www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i4.98

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Study of Antioxidant enzymes- Superoxide dismutase, Catalase and Glutathione Peroxidase level in Breast cancer

Authors
Parijat Suryavanshi[1], Vinod Jain[2], Anoop Singh[3], Gitika Nanda Singh[4]
Department of General Surgery, King George's Medical University, Lucknow, India

Abstract
There are several natural occurring antioxidants in our body which serve to counteract against free radicals generated in our body as byproducts of various metabolic reactions. Any deficiency of these antioxidant in body would lead to increased free radical damage at cellular level which may contribute to mutations leading to oncogenesis. Majority of cancers have been shown to have some imbalance in antioxidants levels. Free radicals- antioxidant imbalance, if proven, may highlight the importance of antioxidant supplementation in prevention and as an adjunct to treatment of cancer. Here we attempt to study antioxidant enzymes namely- superoxide Dismutase, Catalase and Glutathione peroxidase, level in breast cancer patients to find any association with cancer.
Keywords: *Breast cancer, Antioxidants, free radicals, Superoxide Dismutase, Catalase, Glutathione peroxidase.*

Introduction

Breast cancer has ranked number one cancer among Indian females with age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. With an annual incidence of approximately 1,44,000 new cases of breast cancers in India, it has now become the most common female cancer in urban India[2].

Majority of cancers have some imbalance in antioxidant enzyme levels compared with the cell of origin. Normal cells are protected by antioxidant enzymes from the toxic effects of high concentrations of reactive oxygen species generated during cellular metabolism. Even though cancer cells generate reactive oxygen species, it has been demonstrated biochemically that antioxidant enzyme levels are low in most animal and human cancers. Using immunohistochemical techniques, early lesions of human and animal cancers were demonstrated to have low antioxidant enzymes, thus suggesting a role for these enzymes in the genesis of cancer. Majority cancers, except the granular cell variant of human renal adenocarcinoma, examined showed both low catalase and glutathione peroxidase levels, suggesting that most cancer cell types cannot detoxify hydrogen peroxide[3]. These researches could pave way for newer cancer therapies based on modulation of cellular redox state. Present is study is to quantify the levels of Superoxide Dismutase, Catalase and Glutathione peroxidase in blood of breast cancer patients and their association with breast cancer.

Materials and Methods

152 histo-pathologically proven breast cancer patients were included in this study. Patients with documented evidence of any immunosuppressive condition, coexistent other malignancy, history of antioxidant supplementation, exposed to metal industries or on chemotherapy were excluded from this study. Equal number of healthy controls, with no history of any cancer, were included in this study. Informed consent was taken and 5 ml peripheral venous blood sample was drawn under aseptic precautions for analysis. Analysis of antioxidant enzymes level (SOD, Catalase, Glutathione peroxidase) was done in blood lysate. Blood lysate superoxide dismutase (SOD) activity evaluated as per the method of McCord and Fridovich (1969)[4], Blood lysate catalase activity determined as per the method of Aebi and Suter (1974)[5] and Blood lysate Glutathione peroxidase activity determined as per the method of Pagila & Valentine (1967)[6].

The results are presented in mean ± SD and percentages. The Chi-square test used to compare the age groups between cases and controls. The one way analysis of variance used to compare the antioxidant enzymes among the stages of cancer. The binary logistic regression used to find the significant factors associated with breast cancer. The odds ratio (OR) with its 95% confidence interval (CI) calculated. The p - value <0.05 considered significant. All the analysis carried out on SPSS version 16.0 (Chicago, Inc., USA).

Results

A total of 152 breast cancer patients (cases) with 152 controls were included in the study. Table-1 shows the age distribution of cases and controls. More than one third of the cases (38.8%) and controls (39.5%) were between 41-50 years. However, 27% of cases and controls were between 30-40 years. The mean age of cases and controls was 49.32±11.07 and 48.68±10.97 years respectively.

Table 2 shows level of antioxidant enzymes in cases and control and their comparison. The SOD was found to be significantly (p < 0.001) lower among the cases (9.49±6.25, 95%CI=8.49-10.49) compared to controls (27.04±4.39, 95%CI=26.35-27.73) (unit/ml). The catalase was found to be significantly (p < 0.001) lower among the cases (0.09±0.08, 95%CI=0.07-0.10) compared to controls (0.29±0.24, 95%CI=0.26-0.33) (unit/ml). The Glutathione peroxidase was found to be significantly (p < 0.001) lower among the cases (16.96±10.20, 95%CI=15.33-18.60) compared to controls (46.70±14.18, 95%CI=44.43-48.97) (nanomol NADPH oxidase/min/mg protein).

Table 3 shows breast cancer stage wise analysis of antioxidant enzyme levels. In Stage I breast cancer patients (n=6) SOD, catalase and GPx levels are 23.49±12.39 units/ml, 0.12±0.09 units/ml, 28.64±19.04 nanomol NADPH oxidase/min/mg protein, respectively

In Stage II breast cancer patients (n=32) SOD, catalase and GPx levels are 12.32±4.62 units/ml, 0.11±0.09 units/ml, 19.99±10.21 nanomol NADPH oxidase/min/mg protein, respectively. In Stage III breast cancer patients (n=63) SOD, catalase and GPx levels are 9.14±4.79 units/ml, 0.08±0.09 units/ml, 16.45±9.05 nanomol NADPH oxidase/min/mg protein, respectively. In Stage IV breast cancer patients (n=51) SOD, catalase and GPx levels are 6.61±4.85 units/ml, 0.06±0.04 units/ml, 15.03±9.43 nanomol NADPH oxidase/min/mg protein, respectively.

