased risk of breast cancer; the relative risk is approximately two for natural menopause after 55 years of age, compared with menopause before 45 years of age. Early menopause resulting from ovarian ablation is similarly associated with a reduction in risk. For instance, oophorectomy before age 40

years of associated with approximately 50% reduction in risk, compared with natural menopause at 50 years of age, the average age at menopause in the United States.

Exogenous hormones:

Given the recognized importance of ovarian hormones in the etiology of breast cancer, much attention has been focused on the relationship to risk of exogenous hormone use including oral contraceptive pills and post-menopausal hormone replacement therapy. Oral contraceptives have been extensively studied in relation to breast cancer risk, with varying conclusions. Although the majority of studies have not confirmed an overall excess risk associated with oral contraceptive use, a number of studies (including several meta-analyses) have suggested and increased risk association with long term use for early onset cancers, usually defined as cancers occurring prior to 45 years of age.

The relationship of hormone replacement therapy to breast cancer risk was recently assessed in a reanalysis of data from 51 epidemiological studies, encompassing 52,705 women with breast cancer and 1,08,411 controls from 21 countries. This showed a 2.3% (95%, CI- 1.1-3.6) increase in the relative risk of breast cancer for each year of hormone replacement therapy. This corresponded to a relative risk of 1.35 for users of 5 or more years and to a cumulative excess for women who began use of hormones at age 50 of approximately 2 cases per 1000 women for 5 year users, 6 cases per thousand for 10 year users, and 10 cases per 1000 for 15 year users. This increase was comparable with the effect on breast cancer risk of later menopause. The increase was restricted to recent users, with no material excess observed 5 or more years after discontinuation.

Selective Estrogen receptor modulators:

Given the recognized adverse effect of hormone replacement therapy, much recent attention has focussed on Selective Estrogen Receptor Modulators (SERMS), which function as estrogen agonists in some tissues (bone and endometrium) and estrogen antagonists in some (breast). These agents probably offer many of the same advantages as HRT, while eliminating some of the disadvantages (no increase in breast cancer risk). Data indicate that these agents offer substantial advantages in terms of reducing breast cancer size, with the most convincing data derived from the National Surgical Adjuvant Breast and Bowel Project (NSABP). This trial, focused on women at an increased risk of breast cancer found after 69 months of follow-up that those who had received tamoxifen had a 49% reduced risk of invasive breast cancer than placebo-treated women. The beneficial effect pertained to women of all ages,, but was more apparent among women with a history of lobular carcinoma in situ or atypical hyperplasia; in addition, the risk reduction was limited to estrogen receptor positive tumors. Two other trials, one in Britain and the other in Italy, however did not find an effect of tamoxifen on breast cancer risk. This may have reflected limited sample sizes, high drop-out rates or use of other drugs (including HRT) among trial participants.

Diethyl stilbesterol:

Further support for the role of exogenous hormones in the etiology of breast cancer derives from studies of women exposed to diethyl stilbesterol (DES), a drug used between 1938 and 1971 for the treatment of threatened, spontaneous abortions. Nearly all follow-up studies of the mothers have found an increased risk of subsequent breast

cancer, on the order of 30-40%. Dissimilar to other exogenous hormones, the increased risk is not related to how recent the use was and excesses are not observed until 10 or more years after exposure. Although the daughters who were exposed in-utero to DES are at increased risk of vaginal adenocarcinoma, so far they do not appear to be at an increased risk of breast cancer.

Medical history:

Although most studies indicate that women with a history of biopsy-proven benign breast disease are at an increased risk of subsequent breast cancer, the interpretation of the association is complex. The association appears dependant not only on the indications for biopsy, but also on the histological characteristics of the lesions. One study suggested that only proliferative forms of benign breast disease predisposed to subsequent breast cancer risk, with atypical hyperplastic lesions being most predictive. The specific types of benign breast disease associated with the highest risk of subsequent breast cancer, however, have varied across studies, possibly because of difficulties in standard classification of these lesions. A further examination of effects is needed, as well as evaluation of factors that might promote the progression of benign lesions to subsequent cancer.

The appearance of breast mammographically has also been found to be a predictor of subsequent breast cancer risk. An initially proposed parenchymal pattern classification system took into account the amount of breast tissue composed of ductal prominence. More recently, direct measurements of dense areas of the breast have been found to be less subjective and stronger indicators of risk. In one study, breasts with areas of density

of 75% or more were associated with a nearly five fold elevation in risk, a magnitude of risk as great if not greater than most other established risk factors.

It is well recognized that women with fractures or low bone densities, are at a decreased risk of breast cancer, with some evidence that this may reflect their low levels of endogenous hormones.

Dietary factors:

The relationship of dietary factors to breast cancer risk has been extensively studied, with few consistent results emerging. There has been an extensive focus on effects of consumption of dietary fat, stimulated initially by findings that per capita fat intake correlates internationally with breast cancer mortality rates. Numerous epidemiological studies have attempted to confirm this on an individual basis, with most failing to find an association. One meta-analysis of data from case-control studies found that a 100 gram increase in daily total fat intake was associated with a 35% increase in risk. However, results from prospective studies, which are less subject to recall biases, provide no evidence of any such relationship.

In addition to overall fat intake, research has focused on specific types of fat. Several studies have suggested a possible protective effect of olive oil, a mono unsaturated fat, but further studies are needed to confirm the relationship. There has also been interest in a possible protective effect for omega-3 fatty acids (derived from fish), although no definitive results have been obtained.

A variety of other dietary constituents have been hypothesized to affect breast cancer risk. Diets high in fiber have been suggested as protective against breast cancer, possibly

due to inhibition of intestinal re-absorption of estrogens excreted via the biliary system. Whether micronutrients could play a role in breast cancer etiology has also been of interest, especially antioxidants that may provide a cellular defense against reactive oxygen species that damage DNA. Vitamin-A which is also a regulator of cellular differentiation, appears, from both case-control and cohort studies, to be modestly inversely associated with breast cancer risk. In a meta-analysis of case-control studies, a significant protective effect of vitamin-A intake was observed, with stronger relationships apparent for carotenoid vitamin-A (mainly derived from fruits and vegetables) than preformed vitamin-A (retinol, retinyl esters and related compounds from animal sources). Vitamin-C and E have also been examined in relationship to breast cancer risk. Vitamin-C has been of interest not only because it is an anti-oxidant but also because it cab block the formation of carcinogenic nitrosamines. Data from case-control studies provide some evidence for a possible protective effect on breast cancer risk; however, cohort studies show no association.

Selenium, an important component of anti-oxidant enzyme glutathione peroxidase that inhibits cell proliferation has been shown in animal studies to protect against a variety of cancers, including mammary cancers.

Anthropometric factors:

The relationship of body size to breast cancer risk has been extensively investigated, with differing relationships have been observed for pre menopausal and post menopausal diseases. For post-menopausal onset, both weight and body mass index (defined as weight in kilograms divided by the square of height in meters) have been fairly

consistently related to increases in risk. In contrast to relationships with post menopausal breast cancer, body mass appears to be inversely related to pre-menopausal disease, with thin women being at highest risk. Among post menopausal women, body fat distribution also appears to be a factor influencing risk. In a number of studies, women whose fat was distributed abdominally were found to be at a higher risk than those with peripheral fat distribution.

Physical activity:

There has been much recent enthusiasm regarding a potential beneficial effect of physical activity on breast cancer risk, especially given its modifiable nature. The relationship appears to be biologically plausible, given that physical activity has been associated with changes in endogenous hormones, menstrual patterns, body fat distribution patterns and other biological repercussions which could benefit breast cancer risk (change in immunologic parameters, for example). The strongest support for physical activity as a potential preventive mechanism derived from a study of early onset breast cancers in which reductions in risk associated with regular physical activity were found to be independent of the body size. Additional studies, however, have produced conflicting results.

Cigarette smoking:

Although cigarette smoking has been found to result in earlier age at menopause, it has not been generally found to alter breast cancer risk. However, investigators continue to be interested in the effects of smoking at younger ages, hypothesized as a possible etiologic

factor. It has recently been proposed that effects of cigarette smoking might have been missed because of the inclusion of women exposed to passive smoking in the control group of most studies.

Alcohol consumption:

Although the relationship of breast cancer risk to most dietary factors remains unresolved, fairly consistent data have emerged regarding a potential adverse effect of consumption of alcoholic beverages. Longnecker in a meta analysis of 38 case control and cohort studies, showed a progressive increase in the risk of breast cancer with amount of alcohol consumed, with those consuming three or more drinks per day being at a 40% higher risk than non drinkers. Results were consistent across case control and cohort studies. Adjustment for known breast cancer risk factors and dietary variables had little impact on observed relationships.

Prenatal exposures:

Recent interest has focused on the role of variety of prenatal exposures on the role of a variety of prenatal exposures on subsequent breast cancer risk. A number of studies provide support for an increased breast cancer risk among dizygotic twins and a decreased risk for daughters born after a pre eclamptic pregnancy. These birth characteristics have been hypothesized to reflect effect of prenatal estrogenic exposures. There is some evidence for an increase in breast cancer risk among subjects with high birthweights. A few studies suggest an increased breast cancer risk among daughters born of mothers of advanced age although data are not conclusive. A number of reports show a

decrease in breast cancer risk for daughters who were breast fed. Whether this association reflects a protective effect of breast feeding or an adverse effect of supplements has yet to be determined.

Ionizing radiation:

From studies of women exposed to the atomic bombs in Japan and from observations of women exposed to medical treatments involving repeated exposure to radiation it is well established that ionizing radiation to the chest in moderate to high doses (eg. between 1 and 3 Gy.) before the age of 40 years increases breast cancer risk, and the higher the dose, the greater the risk. High rates of breast cancer have also been observed following radiotherapy for Hodgkin disease. Further, second breast cancers have been linked to radiotherapy for primary breast cancer, only among women under the age 45 years at exposure.

