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ORIGINAL ARTICLE

Oxidative stress induced damage in benign and malignant breast diseases: histopathological and biochemical aspects

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Increasing evidences indicate involvement of free radicals in the pathogenesis of benign and malignant breast diseases. Free radicals are highly reactive molecules and react with non–radicals in chain reaction leading to formation of new free radicals. If the defense mechanism of body fails to combat them, these free radicals pose a threat of injuring tissues by reacting with cell lipids. Lipids in the cell membrane undergo degradation to form hydroperoxides, which decompose to form a variety of products including malondialdehyde (MDA). MDA therefore was used as a marker to assess oxidative damage of cells and tissues. The aim of the present study was to assess the status of oxidative stress in the patients of benign and malignant breast diseases. Study has been made on the blood samples of 25 cases of benign breast disease and on an equal number of breast carcinoma patients. 20 healthy subjects were taken as the control cases.

Mean MDA levels were significantly raised with depletion of antioxidant activity in all the patients in comparison to their control group suggesting the role of oxidative damage in the aetiopathogenesis of disease.

Key words: Oxidative damage, benign breast disease, breast carcinoma

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Increasing evidences indicate involvement of free radicals in the pathogenesis of benign and malignant breast diseases. Free radicals are highly reactive molecules and react with non–radicals in chain reaction leading to formation of new free radicals. If the defense mechanism of body fails to combat them, these free radicals pose a threat of injuring tissues by reacting with cell lipids. Lipids in the cell membrane undergo degradation to form hydroperoxides, which decompose to form a variety of products including malondialdehyde (MDA). MDA therefore was used as a marker to assess oxidative damage of cells and tissues. The aim of the present study was to assess the status of oxidative stress in the patients of benign and malignant breast diseases. Study has been made on the blood samples of 25 cases of benign breast disease and on an equal number of breast carcinoma patients. 20 healthy subjects were taken as the control cases.

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Oxidative stress is involved in the development of over a hundred pathologies, including cancer (Pincemail et al, 1998). Generally speaking, oxidative stress is defined as an imbalance between the level of prooxidants (activated oxygen species or AOS) and an organism's defence systems (antioxidants), in favour of the former and resulting in irreversible cell damage. When produced in excess, AOS (some of which are free radicals) can seriously alter the structure of biological substrates such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA). Oxidation of the

constitutive bases of DNA (formation of the oxidised base 8-hydroxy-2'guanosine or 8OHdG) by AOS produced in various ways (action of chemical carcinogens, ionizing radiation, ultraviolet radiation) is thus involved in cancer development to a considerable extent (Pincemail et al, 1999). Recent findings in molecular biology have shown, however, that AOS also play other important roles in cells (Gonzalez, 1999).

We have measured the levels of oxidative stress along with the status of antioxidant enzymes in the serum of the benign breast disease and breast carcinoma patients. Healthy subjects were taken as the control cases to assess the oxidative damage in the patients and also to assess clinico-pathological correlation at the time of their initial presentation.

MATERIALS AND METHODS

The study was conducted with the blood from two groups of patients of benign breast disease, n=25 and malignant disease, n=25 admitted in the University Hospital, Banaras Hindu University, Varanasi. Age and sex matched healthy individuals were selected as controls (n =20). Informed consent was taken from the patients or their attendants in all the cases.

Collection of Blood Samples:

Blood samples were collected from the antecubital vein of the above stated subjects. Venous blood sample about 5 ml was collected in the clean and dry plain vials. Thereafter it was separated by centrifugation at 2000 rpm in a clinical centrifuge for 10-15 minutes. The serum, thus removed, was stored at -20 ^{0}C in a sterile plain glass vial until analyzed.

Estimation of Malondialdehyde

Serum malondialdehyde (marker of lipid peroxidation) levels in the cases and controls were assayed by thiobarbituric acid reactive substances (TBARS) technique of Burge and Aust (1978).

Estimation of total antioxidant status (TAS)

The serum total antioxidant status was determined using Randox assay kit (Miller et al, 1993). The assay based on the principle that ABTS (2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase and H_2O_2 to produce the radical cation $ABTS^+$. This has a relatively stable blue green colour which is measured at 600nm. Antioxidant in the cord blood causes the suppression of this color production to a degree which is proportional to their concentration.

Statistical Analysis:

Statistical analysis was done using statistical software SPSS version 10.0. Data was found to be normally distributed. Paired 't' test, ANOVA test and SNK test were applied and the results were referred as mean ± SD and p value was considered significant at 5% level of significance.

Table 1: Comparison of serum MDA and total antioxidants in cases of benign and malignant breast disease.

Group	No. of patients	MDA (mmol/L) Mean±SD	t-value (p-value)	Total Antioxidant (mmol/L) Mean ±SD	t-value (p-value)
Malignant	25	0.905±0.208	2.638 (0.011)	1.253±0.579	1.140 (0.260)
Benign	25	0.730±0.257		1.451±0.646	

Table 2: Comparison of serum MDA and total antioxidants in cases of malignant breast disease and controls

Group	No. of patients	MDA (mmol/L) (Mean ± SD)	t-value (p-value)	Total Antioxidant (mmol/L) (Mean±SD)	t-value (p-value)
Malignant	25	0.905±0.208	3.125 (0.009)	1.253±0.579	1.739 (0.089)
Control	20	0.714±0.180		1.5042±0.509	

Table 3: Comparison of serum MDA and total antioxidants in cases of benign breast disease and controls

Group	No. of patients	MDA (mmol/L) (Mean±SD)	t-value (p-value)	Total antioxidant (mmol/L) (Mean±SD)	t-value (p-value)
Benign	25	0.730±0.257	0.654 (0.521)	1.451±0.646	0.0426 (0.672)
Control	20	0.714±0.180		1.5042±0.509	

RESULTS

The present study included 50 patients of breast disease, constituting 25 cases of benign and an equal number of malignant cases treated in the Department of Surgery, University Hospital, Banaras Hindu University, Varanasi. Study also included 20 healthy females who acted as age matched controls. The consent of each individual was taken purely for research work.