Table-1: Age distribution of cases and controls

Age in years	Cases (n=152)		Controls (n=152)	
	No.	%	No.	%
30-40	41	27.0	41	27.0
41-50	59	38.8	60	39.5
51-60	31	20.4	30	19.7
>60	21	13.8	21	13.8
Mean±SD	49.32±11.07		48.68±10.97	

Table 2: Comparison of enzyme levels in cases and controls

	Cases	Control	
Superoxide Dismutase (unit/ml)	9.49±6.25	27.04±4.39	p <0.001
Catalase (unit/ml)	0.09±0.08	0.29±0.24	p <0.001
Glutathione Peroxidase (Nanomol NADPH oxidase/min/mg protein)	16.96±10.20	46.70±14.18	p <0.001

Table-3: Comparison of antioxidant enzymes with stages among the cases

Stage	SOD (unit/ml)	Catalase (unit/ml)	GPx(nanomol NADPH oxidase/min/mg protein)
I(n=6)	23.49±12.39[a]	0.12±0.09[a]	28.64±19.04[a,b]
II(n=32)	12.32±4.62[a]	0.11±0.09	19.99±10.21[a,b]
III(n=63)	9.14±4.79[a]	0.08±0.09	16.45±9.05[a]
IV(n=51)	6.61±4.85[a]	0.06±0.04[a]	15.03±9.43[b]
p-value	0.0001*	0.04*	0.01*

Discussion

Although the complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species[7]. Directly or indirectly, these chemical species of oxygen can transiently or permanently damage nucleic acids, lipids, and proteins. Oxidative damage to these cellular macromolecules is implicated in the genesis of diseases, including cancer[8,9]. To protect themselves, body maintains complex systems of multiple types of antioxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST)[10]. These components or enzymes have a major role in counteracting the harmful oxidative damage. Certainly, the genetic polymorphisms of these enzymes and their different expression levels are correlated to the individual's susceptibility to DNA damage and cancer risk.

SOD and Catalase are considered as primary antioxidant enzymes, since they are involve in direct elimination reactive oxygen metabolites. They also act as anti carcinogens and inhibitors at initiation and promotion /transformation stage in carcinogenesis[11]. The second line of defense against ROS is provided by Glutathione enzymes. Glutathione (GSH) is the most abundant thiol in cells that can directly scavenge free radical or act as substrate for Glutathione peroxidase (GPx) or Glutathione-Stransferase (GST) during detoxification of H2O2 and electrophillic compounds. Glutathione peroxidase reduces H2O2 and organic peroxides (ROO) while oxidizing GSH[12]. GSH in conjugation with GPx, plays a central role in defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens. Several studies have shown decrease in antioxidant levels in various cancers including breast cancer[12].

In our study of 152 breast cancer patients and 152 controls, mean age of breast cancer patients were comparable in both groups – cases and controls (49.3 vs 48.6). The blood levels of Superoxide Dismutase, Catalase and Glutathione Peroxidase were found to be significantly low in cases as compared to controls. This finding was found to be statistically significant ($p<0.001$). Sinha et al. and Prabasheela et al. found similar observations in their study[11,13]. Pawlowicz et al., too found similar observation in his study on blood selenium concentrations and glutathione peroxidase activities in patients with breast cancer[14].

In our study, blood level of Superoxide dismutase, Catalase and Glutathione peroxidase is significantly lower in advanced stage of breast cancer as compared to early stages of breast cancer. Several studies correlating antioxidant enzyme levels with breast cancer have shown conflicting results. In a recent study by Yeldu et al.[15] on breast cancer patients of african descent, serum SOD is significantly ($p<0.001$) lower as the in cancer progresses from stage I to IV, while serum activities of CAT, GPX were not significantly ($p>0.05$) different between the stages of the breast cancer. Conversely in a study by Khanzode et al.[16], findings were contradictory. In this study, serum Superoxide Dismutase levels were found to be increased gradually from Stage I to Stage IV. Similar results were present in study by Gibananda et al.[17] in which Superoxide dismutase and Glutathione peroxide levels were increased in breast cancer patients.

Conclusion

In conclusion, our study have found lower level of antioxidant enzymes in breast cancer patients with level significantly correlating with stage of presentation. Higher stage of cancer being

associated with lower level of enzymes. Literature present till date have mixed conclusions. Perhaps such alterations is one of many metabolic alterations in process of either promoting development and/or progression of cancer or could be a consequence of disturbed oxidative - antioxidative balance as tumor develops and progresses. There is a possibility of patient to patient variation in these enzyme levels and quantifying enzyme level as done in our study could help identify patients with low level of antioxidant enzymes who might benefit by antioxidant supplementation

Acknowledgements

The study was conducted within the Department of General Surgery, King George's Medical University, Lucknow, U.P, The authors thank the Department of Surgery .Biochemistry, Radiology and Pathology, King George's Medical University, Lucknow, U.P, for their cooperation and collaboration with this study.

Disclosure: The authors declare no conflict of interest

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