Environmental exposures:

Recent attention has focused on the potential impact of environmental factors on breast cancer risk, with much of interest stemming from recognition that breast cancer mortality rates are high in the industrialized regions. Of particular interest had been the relation to risk of organochloride pesticides, notably DDT and polychlorinated biphenyls which have been demonstrated to induce cytochrome P_{450} enzymes and to affect steroid metabolism.

Multiple primary cancers:

Cancer in one breast is associated with a relative risk of 2-4 for developing a second cancer in the contralateral breast, particularly in women with a family history of breast cancer. Women with breast cancer also experience some increase in the risk of second cancers of the endometrium and ovary, and also possibly of melanoma and of colon, salivary gland and thyroid cancers.

PATHOGENESIS:

Knowledge about the pathogenesis of breast cancer and the interplay between genetic changes and environmental factors is growing rapidly. This significant growth in understanding is based on cancer research at the cellular and molecular levels and work on the Human Genome Project, and is expected to contribute to improved risk assessment, screening and early detection, treatment, and prevention of recurrence.

Cancer can be described as the uncontrolled growth of abnormal cells. Usually cell division is controlled by a network of signals that: promote cell division – oncogenes; slow or stop cell division at the right time - tumor suppressor genes; and repair DNA damage. DNA damage can include gene amplifications, gene deletions, point mutations, loss of heterozygosity, chromosomal re-arrangements, and an abnormal number of chromosomes (NCBI, 2005, and Dickson et al, 2000).

Cancer development can be triggered by mutations of the signals in the network that controls cell division, and can be associated with genetic predisposition exposure to some environmental factor, or both. The most important and best established genes involved in this process are the BRCA-1 gene and the p53 gene found on chromosome 17 and the

BRCA-2 gene found on chromosome 13. For men, the androgen receptor gene has been implicated as a causative factor. Using the techniques of molecular genetics, it has been found that other genes may also be involved in some patients. Some of these genes are altered by mutation, others by amplification or rearrangement, and still others by loss or deletion.

BRCA-1:

The breast cancer susceptibility gene BRCA-1 is located in the region of chromosome 17q21, known as D17S855. It is an autosomal dominant gene that codes for a suppressor protein of 1863 amino acids. This protein contains a zinc finger domain, but it is otherwise distinct from other known proteins. Its precise physiologic function is currently unknown, but it appears to act as a tumor suppressor gene. A number of mutations in this gene have been identified in patients with familial breast and ovarian cancer. The pattern of mutation suggests that BRCA-1 is of etiologic significance in patients with familial breast and ovarian cancer, but its actual role is unknown. Data from the breast cancer linkage consortium predicted the inheritance of this gene conferred a 63% risk of ovarian cancer by age 70, and an 85% risk of breast cancer by age 80.

BRCA-2:

Shortly after the BRCA-1 was isolated, the BRCA-2 gene was described. This breast cancer susceptibility gene is located in chromosome 13q12, and similar to BRCA-1, appears to function as a tumor suppressor gene. Initial data based on high risk families reported the risk of breast cancer in women with BRCA-2 to be 87% by age 80. The

initial risk estimates however for BRCA-1 and BRCA-2 may have been overestimates and not applicable to all patient subsets with either of these genetic mutations.

p53:

This is a tumor suppressor gene with putative roles in DNA replication, transcription and cell cycle control. It inhibits transformation of cells by myc and ras, both of which are well established oncogenes. Somatic alterations of p53 may occur by either deletions or point mutations. This alteration leads to an abnormal accumulation of complexes of abnormal p53 with wild type p53 in the cytoplasm. This abnormal accumulation prevents p53 from entering the nucleus and regulating transcription. These effects are termed dominant negative, and explain why only one allele of p53 needs to be altered instead of the traditional two alleles for a classic tumor suppressor mechanism. Certain familial syndromes also involve this gene, especially the Li-Fraumeni syndrome, in which mutations in p53 are inherited.

Androgen receptor:

Androgen receptor is normally controlled by a gene on the X chromosome. Though it has been implicated in the causation of breast cancer in males, whether or not this gene could be a cause of female breast cancer is not known yet.

Her2/neu: It is a member of class-I growth factor receptor tyrosine kynase family. It has been implicated in the causation of ductal carcinoma in situ – DCIS, and invasive ductal carcinoma. The genetic abnormality is not inherited but acquired, and is thought to confer aggressive biology and refractiveness to chemotherapy.

Breast cancer biology:

Oncogenes and growth factors: Abnormalities of oncogene expression probably influence the cancer cell through specific growth factors or growth factor receptors. This influence can occur either by autocrine mechanisms involving only the cancer cell or through a paracrine mechanism involving adjacent stromal cells. In addition to estrogen and progesterone, numerous growth factors influence the growth of the breast cancer cell. This influence is illustrated by evidence implicating the in vivo amplification and rearrangement of the C-myc93 and C-erbB2 oncogenes in breast cancer. Breast cancer also may be associated with mutations in the H-ras1 mini-satellite locus, which is just downstream from the proto-oncogene H-ras1. Other examples of important growth factors (which have specific receptors on the breast cancer cell membrane) include transforming growth factor α and transforming growth factor β, insulin like growth factors- I and II, epidermal growth factor, somatostatin receptors and retinoic acid receptors.

Estrogen and progesterone receptors:

The demonstration of hormone responsiveness in mammary carcinoma lead to the important principle that neoplasms retain some of the differentiated functions of the tissue of origin. Endocrine organ ablation and hormonal manipulation have been widely used in the treatment of metastatic breast cancer. Steroid hormone action depends on specific binding to high affinity intracellular receptors that have great specificity for the hormone. Binding to defective hormone receptors may prevent a response to hormonal therapy. Quantifying the expression of receptors for estrogen and progesterone is now a

standard practice for all patients with primary breast cancer. Receptor measurements can be performed by immunohistochemistry or one of the several biochemical methods.

Biochemical parameters in breast cancer :

Tumour markers are biochemical indicators of the presence of tumour. Various tumour markers have been studied singly or in combination, in breast malignancies. In this study, the level of three biochemical parameters namely SOD, LDH and GGT were assessed and analyzed for possible correlation to breast cancer.

FREE RADICALS

The occurrence of reactive oxygen species known as pro-oxidants is an attribute of normal aerobic life. The steady state formation of pro-oxidants is balanced by a similar rate of their consumption by anti-oxidants that are enzymatic and/or non-enzymatic. Oxidative stress is associated with a disturbance in the pro-oxidant anti-oxidant balance in favor of pro-oxidant.

A free radical can be defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. It can also be considered as a fragment of a molecule.

As such free radicals can be formed in three ways:

1) By the homolytic cleavage of a covalent bond of a normal molecule with each fragment retaining one of the paired electrons.
2) By the loss of a single electron from a normal molecule
3) By the addition of a single electron to a normal molecule.

The latter, electron transfer, is a far more common process in the biological systems than the homolytic fission, which generally requires high energy input from either high temperatures, UV light or ionizing radiation.

Heterolytic fission in which the electrons of the covalent bond are retained by only one of the fragments of the parent molecule does not result in free radicals but in ions which are charged. Free radicals can be positively charged, negatively charge, or electrically neutral. The unpaired electron and the radical nature of the species are conventionally indicated by writing it with a heavy superscript dot.

The processes by which free radicals and ions are formed are illustrated below.

Radical formation by electron transfer :

$$A + e^- \rightarrow A^{-\bullet}$$

Radical formation by homolytic fission:

$$X : Y \rightarrow X^{\bullet} + Y^{\bullet}$$

Ion formation by heterolytic fission :

$$X : Y \rightarrow X :^{-} + Y^{+}$$

Characteristics of Reactive Oxygen Species :

ROS are oxygen containing compounds that are highly reactive free radicals, or compounds readily converted to these oxygen free radicals in the cell. The major oxygen metabolites produced by one electron reduction of oxygen (superoxide, hydrogen peroxide and hydroxyl radical) are classified as ROS.

Reactive free radicals extract electrons (usually as hydrogen atoms) from other compounds to complete their own orbitals thereby initiating free radical chain reactions. The hydroxyl radical is probably the most potent of ROS. It initiates chain reactions that form lipid peroxides and organic radicals and adds directly to compounds. The superoxide anion is also highly reactive but has limited lipid solubility and cannot diffuse far. However, it can generate the more reactive hydroxyl and hydroperoxy radicals by reacting non-enzymatically with hydrogen peroxide in the Haber-Weiss reaction.

$$O2^{-} + H_2O_2 \xrightarrow{H+} O2 + H2O + {}^{\bullet}OH$$

H_2O_2, although not actually a radical, is a week oxidizing agent that is classified as an ROS because it can generate the hydroxyl radical. Transition metals such as Fe^{2+} or Cu^{+} catalyze formation of the hydroxyl radical from hydrogen peroxide in the non-enzymatic Fenton reaction.

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\bullet}OH + OH^{-} + Fe^{3+}$$

Since H_2O_2 is lipid soluble, it can diffuse through membranes and generate hydroxyl radical at localized Fe^{2+} or Cu^{+} containing sites such as mitochondria. H_2O_2 is also the precursor of hypochlorous acid (HOCl), a powerful oxidizing agent that is produced endogenously and enzymatically by phagocytic cells.

Organic radicals are generated when superoxide or the hydroxyl radical indiscriminately extract electrons from other molecules. Organic peroxy radicals are intermediates of chain reactions such as lipid peroxidation. An additional group of oxygen containing radicals termed RNOS- (reactive nitrogen oxygen species) contain nitrogen as well as oxygen. These are derived principally from the free radical nitric oxide (NO) which is produced endogenously by the enzyme nitric oxide synthase. Nitric oxide combines with oxygen or superoxide to produce additional RNOS.

Major sources of primary ROS in the cell:

ROS are constantly being formed in the cell. Approximately 3-5% of oxygen we consume is converted to oxygen free radicals. Some are produced as accidental by-products of normal enzymatic reactions that escape from the active site of metal containing enzymes during oxidation reactions. Others such as H_2O_2 are physiologic products of oxidases in peroxisomes. Deliberate production of toxic free radicals occurs in the inflammatory response. Drugs, natural radiation, air pollutants and other chemicals also can increase the formation of free radicals in cells.