The average age of breast cancer patients in our study was 48.3 yrs. The youngest patient of breast cancer was 35 yrs old while the oldest patient was of 64 yrs. The average age of patients with benign breast disease was 32.3 yrs. The youngest and oldest being of 14 yrs and 45 yrs respectively. These figures depict that relatively younger subset of female population is exposed to benign breast diseases while the older females were exposed to malignant disease. While more than half of the patients of breast carcinoma were post menopausal , all the patients in benign breast disease group were pre-menopausal.

The most common presentation for breast disease whether benign or malignant in this study was a palpable lump (100%). Even distribution of involvement of side was seen in benign breast disease and malignant group. Majority of the patients in both the groups had left sided disease. Histologically almost all the cases (24 out of 25) of carcinoma breast were infiltrating ductal carcinoma while histopathology of benign breast disease revealed fibroadenoma in more than half of the cases. All the patients in the study had either stage II or stage III disease. 15 (60%) out 25 patients of breast cancer in this study had stage III disease.

The serum concentration of malondialdehyde was significantly higher in carcinoma patients in comparison to those in benign and control group. Raised level of malondialdehyde in breast cancer

patients is marker for higher levels of free radical damage in these patients.

The estimation of total antioxidant which denotes the resistance in body to oxidative damage revealed lower concentration in malignancy as compared to benign group and controls.

The combination of higher concentration of free radicals and lower values of antioxidants concentration in cases of breast cancer creates a picture of increased oxidative stress. Oxidative stress by definition is the difference between pro-oxidants and antioxidants, which in this study is observed to be higher in breast carcinoma as compared to benign breast diseases.

DISCUSSION

Oxidative stress is a condition which modifies the normal intracellular balance between oxidant substances produced during aerobic metabolism and antioxidant system processes which perform the function of neutralization, putting a series of protective mechanisms, of both an enzymatic and non-enzymatic nature, in action. Enzymatic systems include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). In non-enzymatic systems, the most important molecules are glutathione, alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), flavonoids, the phenol compounds and the minerals zinc (Zn), copper (Cu) and selenium (Se) (Halliwell, Gutteridge, 2007). Numerous physiological and pathological processes such as ageing, excessive caloric intake, infections, inflammatory disorders, environmental toxins, pharmacological treatments, emotional or psychological stress, ionizing radiation, cigarette smoke and alcohol increase the bodily concentration of oxidizing substances, known as reactive oxygen species (ROS) or, more commonly, free radicals. These are chemical species which are highly reactive owing to the presence of free unpaired electrons. An increase in free radicals compromises the delicate homeostatic mechanisms which involve neurotransmitters, hormones, oxidizing substances and numerous other mediators. Owing to their structure, which is rich in double bonds, polyunsaturated fatty acids (PUFAs) render cellular membranes vulnerable to damage from free radicals, causing peroxidation. The damage induced by lipid peroxidation renders the cell unstable, and therefore compromises fluidity, permeability, signal transduction and causes receptor, mitochondrial DNA and nuclear alterations.

In the present study, mean concentration of MDA was compared between benign (0.730±0.257 mmol/L) and malignant (0.905±0.208 mmol/L) groups, a statistically significant difference was established (p=0.011). The analysis points to higher concentration of MDA in serum of malignant patients than those in benign breast disease group. Similarly, comparison of mean concentration of MDA of breast cancer patients and control group revealed statistically significant difference (p=0.009). The mean concentration of MDA in patients of breast cancer was 0.905±0.208 mmol/L and in control group 0.714±0.180. This reinforces the higher concentration of free radicals in breast cancer patients.

Total antioxidant status of an individual is a measure of defense against free radical damage. The mean concentration of total antioxidants was lower in carcinoma breast patients (1.253±0.579 mmol/L) than their benign counter part (1.451±0.646 mmol/L) and healthy control (1.5042±0.509 mmol/L). These findings depict a weak protection offered by body to the exposure of oxidative damage by free radicals and points

towards the significant role of free radicals in carcinogenesis.

CONCLUSION

Our study indicates that there is an association between increased oxidative stress and the benign breast disease and breast carcinoma disorders. Morphological, biochemical and molecular studies reveal that oxidative stress plays a primary role in the development of degenerative changes in the cells and tissues of our body. Since all the cells and tissues of our body are also equipped with anti oxidative enzymes like super oxide dismutase (SOD), glutathione peroxidase (GPX),glutathione reductase (GRd) and substances like reduced glutathione (GSH), they dispose the free radicals as and when they are generated thereby protecting the cells and tissues from the oxidative attack. Normally a balance is maintained between the oxidative attack of the free radicals and the antioxidative defense system prevailing in the cells and tissues of body. But when the balance is tilted more towards the generation of free radicals, then degenerative changes cause many degenerative diseases including cancer.

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