Relationship of lipid profile and antioxidant enzymes in women with breast cancer

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Abstract
Introduction- In breast cancer patients, we examined the byproducts of lipid profile and the antioxidant enzyme activity. Female's levels of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol are associated with the development of breast cancer, allowing us to investigate their potential significance in breast cancer prevention.

Materials and methods - 44 breast cancer patients were included in the study. 44 healthy female as control and 44 female newly diagnosed breast cancer patients were involved in this study. Serum samples of Breast cancer patients were taken from women.

Results - 44 breast cancer patients in total took part in this prospective research study. Compared to the healthy control patients, the breast cancer patients had decreased catalase activity, but higher levels of superoxide dismutase, glutathione peroxidase, and glutathione transferase. Patients with breast cancer had higher serum levels of lipid profile. Furthermore, neither the blood levels of HDL-C nor the LDL-C were significantly high between breast cancer patients and healthy people.

Conclusion -This work supports the idea that the generation of lipid in contents in tumor associated patients itself is of main relevance in the carcinogenesis by showing that the antioxidant defense system is altered in malignant breast tissues.

Key words- Breast Cancer, Antioxidants, Catalase , Superoxide Dismutase

1.0 Introduction

Oxidative stress is the term for a change in the pro-oxidant/antioxidant balance that favors the pro-oxidant side[1]. According to several lines of research[2,3,4], cellular oxidative stress plays a significant role in the development of cancer. Current theories suggest that pro-oxidant states contribute to the promotion of tumours[5,6]. The mechanisms by which pro-oxidant conditions may impact the fate of cancers remain poorly described despite many years of ongoing research. It has been demonstrated that a variety of experimental liver cancers exhibit increased antioxidant activity and decreased potential for lipid contents [7,8]. As a result, it has been hypothesized that the formation of reactive oxygen species and altered antioxidant functions enhance the neoplastic growth of started cells[9,10,11]. However, it is unclear if the increased antioxidative capacity applies to other forms of experimental tumors, not to mention the wide variety of human malignancies.

Breast cancer is regarded as an opulent illness that typically results in a high fat diet and obesity and it is linked to obesity and weight gain is still a mystery [12]. Women's levels of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol are associated with the development of breast cancer, allowing us to investigate their potential significance in breast cancer prevention. Several cellular defense systems, including enzymatic (glutathione peroxidase, superoxide dismutase) and non-enzymatic (vitamin E, vitamin C, glutathione) elements, regulate the amounts of free radical molecules in the body[13]. Total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, total antioxidant capacity (TAC), and glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity is altered in the serum of breast cancer patients (n = 30) and healthy subjects (n = 100) and were the main objectives of the current study. The antioxidant defense system in breast cancer has not been thoroughly investigated, despite the large number of studies on lipid levels in patients having tumor tissues. In the current work, we looked at the levels of antioxidant enzyme activity in human breast cancer patients.

2.0 Materials and methods

The study protocol was approved by the hospital ethical board of Index Medical College, Hospital and Research Centre, Indore (M.P) - 452016, and conducted in the same hospital fromJan 2022 to Aug. 2022 and all participants provided their informed consent to participate. Serum samples of Breast cancer patients were taken from women. 44 breast cancer patients were included in the study. 44 healthy female as control and 44 female newly diagnosed breast cancer patients were involved in this study. Patients with breast cancer were classified using the TNM system. There were 14, 8 and 8 patients classified as stages II, III and IV, respectively. All patients were studied prior to treatment. None of the patients were using oral contraceptives, hormones, or vitamins, and all were non-smokers.

The total number of parameters from each sample that were ultimately evaluated. All the patients were suffering with breast cancer and having oestrogen and progesterone receptor positive ductal carcinomas according to histology department of Index Medical College, Hospital and Research Centre, Indore (M.P) - 452016. For this a portion of the tumor sample was used and another portion was used for histology. For comparison, a sample of the mammary gland that wasn't malignant was extracted.

2.1 Collection of samples

2.1.1Blood sample

Blood samples from both breast cancer patients and healthy subjects were collected after an overnight fast (1224 h) into tubes. The samples were centrifuged at 3000x g for 15 min, serum was removed with pipette, and stored at -80^{0}C for the following assays.

2.1.2 Methods

The assays for measurement of superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) and other were performed as per the manual provided in the Cayman Chemical Company, India. Serum total cholesterol (TC), triglyceride (TG), HDL- cholesterol (HDL-C), LDL-cholesterol (LDL-C) and biochemical parameters were measured Enzymatically on the Mispa Nano autoanalyzer using a commercial kit (Agappe, Switzerland).