1. **Co-Q**

One of the major sites of superoxide generation is Coenzyme-Q in the mitochondrial electron transport chain. The one electron reduced form of Co-Q (Co-$QH^{\bullet}$) is free within the membrane and can accidentally transfer an electron to dissolved oxygen thereby forming superoxide.

2. **Oxidases, oxygenases and peroxidases**

Most of the oxidases, peroxidases and oxygenases in the cell bind oxygen and transfer single electrons to it via a metal. Free radical intermediates of these reactions may be accidentally released before the reduction is complete.

Cytochrome P_{450} enzymes are a major source of free radicals leaked from reactions. Induction of P_{450} enzymes by alcohol, drugs or chemical toxicants leads to increased cellular injury. When substrates for cytochrome P_{450} enzymes are not present, its potential for destructive damage is diminished by repression of gene transcription. Hydrogen peroxide and lipid peroxides are generated enzymatically as major reaction products by a number of oxidases present in peroxisomes, mitochondria and endoplasmic reticulum. For example, monoamine oxidase, which oxidatively degrades the neurotransmitter dopamine, generates hydrogen peroxide at the mitochondrial membrane of certain neurons. Peroxisomal fatty acid oxidase generates hydrogen peroxide rather than FAD (2H) during the oxidation of very long chain fatty acids. Xanthine oxidase, an enzyme of purine degradation, that can reduce O_2 to O_2^- or hydrogen peroxide in the cytosol, is thought to be a major contributor to ischemia reperfusion injury. Lipid peroxides are also formed enzymatically as intermediates in the pathways for synthesis of eicosanoids including leucotrienes and prostaglandins.

3. Ionizing radiation

Ionizing radiation has a high enough energy level that it can split water into the hydroxyl and hydrogen radicals, thus leading to radiation damage to the skin, mutations, cancer and cell death. It may also generate organic radicals through direct collusion with organic cellular components.

Oxidative mechanisms in carcinogenesis:

Carcinogenesis is a complex multistage process often taking decades until malignancy appears. Conventionally, the carcinogenic process has been divided into three main stages: initiation, promotion and progression.

Initiation requires an irreversible genetic damage causing mutations in transcribed genes. Promotion consists of a potentially reversible oxidant mediated conversion step followed by a clonal expansion of the initiated cells into benign tumors, which can progress to malignancy when they acquire many additional genetic changes. Those genetic changes include modification of DNA bases, insertions and deletions, genetic instability consisting of loss of heterozygosity, chromosomal translocations and sister-chromatid exchanges, activation of oncogenes and suppression of tumor suppressor genes. Although cell initiation is a frequent occurrence, tumor promotion and progression usually require a long time because of all the genetic changes that have to accumulate within the same few cells.

It has been known for many years that antioxidants inhibit formation of tumors, even though they might not decrease DNA adducts, thought to be the initiating lesions. Hence, antioxidants are likely to interfere with the oxygen formation during promotion and/or progression of tumor development. Reactive oxygen species are generated during all stages of carcinogenesis, especially during the long time required to take an initiated cell to a fully disseminated cancer. This long period between the initiation stage and cancer

development provides a wide open window of opportunity to interfere with and suppress the carcinogenic process.

The importance of oxidative stress to human cancers is underscored by the existence of cancer prone syndromes characterized by the formation of high levels of oxidants and/or impairments in their degradation or repair of DNA damage they evoke. Those human congenital syndromes include Fanconi's anemia, xeroderma pigmentosum, Bloom's syndrome, ataxia telangectasia, Wilson's disease and hemochromatosis among others.

Approximately twenty types of oxidatively altered DNA molecules have been identified. The non-specific binding of Fe^{2+} to DNA facilitates localized production of the hydroxyl radical, which can cause base alterations in the DNA. it can also attack the deoxyribose backbone and cause strand breaks. This DNA damage can be repaired to some extent by the cell or minimized by apoptosis of the cell.

SUPEROXIDE DISMUTASE:

Superoxide dismutase (SOD) is a key antioxidant in nearly all cells exposed to oxygen. ROS are cleared from the cell by superoxide dismutase (SOD), catalase, or glutathione (GSH) peroxidase. The enzyme superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.

$M^{(n+1)+} - SOD + O_2^- \rightarrow M^{n+} - SOD + O_2$ where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2) ; Ni (n=2).

$M^{n+} - SOD + O_2^- + 2H^+ \rightarrow M^{(n+1)+} - SOD + H_2O_2$

In humans, three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular. The first is a dimer (consists of two units), while the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive centre. The genes are located on chromosomes 21, 6 and 4, respectively (21q22.1, 6q25.3 and 4p15.3-p15.1).

The physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die several days after birth, amidst massive oxidative stress[Li, et al., Y. (1995). "Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase.". Nat. Genet. 11: 376-38]. Mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma [3 Elchuri, et al., S. (2005). "CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life.". Oncogene 24: 367-380.], an acceleration of age-related muscle mass loss[4 Muller, et al., F. L. (2006). "Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy.". Free Radic. Biol. Med 40: 1993-2004]. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan

The cytosol of virtually all eukaryotic cells contain an SOD enzyme with copper and zinc (Cu-Zn-SOD). The Cu-Zn enzyme is a homodimer of molecular weight 32,500. The two subunits are joined primarily by hydrophobic and electrostatic interactions. The ligands of copper and zinc are histidine side chains. Most mitochondria including human mitochondria and bacteria such as E. coli contain Manganese-SOD. The ligands of the

manganese ions are 3 histidine side chains, an aspartate side chain and a water molecule or hydroxy ligand depending on the Mn oxidation state (respectively II and III).

Many bacteria also contain a form of the enzyme Fe-SOD. The active sites of Mn and Fe superoxide dismutases contain the same type of amino acids side chains.

LACTATE DEHYDROGENASE

EC 1.1.1.27; L lactate; NAD + oxidoreductase.

It is a hydrogen transfer enzyme that catalyzes the oxidation of L- lactate to pyruvate with the mediation of NAD + as a hydrogen acceptor.

$$CH3CHOHCOO^- + NAD^+ \leftrightarrow CH3COCOO^- + NADH + H^+$$

(Lactate) (Pyruvate)

This reaction is reversible, and the reaction equilibrium strongly favors the reduction of pyruvate t o lactate – the reverse reaction.

The enzyme has a molecular weight of 13400 and is composed of four peptide chains of 2 types M (or A) and H (or B), each under separate genetic control.

The subunit compositions of the five isoenzymes, in order of decreasing anodal mobility in an alkaline medium are LD-1 (H4); LD-2 (H3M); LD -3 (H2M2); LD4 (HM3) and LD 5 (M4). A different sixth LD isoenzyme, LD-X composed of four X subunits is present in

post pubertal testis. A seventh LD, called LD-6, has been identified in the sera of severely ill patients.

LD activity is present in all cells of the body and is invariably found only in the cytoplasm of the cell. Enzyme concentration in various tissues is about 500 times greater than normally found in serum. Therefore leakage of the enzyme from even a small mass of damaged tissue increases the observed serum activity of LD to a significant extent.

Different tissues show different isoenzyme composition. In cardiac muscle, kidneys and erythrocytes, LD-1 and LD-2 predominate, whereas in liver and skeletal muscles LD-4 and LD-5 predominate (cathodal). Isoenzymes of intermediate mobility account for the LD activity from many tissues, eg., spleen, lungs, lymph nodes, lymphocytes and platelets.

Clinical significance

Serum LD is relevant in the diagnosis of myocardial infarction, hemolytic anemia, and neoplastic conditions such as ovarian dysgerminoma and testicular germ cell tumor. For monitoring purposes, LD is relevant in predicting the survival duration and rate in Hodgkin's disease and non-Hodgkin's lymphoma and in the follow-up of dysgerminoma. Hemolysis, if severe, produces an LD isoenzyme pattern similar to myocardial infarction. Megaloblastic anemia causes the erythrocyte precursor cells to break down in the bone marrow (ineffective erythropoiesis) resulting in the release of large quantities of LD-1 and LD-2 isoenzymes. Marked elevations of total LD activity in serum- up to 50 times

the upper reference limit has been observed in megaloblastic anemias. Elevations of LD activity are observed in liver disease. Elevations are especially high (ten times normal) in toxic hepatitis with jaundice. Slightly lower values are observed in viral hepatitis and in infectious mononucleosis. LD activity is normal or at most twice the upper reference limit in cirrhosis and obstructive jaundice. Serum LD-5 is often notably elevated in patients with either primary liver disease or liver anoxia secondary to decreased oxygen perfusion. Patients with malignant disease show increased LD activity in serum; up to 70% of patients with liver metastasis and 20%-60% of patients with other non-hepatic metastasis have elevated total LD activity. Elevated LD-1 is observed in germ cell tumors such as teratoma, seminoma of testis and dysgerminoma of the ovary. LD-1 appears to be a useful predictor of outcome in patients with testicular germ cell tumors.

GAMMA GLUTAMYL TRANSPEPTIDASE

EC 2.3.2.2; γ-glutamyl – peptide : amino acid γ-glutamyl transferase

Peptidases are enzymes that catalyze the hydrolytic cleavage of peptides to form amino acids or smaller peptides. GGT is present in proximal renal tubule, liver, pancreas, and intestine. The enzyme is present in cytoplasm (microsomes) but the larger fraction is located in the cell membrane and may transport amino acids and peptides into the cell across the cell membrane in the form of gamma glutamyl peptides. It may also be involved in some aspects of glutathione metabolism. GGT activity in serum comes primarily from liver. The enzyme in serum is heterogeneous with respect to both net molecular charge and size. These forms appear to be derived from post translational

modifications of a single type of enzyme molecule rather than to be due to the existence of true isoenzymes.

Clinical significance:

Though renal tissue has the highest concentration of GGT, the enzyme present in serum appears to originate primarily from the hepatobiliary system. GGT is a sensitive indicator of the presence of hepatobiliary disease, being elevated in most subjects with liver disease regardless of cause, but its usefulness is limited by the lack of specificity.

Highest elevation : post hepatic biliary obstruction

High elevations : primary or secondary liver neoplasm.

Moderate elevation : infectious hepatitis

In acute and chronic pancreatitis, and in some pancreatic malignancies (especially if associated with hepatobiliary obstruction), enzyme activity may be five to fifteen times the upper reference limit. Elevated activities of GGT are found in the sera of individuals with alcoholic hepatitis. Increased concentrations of the enzyme are also found in the serum of individuals receiving anticonvulsant medications such as phenytoin, phenobarbitol.

Reference interval of GGT

Females : up to 38 U/L

Males : up to 55 U/L

AIM OF THE STUDY

On reviewing the role of reactive oxygen species in the causation of carcinogenesis and the changes in biochemical parameters in serum of patients with carcinoma breast, this study has been taken up with keen interest to establish the following aims

- To determine the reference ranges for the following biochemical parameters in female individuals of age between 35 and 75- serum lactate dehydrogenase, gamma glutamyl transpeptidase, total protein, albumin, albumin-globulin ratio, and blood superoxide dismutase
- To determine the levels of these parameters in individuals with carcinoma breast
- To determine whether the levels of these biochemical parameters differ significantly in individuals with carcinoma breast when compared to apparently healthy individuals.

MATERIALS AND METHODS

The study was done during the period February 2007 to June 2007. It was carried out in two groups, namely apparently healthy female controls and females with newly diagnosed carcinoma breast.

Control Group:

The group comprised of 27 apparently healthy female subjects with no significant medical illness, and they were selected from the patients attending the general outpatient department of Madras Medical College, Chennai.

Test Group: This group comprised of 50 females recently diagnosed with carcinoma breast through tissue diagnosis, being managed under the general surgery wards of Madras Medical College.

Inclusion criteria:

Females with confirmed diagnosis of carcinoma breast through tissue diagnosis, any stage of carcinoma, of any age-group.

Exclusion criteria:

1. Individuals who have undergone any surgical intervention for the carcinoma breast other than tissue biopsy

2. Individuals who have received chemotherapy or radiotherapy for the carcinoma breast.
3. Individuals diagnosed to have diabetes mellitus, or liver disease, or pancreatic diseases
4. Individuals with a history of recent acute myocardial infarction.

Sample collection

5ml of peripheral venous blood was withdrawn under sterile conditions with disposable syringes from all the 77 subjects of the study. 1.5 ml of blood was transferred into a test tube containing EDTA, for superoxide dismutase estimation. After thorough mixing, the contents were transferred into a 2 ml Eppendorff tube and labeled properly. These Eppendorff tubes were then stored at a temperature of -20° C till the samples were analyzed.

The remaining blood of 3.5 ml from each subject was transferred to another test tube without any anti-coagulant. Serum separated from this tube was pipetted into a centrifuge tube and was centrifuged at 2000 rpm for 5 minutes to obtain clear fluid without cells. The clear serum was then analyzed for the estimation of serum Lactate Dehydrogenase, serum Gamma glutamyl transpeptidase, serum total proteins, and serum albumin.

The biochemical parameters undertaken for the study were determined by semi auto analyzer ERBA CHEM- 5 plus V2 using the following methodology:

ESTIMATION OF SUPEROXIDE DISMUTASE IN WHOLE BLOOD

Principle: The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($O2^{\bullet}$), produced during oxidative energy processes to hydrogen peroxide and molecular oxygen.

This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2- (4- iodophenyl) – 3- (4 -nitrophenol)- 5 phenyl tetrazolium chloride (I.N.T) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T. under the conditions of the assay.

$$\text{Xanthine} \xrightarrow{\text{XOD}} \text{Uric acid} + O_2^{\bullet}$$

$$\text{INT} \xrightarrow{O2^{\bullet}} \text{Formazan dye}$$

Or,

$$O_2^{\bullet} + O_2^{\bullet} + 2H+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Sample preparation

EDTA whole blood samples were used. 0.5ml of whole blood was centrifuged for 10 minutes at 3000 rpm and then plasma discarded. The erythrocytes were washed with 3 ml

of 0.9% NaCl solution and centrifuged for 10 minutes at 3000 rpm. The supernatant was discarded. This NaCl wash and centrifugation was repeated four times. The washed centrifuged erythrocytes was made up to 2 ml with cold redistilled water mixed and left to stand at +4 oC for 15 minutes. The lysate was diluted with 0.01 mol/L phosphate buffer pH7 so that the percentage inhibition falls between 30% and 60%.

Reagent composition:

Contents		Initial concentration of solutions
R1a.	Mixed substrate	
	Xanthine	0.05 mmol/L
	I.N.T.	0.025 mmol/L
R1b.	Buffer	
	CAPS	40 mmol/L, pH 10.2
	EDTA	0.94 mmol/L
R2.	Xanthine Oxidase	80 U/L
CAL	Standard	4 U/mL

Stability and preparation of reagents

The contents of one vial of R1a. was mixed with 20 ml of R1b buffer. This was stored at +2 ^{o}C to +8 ^{o}C. R2 Xanthine oxidase one vial was reconstituted with 10 ml of redistilled water, and was stored at +2 ^{o}C to +8 ^{o}C. One vial of standard was reconstituted with 10 ml of redistilled water. Subsequent dilutions of this standard were prepared with Ransod sample diluents. The following dilutions were made of the Standard CAL (S6) to produce a standard curve:

Volume of	Volume of standard solution	Sample diluent
S6	Undiluted standard	---
S5	5ml of S6	5 ml
S4	5ml of S5	5 ml
S3	5ml of S4	5 ml
S2	3ml of S3	6 ml

S1 = sample diluent

Procedure

The semiautoanalyzer was programmed to the following settings:

Wavelength 505 nm

Cuvette 1 cm path length

Temperature 37 ^{o}C

Measurement against air

The following were pipette into separate cuvettes and mixed.

	Sample diluent	Standards S2-S6	Diluted sample
Diluted sample	---	---	0.05 ml
Standard	---	0.05 ml	---
Ransod sample diluent	0.05 ml	---	---
Mixed substrate (R1)	1.7 ml	1.7 ml	1.7 ml

Xanthine oxidase (R2) 0.25 ml was added to each of the above cuvettes.

The initial absorbance A1 was read after 30 seconds and the final absorbance A2 after 30 minutes.

Calculation:

$$\frac{A2-A1}{3} = \Delta A \text{ per minute of standard or sample}$$

Sample diluent rate (S1 rate) = rate of uninhibited reaction = 100 %

All standard rates and diluted sample rates were converted into percentages of the sample diluent rate and subtracted from 100 % to give a percentage inhibition.

$$100 - \frac{(\Delta AStd/min \times 100)}{(\Delta AS1/min)} = \text{percentage inhibition}$$

$$100 - \frac{(\Delta ASample/min \times 100)}{(\Delta AS1/min)} = \text{percentage inhibition}$$

Percentage inhibition for each standard against log10 was plotted. The percentage inhibition of the sample was obtained from the curve.

LACTATE DEHYDROGENASE

Method: UV Kinetic (IFCC and SFBC) Method

Kit Used: Autopak of Bayer Diagnostics

Principle:

Lactate Dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate and NADH to NAD. LDH activity in serum/plasma is directly to proportional to the rate of decrease in absorbance of NADH at 340 nm .

Pyruvate + NADH + H^+ ↔ Lactate + NAD^+

Sample collection, storage & stability

Serum was used for estimation of LDH, and was stored at 2^{o}C to 8 ^{o}C for one week.

Reagents:

Reagent 1 (co enzyme) :

NADH	240 µmol / L

Reagent 1A (Buffer)

Tris buffer, pH 7.2	80 mmol/ L
Sodium Chloride	200 mmol/ L
Pyruvate	1.6 mmol / L

Reagent reconstitution

The reagents were allowed to attain the room temperature. 3 ml of reagent 1A was added into one bottle of reagent 1, mixed by gentle swirling till complete dissolution. The reconstituted reagent mixture was used after 5 minutes. The reconstituted reagent was stored at 2 ^{o}C to 8 ^{o}C

Procedure

The reconstituted reagent was brought to room temperature prior to use.

Assay parameters:

Reaction Type	: Kinetics
Reaction slope	: Decreasing
Wave length	: 340 nm
Flow cell Temp	: 37 ^{o}C
Delay time	: 60 secs
No . of readings	: 4
Interval	: 60 secs
Sample volume	: 20 µ L
Reagent volume	: 1 ml
Path length	: 1 cm
Factor	: 8095
Zero setting with	: distilled water

To 1 ml of the reconstituted reagent 20 µL of serum was added and the decrease in absorbance was measured at 340 nm immediately.

Test:

Reconstituted reagent	1 ml
Sample	20 µ L

Reference values:

Serum: 200 to 400 U/L (37 ^{o}C).

GAMMA GLUTAMYL TRANSPEPTIDASE ASSAY

Method : KINETIC (SZASZ) method

Kit used: Autopak of Bayer Diagnostics

Principle:

Gamma – Glutamyl Transpeptidase catalyzes the transfer of the Gamma–Glutamyl group from the substrate gamma–glutamyl para–nitroanilide to glycylglycine releasing free paranitroaniline which absorbs light at 405 nm. Enzyme activity is proportional to the increase in absorbance at this wavelength.

GPNA + Glycylglycine $\rightarrow$ L γ Glutamyl glycylglycine + p-nitroaniline

GPNA = L-γ Glutamyl para–nitroanilide

Reagents:

Reagent-1 (substrate) :

Glycylglycine	94 mmol/L
L-γ Glutamyl para–nitroanilide	3.2 mmol/L

Reagent-1A (buffer)

Tris buffer pH 8.2	200 mmol/L
Surfactant	0.2

Reagent reconstitution:

The reagents were brought to room temperature. 3 ml of reagent-1A was added to one bottle of reagent-1, and mixed by gentle swirling till complete dissolution. The reconstituted reagent mixture was used after 5 minutes. The reconstituted reagent was stored at 2 ^{o}C to 8 ^{o}C

Procedure:

The samples and the reconstituted reagent were brought to room temperature prior to use.