In order to estimate the initial velocities, care was taken to carry out all enzyme activity tests under ideal circumstances with regard to incubation time and protein concentration. By inhibiting epinephrine autoxidation, superoxide dismutase (Cu/Zn form) was measured spectrophotometrically[14,15]. The rate at which 15 mmol/1 hydrogen peroxide dissolves at 240 nm was used to measure catalase activity [16]. Purified enzyme preparations were used to create standard curves for the activities of catalase and superoxide dismutase, and the enzyme activities were then expressed as micrograms of the purified enzyme preparation per milligrams of protein. In the analysis, 1 pg of superoxide dismutase preparation (bovine erythrocytes) was equivalent to 3.6 U and 1 pg of catalase preparation (bovine liver catalase) to 2.5 U. The glutathione transferase (with l-chloro-2,4-dinitrobenzene as the substrate) and glutathione peroxidase (with cumene hydroperoxide as the substrate) activities were determined spectrophotometrically by the methods described. The activities of superoxide dismutase and catalase were assessed, while assays for glutathione peroxidase and glutathione transferase were conducted using the 10 000 g supernatant fluid. The biuret method was used to determine the protein content, with bovine serum albumin serving as the reference protein[17].

2.1.3 Statistical analysis

The data for biochemical analysis were expressed as mean±SE. Mann Whitney U-test was used to determine the significance of biochemical parameters among the patient and healthy groups. P value of <0.05 was considered as significant. Data were analyzed using the statistical package program SPSS 10.0.

3.0 Results

A total of 44 healthy controls and 44 women were included in the study with histologically proven cases of breast cancer. In our study, 64% patients were rural and 36% were urban. Mean age of controls were 42.8±4.6 years; and cases were 51.5±15.1(*P*=0.106) years respectively ranging from 37 years to 69 years. The age distribution was almost equal among cases and controls (Table 1). The levels of lipid and the activities of antioxidant enzymes were measured in breast cancer patients.

In the cancerous patients, catalase activity was lower, while those of glutathione peroxidase and superoxide dismutase were slightly increased when compared to the normal healthy control group. The activity of the enzymes related to hexose monophosphate shunt was significantly elevated in the cancerous tissue. Majority of patients (14/44) presented in stage II of breast cancer and 17 in both stage III and stage IV; and 13 were in stage I indicating that even rural patients presented in advanced stages.Total cholesterol, triglycerides, LDL-C levels, and Pearson correlation coefficients were calculated between the parameters of lipids and antioxidant enzymes in the cancerous patients and healthy controls. The activity of both SOD and GSH-peroxidase were measured in serum of cases and was found to be increased in cases as compared. The above data were statistically significant (p<0.001). The analysis revealed a couple of interesting correlations: Glutathione transferase correlated positively with glutathione peroxidase activity both in cancerous patients (r = 0.50, P = 0.04, n = 22) and healthy controls (r = 0.51, P = 0.001, «= I5), and healthy controls there was also a significant correlation

between catalase activity and the content of lipids (0-0.68, P = 0.001, n=22).
HDL-cholesterol, an important component of metabolic syndrome, were compared among cases and controls. Both total cholesterol and triglyceride levels were found to be increased in cases. Total cholesterol level among controls and cases was 132±14 and 298±51; and triglycerides level was 121±18 and 183±76. Even though HDL-C among controls and cases (43±6 and 32±11 respectively) was in normal range, serum HDL-C levels of cases were significantly lower than controls. The above data were statistically significant ($p<0.01$). However, LDL-cholesterol among controls and cases (75±26 and 87±36) was not found to be altered statistically significant ($p>0.01$). Our data thus suggest that breast cancer had comparatively higher total cholesterol and triglycerides.

Table 1- Activities of antioxidant enzymes and lipid profile in patients having breast cancer as compare to healthy patients

	Characteristics & parameters	Healthy patients (n=44)	Breast cancer patients(n=44)	*P* value
1	**Age**	42.8±4.6	51.5±15.1	**0.106**
2	**Age at menarche**	17±5	19±5	
3	**Premenopausal status**	11	13	
4	**Post menopausal status**	33	31	
5	**Total cholesterol**	132±14	298±121	**<0.001**
6	**TAG**	121±18	183±76	**<0.001**
7	**HDL-C**	43±6	32±11	**<0.001**
8	**LDL-C**	75±26	87±36	**0.04**
9	**Superoxide dismutase**	0.19±0.3	0.50±0.12	**0.01**
10	**Glutathione peroxidase**	1.0±0.1	1.6±0.5	**<0.001**
11	**Glutathione transferase**	0.5±0.1	0.7±0.1	**<0.001**
12	**Catalase**	112.25±21.4	63.5± 13.1	**0.001**

Note:-

- Mann–Whitney U test was used as a non-parametric method to analyses group differences.
- p value <0.05 was considered significant
- Diagnostic values of various parameters are expressed as the mean ± SD in between the groups and are also expressed as the median and range. TAG :- Tri acyl glycerol, HDL:- High density cholesterol, LDL:- Low density cholesterol.