The following general system parameters were used:

General System Parameters:

Reaction Type	: Kinetics
Reaction slope	: Increasing
Wave length	: 405 nm
Flow cell Temp	: 30 ^{o}C
Delay time	: 60 secs
No. of readings	: 4
Interval	: 60 secs
Sample volume	: 100 µ L
Reagent volume	: 1 ml
Path length	: 1 cm
Factor	: 1111
Zero setting with	: distilled water

The semiautoanalyzer was calibrated to the above mentioned system parameters. 1 ml of the reconstituted reagent and 100 µL of the sample were dispensed into a test tube, mixed and analyzed in the semiautoanalyzer immediately.

TOTAL PROTEIN

Method: BIURET method

Kit used: Bayer AUTOPAK

Principle:

Peptide bonds of protein form a blue violet colored complex with cupric ions in an alkaline medium. The intensity of colour is proportional to the number of peptide bonds and the colour is read at 540 nm

(530 to 570 nm) . The final colour is stable for 8 hours.

Sample used: Serum.

Reagents:

Reagent 1 (biuret reagent):

sodium hydroxide	3.8 mol/l
potassium sodium tartarate	0.1 mol/l
cupric sulphate	33 mmol/l
potassium iodide	30 mmol/l

reagent 1A (surfactant)

surfactant	20g /L

standard (total protein 6g/ dl)

BSA	60g/dl

Reagent reconstitution

The reagents were allowed to attain the room temperature. 41 ml of distilled water was added to one bottle of reagent 1 and then the contents of one bottle of reagent 1A was added, mixed gently to avoid foaming.

Procedure

The reconstituted reagent was brought to room temperature prior to use.

System parameters

Reaction type : end point

Reaction slope : increasing

Wavelength : 546 nm (530 – 570 nm)

Flow cell temp. : 30

Incubation : 20 min at room temp

Reagent volume : 1 ml

Std. Concentration : 6 g/ dl

Zero setting with : reagent blank

1 ml of reagent was taken in all test tubes. For standard, 10 µL of standard was added. For test, 10 µL of samples were added and incubated for 20 min at room temperature and the readings were taken at 546 nm.

ALBUMIN

Method : BromoCresol green method

Principle:

Albumin in a buffered solution reacts with the anionic bromoCresol green (BCG) with a dye binding reaction to give a proportionate green colour which is measured at 628 nm (600n- 650 nm) . The final colour is stable for 10 minutes.

Sample: Serum.

Reagents:

Reagent 1 (bromoCresol green)

Succinic acid 94 mmol / L

Sodium hydroxide	10.2 mmol / L
BCG	0.149 mmol / L

Standard (albumin 5 g / dL)

BSA	50 g / L

Reagent reconstitution: Albumin reagent is ready to use .

Procedure:

The reagent was brought to room temperature before use.

General system parameters:

Reaction type	: end point
Reaction slope	: increasing
Wavelength	: 628 nm (600 – 650 nm)
Flowcell temperature	: 30
Incubation	: 1 minute, room temperature
Sample volume	: 10 µL
Reagent volume	: 1 mL
Standard concentration	: 5g /dl
Zero setting with	: reagent blank

The instrument was set using the above parameters .

To 1 ml of reagent 10 µL of samples were added and incubated for 1 minute at room temperature and readings were taken.

RESULTS

The mean age of the controls was 51.88±8.59 and the mean age of the cases was 52.28±10.93. The biochemical parameters namely the antioxidant enzyme superoxide dismutase, gamma glutamyl transferase and lactate dehydrogenase, serum total protein, serum albumin, albumin-globulin ratio, along with the data on the biological parameters such as family history of breast cancer, parity, age at first child birth, duration of exposure to estrogen, obtained from the 77 subjects taken up for this study are given in Table-1. Subjects 1 to 27 are the controls and 28 to 77 are the cases.

Table-2 shows the compilation of biochemical parameters for the controls. The mean and standard deviation were ; for serum total protein 7.4±0.6, for serum albumin 4.3±0.5, A:G ratio 1.4 ±0.2 , LDH 213±112, GGT 20 ±6, SOD 189.8 ±14.8.

Table-3 shows the compilation of biochemical parameters for the cases. The mean and standard deviation were ; for serum total protein 6.5±0.5, for serum albumin 3.6±0.5, A:G ratio 1.3 ±0.4, LDH 423±198, GGT 20 ±6, SOD 151.7 ±10.7.

Table-4 shows the comparison of biochemical parameters among the cases and controls. Figure-1 to Figure-6 illustrate the distribution of these values. The statistical significance of the difference in the mean values of the biochemical parameters between the cases and the controls was calculated using Mann-Whitney U test for LDH and Students' t-test for the other parameters. The p value for serum total protein, albumin, LDH, GGT and blood SOD was 0.00 where as for A:G ratio it is 0.07

Table-5 shows the comparison of biological risk factors among the cases and controls. The statistical significance was determined by Chi-square test. The p value for positive family history was 0.54, for nulliparity 0.15, for age more than 30 at first child birth 0.29,for duration of exposure to estrogen more than 30 years 1.00 .

Table-6 shows compilation of biochemical parameters in cases with clinical stage-2 carcinoma breast. The mean and were standard deviation were; for serum total protein 6.7±0.3, for serum albumin 3.8±0.4, A:G ratio 1.4 ±0.4 , LDH 410±228, GGT 27 ±7 SOD 160.5 ±7.9.

Table-7 shows compilation of biochemical parameters in cases with clinical stage-3 carcinoma breast. The mean and were standard deviation were; for serum total protein 6.4 ±0.5, for serum albumin 3.5±0.5, A:G ratio 1.3 ±0.4 , LDH 439±190, GGT 29±6, SOD 147.3±6.3.

Table-8 shows comparison of biochemical parameters in cases with stage-2 and stage-3 breast cancer. Statistical significance of the difference between the parameters was calculated using Mann-Whitney U test for LDH and Students' t-test for the other parameters. The p value for serum protein, albumin, A:G ratio, LDH ,GGT and blood SOD were 0.05,0.06, 0.49,0.18,0.28 and 0.00 respectively.

DISCUSSION

The reference ranges for the analyzed biochemical parameters of the apparently healthy female controls with a mean age of 51.88±8.59 years are as follows:

Total protein	7.4±0.6 g/dL
Serum albumin	4.3±0.5 g/dL
A:G ratio	1.4±0.2
Serum LDH	213±112 IU/L
Serum GGT	20±6 IU/L
Blood SOD	189.8±14.8 U/mL

These mean values fall within the accepted reference ranges, and therefore could be accepted as valid for this study.

Comparison of the levels of various biochemical parameters under study, between the cases and controls is shown in Table-4. From this table, it is found that serum total protein and albumin have been found to be significantly lowered in cases with a p value of 0.00 (mean for protein 6.5±0.5, mean for albumin 3.6±0.5) compared to controls (mean value for protein 7.4±0.6, mean for albumin 4.3±0.5) but A:G ratio is not significantly altered.(mean for cases 1.3 ±0.4 and for controls 1.4±0.2)

The levels of LDH has been found to be significantly elevated in the cases with a mean value of 423±198 compared to controls mean value 213±112 IU/L. (*p value <0.00)* Lactate dehydrogenase is an oxidation reduction enzyme which reversibly catalyzes the reaction between lactic acid and pyruvic acid in the final step of the glycolytic pathway. It is distributed widely in body tissues such as RBCs , liver, cardiac and skeletal muscle , and in brain, and is raised in a variety of physiological and pathological states. Serum

LDH is thus derived from the tissues or from blood and its level is an indicator of destruction of these cells. LDH levels are increased in malignancies because of increased production of enzymes by tumor cells, change in the permeability of the cells, allowing leakage of soluble enzymes into circulation, consequent to the blockade of the duct system through which the enzyme passes. Release of LDH from dying tumor cells and induction of LDH synthesis in the normal tissues of the host by the tumor also contribute to raised LDH levels.

The levels of serum GGT has been found to be significantly elevated (*p value <0.00)* in the cases. GGT is a membrane-bound glycoprotein enzyme present in normal human serum. It initiates cleavage of extra cellular glutathione and has been shown to promote oxidative damage to cells. Glutathione level decreases when there is increase in transpeptidation. So this is believed to be one of the mechanisms of free radical damage to tissues. Also studies have shown that in the presence of chelated iron, pro oxidant species can be originated during GGT mediated metabolism of glutathione and that a process of lipid peroxidation can be started eventually in suitable lipid membranes. Recently GGT has been shown to promote oxidative damage to cells. In a study by G Wagner et al (enhanced gamma glutamyl transpeptidase expression and superoxide production in MPV 17 -/- glomerulosclerosis in mice) for murine model of glomerulosclerosis involving loss of gene coding for a peroxisomal protein , enzyme activity and mRNA expression of membrane bound GGT were increased , while plasma glutathione peroxidase and superoxide dismutase levels were lowered. Inhibition of gamma-glutamyl transpeptidase by acivicin reverted the lowered plasma glutathione

peroxidase and superoxide dismutase activities, indicating reciprocal control of gene expression for these enzymes.

The levels of blood SOD has been found to be significantly decreased (*p value <0.00)* in the cases. Evidence is increasing that free- radicals are implicated in cancer initiation and promotion. Increased incidence of cancer with advancing age may be due, at least in part, to the increasing level of free radical reactions with age, and the diminishing ability of the immune system to eliminate the altered cells and to detoxify the free radicals. Normally, erythrocytes contain enough scavengers such as superoxide dismutase and glutathione peroxidase to protect against free radical injury because the activities of SOD and glutathione peroxidase are higher in erythrocytes than in other tissues in the body. It is known that oxygen radical scavenging enzymes can respond to conditions of increased oxidative stress with compensatory increases in activity. However, in this study, there is no indication towards the corresponding adaptation in activity of SOD in persons with cancer breast. The decreased activity of the antioxidant enzyme SOD may reflect the 1)

1) known sensitivity of these enzymes to radical induced inactivation

2) exhaustion of free radical scavenging enzymes

3) decreased expression of anti oxidant enzymes by ROS

In comparing the levels of the biochemical markers among the subjects with stage-2 and stage-3 breast cancers, only SOD levels were lowered which is significant (*p value* 0.00) - mean blood level of 147.3 IU/mL in stage-3 compared to the mean blood level of 160.5 IU/mL in stage-2. This again could be indicative of further radical induced inhibition of SOD activity in advanced stages of breast cancer.

Differences in positive family history of breast cancer, parity, age at first childbirth, and duration of exposure to estrogen among the cases and controls were not statistically significant. The p values were 0.59, 0.15, 0.29 and 1.00 respectively.

There have been studies, though, that have found that family history of breast cancer, nulliparity, age more than thirty at first childbirth and increased duration of exposure to estrogen are associated with increased risk of breast cancer. Contrary observations in this study would be attributed to the sample size and the study design.

SUMMARY

Carcinoma breast is the most common malignancy in females worldwide. There are various risk factors for developing breast cancer; positive family history, nulliparity, age more than 30years at first child birth, exposure to ionizing radiation etc.

The imbalance between free radicals and the anti-oxidants in the causation of breast cancer is being researched. Free radicals has been implicated in initiation and promotion of carcinogenesis.This study was undertaken to evaluate if the levels of biochemical markers LDH and GGT, and the level of antioxidant enzyme SOD are altered in cases with carcinoma breast, when compared to the levels in apparently normal controls.

In humans, three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 in mitochondria and SOD3 in extracellular matrix. SOD causes detoxifies super oxide to hydrogen peroxide. LDH is a glycolytic enzyme with five iso enzymes. The level of LDH is increased in various malignancies. GGT helps in transportation of amino acids in the cells .

The levels of LDH and GGT were found to be significantly elevated in the cases, whereas the levels of SOD were found to be significantly lowered in the cases. The SOD levels were lower in cases with stage-3 carcinoma breast than in cases with stage-2 carcinoma breast.

CONCLUSION

The levels of biochemical markers LDH and GGT have been found to be significantly elevated. The levels of antioxidant enzyme SOD significantly lowered in cases with carcinoma breast.

The association of the levels of these biochemical parameters with the clinical course of the carcinoma breast, is yet to be established. Further, the levels of these biochemical markers in non malignant breast tumours need to be studied. Definite association of these markers with carcinoma breast might open up the scope for use of these biochemical markers as adjuvant in breast cancer screening. This also would warrant further studies of these biochemical markers in patients post surgery and/or chemotherapy and/or radiotherapy, which could open up possibilities of these biochemical markers to be used in estimation of these in the diagnosis, management and follow-up of individuals at risk of and those diagnosed to have carcinoma breast which will considerably help in reducing morbidity and mortality associated with cancer breast.

SCOPE FOR FURTHER STUDY

Based on the results of this study, further research could be undertaken

- To study the role of oxidative stress and deficiencies of antioxidant enzymes in the pathogenesis of breast cancer.
- To study the role of antioxidant enzymes in the prevention of carcinoma breast.
- To study the pre treatment and serial post treatment levels of the biochemical markers LDH, GGT and SOD to find out whether the levels of these biochemical markers could be effectively used for follow-up monitoring of individuals with breast cancer after intervention.

TABLE-1 MASTER

Sl no	Group	Age	Hormone exposure	menopause	Marietal status	Parity	Age at 1st child birth	Family history	Staging	Protein g/dL	Albumin g/dL	A:G	LDH IU/L	GGT IU/L	SOD U/mL
1	0	44	30	No	Married	4	24	No	NA	7.4	4.4	1.47	312	27	171.2
2	0	47	30	Yes	Married	4	22	No	NA	7.2	4.3	1.48	453	17	181.6
3	0	54	31	Yes	Married	3	20	No	NA	6.9	4.1	1.46	356	18	183.2
4	0	50	28	Yes	Married	3	25	No	NA	6.7	3.9	1.39	330	16	201.6
5	0	50	30	Yes	Married	2	22	No	NA	6.4	4	1.67	440	21	203.2
6	0	44	31	No	Married	3	23	No	NA	7.3	4.4	1.52	348	18	168.9
7	0	57	34	Yes	Married	5	21	No	NA	8.1	5	1.61	131	23	192.8
8	0	50	33	Yes	Married	2	28	No	NA	7.8	4.8	1.60	100	31	198
9	0	60	36	Yes	Married	2	24	No	NA	8	4.4	1.22	110	16	174.6
10	0	50	32	Yes	Married	3	23	No	NA	7.9	4.5	1.32	152	17	168.9
11	0	53	31	Yes	Married	2	22	No	NA	7.3	4.6	1.70	120	18	186.4
12	0	60	32	Yes	Married	3	21	No	NA	7.9	4.4	1.26	170	16	197.3
13	0	55	37	Yes	Married	4	19	No	NA	6.8	4.1	1.52	115	15	187.8
14	0	55	34	Yes	Married	2	26	No	NA	7.9	4.5	1.32	186	15	179.9
15	0	52	33	Yes	Married	2	18	No	NA	7.3	4.4	1.52	127	16	198.9
16	0	42	27	No	Married	3	22	No	NA	7.9	4.4	1.26	112	24	206.7
17	0	55	31	Yes	Married	3	21	No	NA	6.8	3.9	1.34	188	20	212.3
18	0	44	26	Yes	Unmarried	0	0	No	NA	7.9	4.5	1.32	150	29	222.3
19	0	53	31	Yes	Married	3	27	No	NA	7.1	4	1.29	181	15	214.8
20	0	47	32	No	Married	2	24	No	NA	5.9	2.6	0.79	131	17	186.7
21	0	40	27	No	Married	4	17	No	NA	8	4.4	1.22	151	16	178.9
22	0	50	33	Yes	Married	3	23	No	NA	7.9	4.8	1.55	130	40	192.6
23	0	77	37	Yes	Married	3	21	No	NA	8.4	5	1.47	118	16	169.8
24	0	45	29	No	Married	5	16	No	NA	6.8	4.7	2.24	291	19	178
25	0	55	33	Yes	Married	6	19	No	NA	7.5	4.3	1.34	145	16	173.9
26	0	40	25	No	Married	2	21	No	NA	7.4	4.6	1.64	316	20	198.6

27	0	72	35	Yes	Married	9	25	No	NA	7.2	4.3	1.48	390	16	194.9
28	1	50	32	Yes	Married	3	19	No	2A	6.1	3.2	1.10	332	29	162.6
29	1	75	35	Yes	Married	4	18	No	3B	5.8	3.7	1.76	267	32	142.8
30	1	70	36	Yes	Married	4	25	No	2B	6.3	4.4	2.32	375	25	156.8
31	1	48	34	Yes	Married	2	22	No	3B	6	4	2.00	343	31	146.7
32	1	54	40	No	Married	3	19	No	3A	7.3	3.8	1.09	354	20	148.9
33	1	65	35	Yes	Married	4	22	No	2B	6.7	3.6	1.16	704	35	164.2
34	1	60	34	Yes	Married	2	21	No	3A	6.4	3.5	1.21	510	40	150.6
35	1	67	32	Yes	Married	6	18	No	2B	7	4.4	1.69	381	21	168.9
36	1	47	32	No	Married	1	25	No	2A	6.2	4.1	1.95	718	37	171.6
37	1	50	34	Yes	Married	2	31	No	1	6	3.9	1.86	291	28	181
38	1	60	35	Yes	Married	2	25	No	3A	5.6	3.5	1.67	704	33	143.7
39	1	45	35	No	Married	2	30	No	3A	6.4	4.1	1.78	1108	35	146.9
40	1	35	20	No	Married	2	18	No	3A	7	4	1.33	506	21	161.2
41	1	49	33	Yes	Married	2	28	No	2A	6.9	3.8	1.23	992	34	166
42	1	45	31	No	Married	4	18	No	3B	6.1	2.5	0.69	518	27	151.2
43	1	50	28	Yes	Married	2	18	No	3C	5.8	2.5	0.76	721	31	138.7
44	1	42	26	No	Married	3	19	No	3B	6.7	3	0.81	251	18	141.2
45	1	50	33	Yes	Married	3	18	No	3B	6.3	2.9	0.85	445	25	146.3
46	1	47	30	Yes	Married	2	21	No	3B	6	3.1	1.07	405	31	152
47	1	63	39	Yes	Unmarried	0		No	3B	7.1	2.8	0.65	915	37	150.2
48	1	35	20	No	Married	2	20	No	3A	6.5	3.2	0.97	320	28	146.7
49	1	56	36	Yes	Married	4	31	No	3B	6.4	3.7	1.37	494	33	139.1
50	1	48	29	Yes	Married	2	28	Elder sister	3A	6.2	3.8	1.58	520	28	148.2
51	1	39	20	Yes	Married	2	19	No	3A	5.8	3	1.07	432	27	150.6
52	1	49	32	Yes	Married	2	20	No	3A	5.6	3.8	2.11	420	30	156.5
53	1	40	25	No	Married	1	28	No	3A	6.4	4.1	1.78	436	25	136.8
54	1	53	38	Yes	Married	2	28	No	2B	6.8	3.3	0.94	267	26	153.8
55	1	55	35	Yes	Married	6	19	No	2B	6.9	3.4	0.97	320	31	161.7