4.0 Discussion

According to certain theories, the development of breast cancer may be influenced by a high dietary fat consumption and pro-oxidant conditions[18]. Animal studies have demonstrated that high-fat diets encourage chemically produced and transplanted mammary cancers [19,20,21]. Cancerous breast tissue is persistently high in polyunsaturated fatty acids. Studies on the part of pro-oxidant states in carcinogenesis have been published throughout the past few years. Since the findings appear to differ from study to study [22,23,24], it is challenging to interpret the findings. The research that have been released thus far do not support a broad hypothesis on the role of oxidative stress in the pathobiology of cancer, and the discrepancy is likely caused by the heterogeneity of the tumor tissues. Increased production of lipid products has been observed in some tumor types, such as breast cancer[25] and colon cancer [26]. As a result, in epidemiological studies, the occurrence of breast cancer has been linked to high dietary fat intake [27]. Regarding breast cancer, a relatively consistent finding is the high content of polyunsaturated fat in cancerous tissues [28]. The greater lipid content and high levels of polyunsaturated fatty acids in malignant breast tissue may be due to changes in cellular lipid metabolism in favor of cellular proliferation. The activity of the antioxidant enzyme catalase was lowered in the malignant tissue, and this finding is consistent with that of numerous other diseased conditions, indicating that the cancerous tissue's ability to process hydrogen peroxide may have been altered[29]. Elevated superoxide dismutase, and glutathione peroxidase activities occurred concurrently with the decline in catalase activity. The balance between Cu, Zn-superoxide dismutase, and catalase has been proposed to be a determining factor for cellular sensitivity to a burst of extracellular active oxygen [30], hence this may have biological significance. So, Breast cancer in women has been associated with changes in serum lipid and lipoprotein levels. Previous study reported that prevalence of decreased serum HDL- cholesterol levels with higher cholesterol and triglyceride levels is increasing in parallel with increasing breast cancer incidence worldwide [30,31].

In our study, when compared with breast cancer patients, total serum cholesterol and triglyceride levels were increased in cases; whereas HDL-cholesterol, though being in the normal range, was found to be decreased in cases as compared to controls. However, LDL-C level did not differ between the two groups. Considering the data presented in this study, we suggest that free radicals induce peroxidation of unsaturated fatty acid in patients with breast cancer and that obesity might be associated with development of breast cancer.

Free radical generation is controlled by a large number of antioxidant systems that act as protection against free radicals. The disturbance of the pro-oxidant/ antioxidant balance, resulting from increased free radical production, antioxidant enzyme inactivation, or excessive antioxidant consumption, is a causative factor in oxidative damage. Therefore, measurement of the total antioxidant capacity in biological samples along with activities of various antioxidant enzymes

has been developed. The level of and activity of both SOD and GSH-peroxidase decreased in breast cancer patient as compared to the healthy control. Our report was in accordance with the previous studies[32,33]. Decrease in antioxidants may be due to enhanced accumulations of free radicals.

To explain our findings, and as it was previously documented[34], the breast cancer patients in the current investigation had higher serum levels of lipid profile. This shows that the growth of malignant tissue causes a rise in serum concentrations of lipid peroxidation products, which may, potentially, be caused by a rapid turnover of membrane lipids. Given that polyunsaturated fatty acids are more effectively absorbed into cellular lipid than saturated fatty acids, this may also be a factor in the high quantities of these fatty acids seen in malignant breast tissue. Research indicates that membrane lipid turnover is enhanced in malignant breast tissue. The rate of glycolysis has been found to be enhanced in malignant tissue, and this study's findings that the hexose monophosphate shunt is more active than usual support changes in glucose metabolism[35,36].

5.0 Conclusion

In light of the findings of this investigation, we hypothesized that free radicals cause increase in the levels of lipid profile contents in breast cancer, which is accompanied by impaired enzymatic antioxidant activity, and that may rise in response to inflammation. To investigate the correlation between oxidative stress, lipid profile, and antioxidant status in connection to breast cancer patients and healthy controls, research involving more patients.

6.0 References

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