56	1	39	26	No	Married	2	18	No	3C	7.2	3.2	0.80	390	31	140.4
57	1	70	34	Yes	Married	1	35	No	3B	6.7	4.2	1.68	502	35	151.8
58	1	52	36	Yes	Married	3	22	No	3A	6.8	3.8	1.27	461	37	141.1
59	1	45	30	No	Married	0		No	3B	5.9	4	2.11	480	23	148.9
60	1	70	33	Yes	Married	0		No	2A	7.2	4.3	1.48	330	25	146.8
61	1	50	35	No	Married	2	30	No	3B	6.4	3.9	1.56	240	29	147.8
62	1	41	18	Yes	Married	2	20	No	2B	6.7	4.1	1.58	281	21	145.3
63	1	66	35	Yes	Married	2	19	Maternal aunt	3B	5.7	3.1	1.19	307	33	138.7
64	1	60	32	Yes	Married	5	17	No	3C	7.1	3.7	1.09	345	19	145.3
65	1	40	27	No	Married	1	25	No	3A	6.8	4	1.43	275	22	147.9
66	1	47	32	No	Married	3	18	No	2B	6.5	3.9	1.50	256	31	161.7
67	1	75	34	Yes	Married	2	21	No	3B	6.7	4	1.48	332	24	145.4
68	1	50	30	Yes	Married	1	32	No	2B	6.9	3.8	1.23	312	32	158.9
69	1	52	37	No	Married	3	20	No	2A	7.1	3.1	0.78	254	16	169.6
70	1	35	20	No	Unmarried	0	0	No	3B	6.8	3.3	0.94	330	26	165.8
71	1	75	27	Yes	Married	4	20	No	3B	6.3	3	0.91	261	28	154.1
72	1	54	32	Yes	Married	5	17	No		5.8	2.7	0.87	432	37	127.8
73	1	38	24	No	Married	2	15	No	3A	6.1	2.5	0.69	304	33	145.2
74	1	60	35	Yes	Married	6	20	No	3B	7.1	3.8	1.15	316	42	147
75	1	48	32	No	Married	4	19	No	1	6.8	3.9	1.34	186	27	170
76	1	44	29	No	Married	4	20	No	2A	7	3.9	1.26	221	18	158.6
77	1	56	28	Yes	Married	4	18	No	3A	7.2	4	1.25	286	34	142.3

TABLE-2 BIOCHEMICAL PARAMETERS IN CONTROLS

Sl no	Age	Protein g/dL	Albumin g/dL	A:G	LDH IU/L	GGT IU/L	SOD U/mL
1	44	7.4	4.4	1.47	312	27	171.2
2	47	7.2	4.3	1.48	453	17	181.6
3	54	6.9	4.1	1.46	356	18	183.2
4	50	6.7	3.9	1.39	330	16	201.6
5	50	6.4	4	1.67	440	21	203.2
6	44	7.3	4.4	1.52	348	18	168.9
7	57	8.1	5	1.61	131	23	192.8
8	50	7.8	4.8	1.60	100	31	198
9	60	8	4.4	1.22	110	16	174.6
10	50	7.9	4.5	1.32	152	17	168.9
11	53	7.3	4.6	1.70	120	18	186.4
12	60	7.9	4.4	1.26	170	16	197.3
13	55	6.8	4.1	1.52	115	15	187.8
14	55	7.9	4.5	1.32	186	15	179.9
15	52	7.3	4.4	1.52	127	16	198.9
16	42	7.9	4.4	1.26	112	24	206.7
17	55	6.8	3.9	1.34	188	20	212.3
18	44	7.9	4.5	1.32	150	29	222.3
19	53	7.1	4	1.29	181	15	214.8
20	47	5.9	2.6	0.79	131	17	186.7
21	40	8	4.4	1.22	151	16	178.9
22	50	7.9	4.8	1.55	130	40	192.6
23	77	8.4	5	1.47	118	16	169.8
24	45	6.8	4.7	2.24	291	19	178
25	55	7.5	4.3	1.34	145	16	173.9
26	40	7.4	4.6	1.64	316	20	198.6
27	72	7.2	4.3	1.48	390	16	194.9

MEAN	51.889	7.3963	4.3444	1.4452	213.07	19.704	189.77
SD	8.5904	0.5932	0.4827	0.245	112.37	5.9408	14.825

TABLE-3 BIOCHEMICAL PARAMETERS IN CASES

P Sl no	Protein g/dL	Albumin g/dL	A:G	LDH IU/L	GGT IU/L	SOD U/mL
1	6.1	3.2	1.10	332	29	162.6
2	5.8	3.7	1.76	267	32	142.8
3	6.3	4.4	2.32	375	25	156.8
4	6	4	2.00	343	31	146.7
5	7.3	3.8	1.09	354	20	148.9
6	6.7	3.6	1.16	704	35	164.2
7	6.4	3.5	1.21	510	40	150.6
8	7	4.4	1.69	381	21	168.9
9	6.2	4.1	1.95	718	37	171.6
10	6	3.9	1.86	291	28	181
11	5.6	3.5	1.67	704	33	143.7
12	6.4	4.1	1.78	1108	35	146.9
13	7	4	1.33	506	21	161.2
14	6.9	3.8	1.23	992	34	166
15	6.1	2.5	0.69	518	27	151.2
16	5.8	2.5	0.76	721	31	138.7
17	6.7	3	0.81	251	18	141.2
18	6.3	2.9	0.85	445	25	146.3
19	6	3.1	1.07	405	31	152
20	7.1	2.8	0.65	915	37	150.2
21	6.5	3.2	0.97	320	28	146.7
22	6.4	3.7	1.37	494	33	139.1
23	6.2	3.8	1.58	520	28	148.2
24	5.8	3	1.07	432	27	150.6
25	5.6	3.8	2.11	420	30	156.5
26	6.4	4.1	1.78	436	25	136.8
27	6.8	3.3	0.94	267	26	153.8
28	6.9	3.4	0.97	320	31	161.7
29	7.2	3.2	0.80	390	31	140.4
30	6.7	4.2	1.68	502	35	151.8
31	6.8	3.8	1.27	461	37	141.1
32	5.9	4	2.11	480	23	148.9
33	7.2	4.3	1.48	330	25	146.8

TABLE-3 BIOCHEMICAL PARAMETERS IN CASES

34	6.4	3.9	1.56	240	29	147.8
35	6.7	4.1	1.58	281	21	145.3
36	5.7	3.1	1.19	307	33	138.7
37	7.1	3.7	1.09	345	19	145.3
38	6.8	4	1.43	275	22	147.9
39	6.5	3.9	1.50	256	31	161.7
40	6.7	4	1.48	332	24	145.4
41	6.9	3.8	1.23	312	32	158.9
42	7.1	3.1	0.78	254	16	169.6
43	6.8	3.3	0.94	330	26	165.8
44	6.3	3	0.91	261	28	154.1
45	5.8	2.7	0.87	432	37	127.8
46	6.1	2.5	0.69	304	33	145.2
47	7.1	3.8	1.15	316	42	147
48	6.8	3.9	1.34	186	27	170
49	7	3.9	1.26	221	18	158.6
50	7.2	4	1.25	286	34	142.3

MEAN	6.502	3.586	1.30738	423	28.82	151.71
SD	0.48634	0.51429	0.41807	197.812	6.063	10.671

Table-4 COMPARISON OF BIOCHEMICAL PARAMETERS IN CASES AND CONTROLS

Sl No	Study group	Protein Mean ± SD g/dL	Albumin Mean ± SD g/dL	A:G Mean ± SD	LDH Mean ± SD IU/L	GGT Mean ± SD IU/L	SOD Mean ± SD U/mL
1	Controls (n=27)	7.4±0.6	4.3±0.5	1.4±0.2	213±112	20±6	189.8±14.8
2	Cases (n=50)	6.5±0.5	3.6±0.5	1.3±0.4	423±198	29±6	151.7±10.7
p-value		0.00	0.00	0.07	0.00	0.00	0.00
Level of significance*		S	S	NS	S	S	S

*S = SIGNIFICANT

NS= NOT SIGNIFICANT

Table-5 DISTRIBUTION OF BIOLOGICAL RISK FACTORS AMONG CASES AND CONTROLS

Sl No	Study group	Family history of CA Breast %	Nulliparity %	First child birth at > 30 yrs %	Exposure to Estrogen > 30 yr %
1	Control (N=27)	0	10	8	64
2	Cases (N=50)	4	3.7	0	33.3
p-value		0.54	0.15	0.29	1.00
Level of significance*		NS	NS	NS	NS

*NS= NOT SIGNIFICANT

Table-6 COMPILATION OF BIOCHEMICAL PARAMETERS IN STAGE-2 CA BREAST

Sl no (Master)	Staging	Protein g/dL	Albumin g/dL	A:G	LDH IU/L	GGT IU/L	SOD U/mL
28	2A	6.1	3.2	1.10	332	29	162.6
36	2A	6.2	4.1	1.95	718	37	171.6
41	2A	6.9	3.8	1.23	992	34	166
60	2A	7.2	4.3	1.48	330	25	146.8
69	2A	7.1	3.1	0.78	254	16	169.6
76	2A	7	3.9	1.26	221	18	158.6
30	2B	6.3	4.4	2.32	375	25	156.8
33	2B	6.7	3.6	1.16	704	35	164.2
35	2B	7	4.4	1.69	381	21	168.9
54	2B	6.8	3.3	0.94	267	26	153.8
55	2B	6.9	3.4	0.97	320	31	161.7
62	2B	6.7	4.1	1.58	281	21	145.3
66	2B	6.5	3.9	1.50	256	31	161.7
68	2B	6.9	3.8	1.23	312	32	158.9
MEAN		6.7	3.8	1.4	410	27	160.5
SD		0.3	0.4	0.4	228	7	7.9

TABLE-7 COMPILATION OF BIOCHEMICAL PARAMETERS IN STAGE-3 CA BREAST

Sl no (Master)	Staging	Protein g/dL	Albumin g/dL	A:G	LDH IU/L	GGT IU/L	SOD u/mL
32	3A	7.3	3.8	1.09	354	20	148.9
34	3A	6.4	3.5	1.21	510	40	150.6
38	3A	5.6	3.5	1.67	704	33	143.7
39	3A	6.4	4.1	1.78	1108	35	146.9
40	3A	7	4	1.33	506	21	161.2
48	3A	6.5	3.2	0.97	320	28	146.7
50	3A	6.2	3.8	1.58	520	28	148.2
51	3A	5.8	3	1.07	432	27	150.6
52	3A	5.6	3.8	2.11	420	30	156.5
53	3A	6.4	4.1	1.78	436	25	136.8
58	3A	6.8	3.8	1.27	461	37	141.1
65	3A	6.8	4	1.43	275	22	147.9
73	3A	6.1	2.5	0.69	304	33	145.2
77	3A	7.2	4	1.25	286	34	142.3
29	3B	5.8	3.7	1.76	267	32	142.8
31	3B	6	4	2.00	343	31	146.7
42	3B	6.1	2.5	0.69	518	27	151.2
44	3B	6.7	3	0.81	251	18	141.2
45	3B	6.3	2.9	0.85	445	25	146.3
46	3B	6	3.1	1.07	405	31	152
47	3B	7.1	2.8	0.65	915	37	150.2
49	3B	6.4	3.7	1.37	494	33	139.1
57	3B	6.7	4.2	1.68	502	35	151.8
59	3B	5.9	4	2.11	480	23	148.9
61	3B	6.4	3.9	1.56	240	29	147.8
63	3B	5.7	3.1	1.19	307	33	138.7
67	3B	6.7	4	1.48	332	24	145.4
70	3B	6.8	3.3	0.94	330	26	165.8
71	3B	6.3	3	0.91	261	28	154.1
74	3B	7.1	3.8	1.15	316	42	147
43	3C	5.8	2.5	0.76	721	31	138.7
56	3C	7.2	3.2	0.80	390	31	140.4
64	3C	7.1	3.7	1.09	345	19	145.3
MEAN		6.4	3.5	1.3	439	29	147.3
SD		0.5	0.5	0.4	190	6	6.3

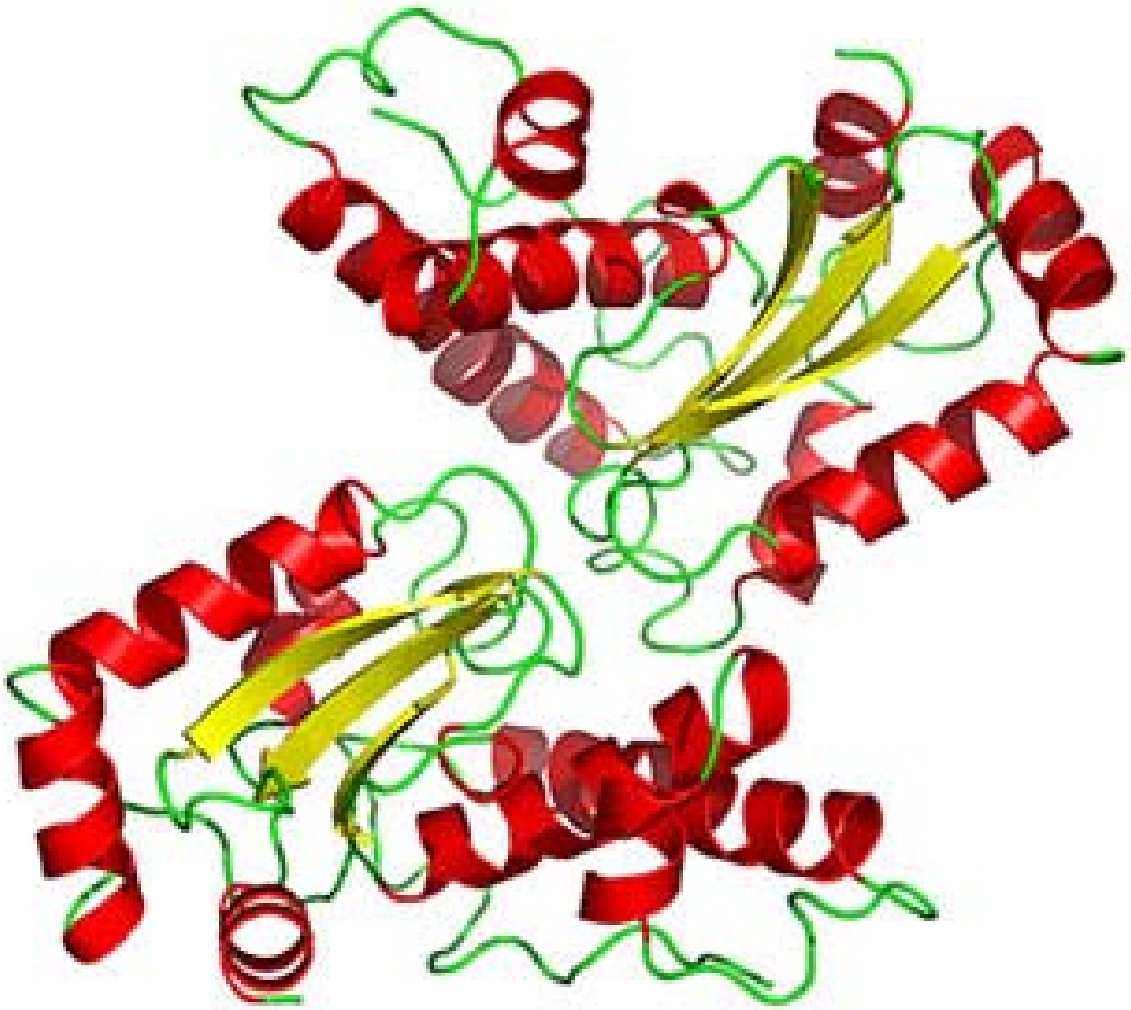
TABLE-8 COMPARISON OF BIOCHEMICAL PARAMETERS IN Stage-2 AND Stage-3 CA BREAST

Sl No	Study group	Protein Mean±SD g/dL	Albumin Mean±SD g/dL	A:G Mean±SD	LDH Mean±SD IU/L	GGT Mean±SD IU/L	SOD Mean±SD U/mL
1	Stage 2	6.7±0.3	3.8±0.4	1.4±0.4	410±228	27±7	160.5±7.9
2	Stage 3	6.4±0.5	3.5±0.5	1.3±0.4	439±190	29±6	147.3±6.3
p-value		0.05	0.06	0.49	0.18	0.28	0.00
Level of significance*		S	NS	NS	NS	NS	S

* S=Significant

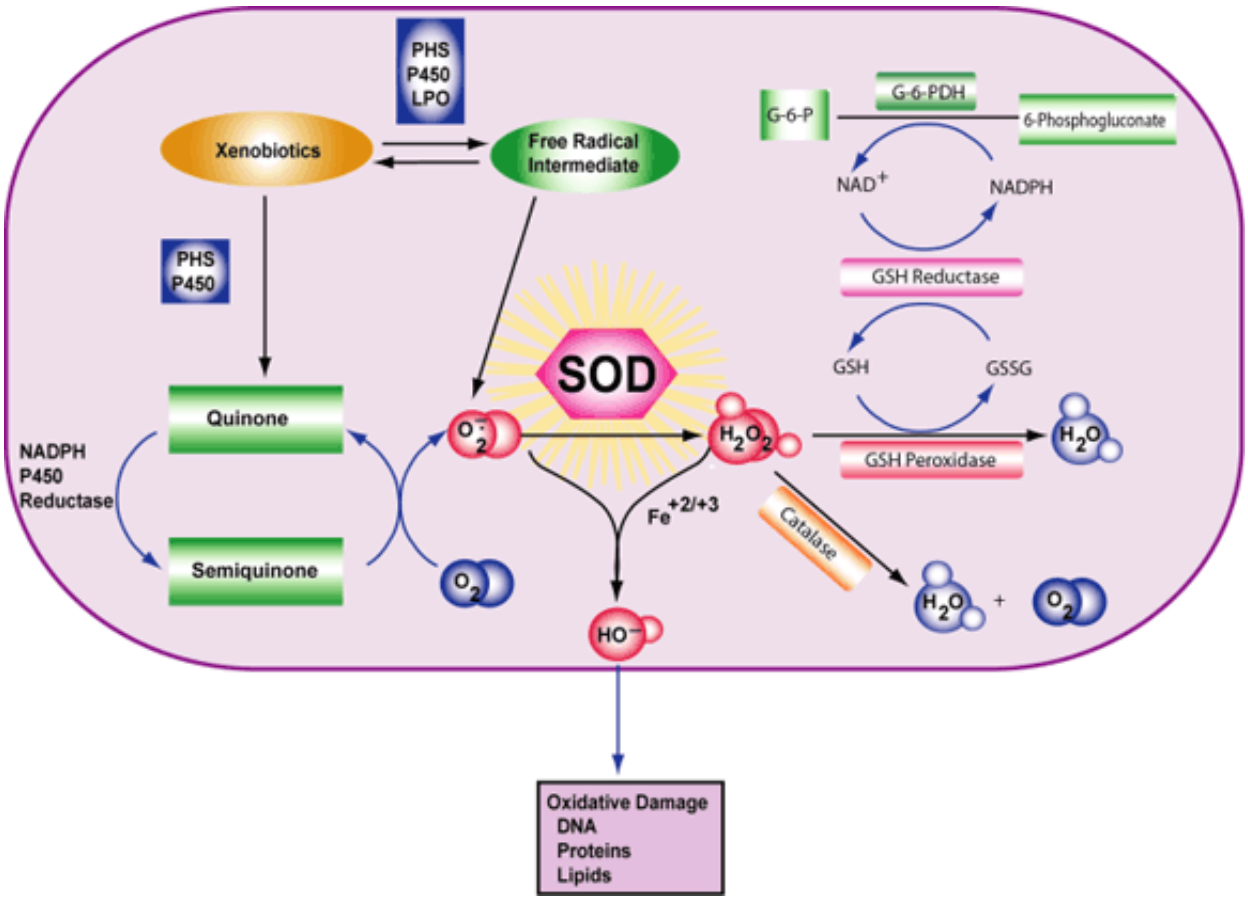
NS= Not significant

SOD STRUCTURE



(courtesy www.3dchem.com)

ROLE OF SOD IN FREE RADICAL SCAVENGING



(courtesy www.sigma aldrich.com)

LDH STRUCTURE (courtesy www.csrii.iit.edu)