Oxidative Stress and Its Relationship With Adenosine Deaminase Activity in Various Stages of Breast Cancer

Mridula Mahajan · Nitika Tiwari · Ritu Sharma ·
Sukhraj Kaur · Neetirajan Singh

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Abstract Reactive oxygen species (ROS) cause damage to the DNA producing mutations and formation of tumours such as carcinoma of breast. Tumour cells are known to produce ROS at a greater pace than the non-transformed cells. The increased production of reactive oxygen species causes oxidative stress leading to cell proliferation and hence increased inflammatory conditions. The present study was aimed to investigate the role of oxidative stress in the pathogenesis of breast cancer. Females suffering from breast cancer had significantly decreased Superoxide dismutase (SOD) and reduced glutathione (GSH) levels in comparison to normal females. The compromised antioxidant defence system produces the oxidative stress which in turn creates the inflammatory response shown by concomitant increased adenosine deaminase (ADA) activity in female patients. ADA diminishes the protective molecule adenosine. There were significant variations ($p < 0.01$) in ADA activity with different clinical stages (stage 1–4) of breast cancer suggesting thereby that estimation of ADA activity can be used as a diagnostic tool to detect the stage of cancer along with cytological studies. Mastectomy was performed and post-operatively serum SOD and ADA activity and plasma GSH levels were estimated. There was a statistically significant increase in activity of SOD and levels of GSH while serum ADA activity decreased significantly, suggesting thereby that oxidative stress is

responsible for increased cell proliferation and hence the inflammatory conditions in CA breast that got ameliorated post-operatively.

Keywords Breast cancer · Adenosine deaminase · GSH · SOD

Introduction

Cancer is a multistep disease process [1] and is one of the major causes of death worldwide [2]. The term breast cancer refers to the malignant tumor that has developed from cells in the breast. It mostly begins in the ducts of the breast and sometimes in the lobules and rarely in the stromal tissues such as fatty and fibrous connective tissue [3]. Most common sign of this disease is a lump in the breast, spontaneous clear or bloody discharge from nipple, change in size or shape of breast and flattening or redness or pitting of the skin around the breast [4]. Inflammation of breast tissue and fibroadenoma are most common causes of benign breast disease which should be continuously monitored [5].

Experimental and epidemiological evidences implicate the involvement of oxygen derived free radical in the pathogenesis of breast cancer. These free radicals are able to cause damage to membrane, mitochondria and macromolecules including proteins, lipids and DNA and actively take part in cell proliferation. This cascade in turn generates the inflammatory response and causes the progression of the disease. Hence it is very important to check oxidative stress at initial stages.

Elevated activity of SOD has been reported in females suffering from different stages of breast carcinoma (without metastasis) in comparison to normal healthy females [6]. On the contrary, in another study, low activity of SOD

M. Mahajan (✉) · N. Tiwari · R. Sharma · S. Kaur
Department of Biochemistry, Government Medical College,
Amritsar, Punjab, India
e-mail: mahajan.mridula@gmail.com;
mahajanmridula@yahoo.com

N. Singh
Department of Surgery, Guru Nanak Dev Hospital Amritsar,
Amritsar, India

has been reported in this respect [7]. Similar trend has been reported in case of reduced glutathione concentration. Increased [8–10] as well as decreased [11–13] levels of GSH have been reported in patients of breast cancer. This clearly emphasises the need to further evaluate the role of these parameters in breast cancer because increased oxidative stress gives rise to inflammation which could further aggravates the disease. Breast carcinoma involves a cascade of events that are highly inflammatory. Adenosine deaminase enzyme (ADA) catalyzes the conversion of adenosine to inosine which finally gets converted to uric acid [14]. Adenosine is an important signalling molecule that exerts major anti-inflammatory actions in tumorous conditions such as inhibition of tumor infiltration in lymphoid cells [15]. Increased ADA activity could lead to scavenging of important molecule i.e. adenosine. Increased activity of ADA has been reported in breast cancer patients in different stages as compared to controls [16–18]. It is evident that oxidative stress is the generator of inflammatory conditions in breast cancer; hence there is need to establish the robust biomarkers for diagnosis and prognosis of the disease. The present study was aimed to elucidate the role of oxidative stress in relation to ADA in breast cancer.

Materials and Methods

The present study included 60 mammographically, somo-mammographically and cytopathologically diagnosed female patients of carcinoma breast and sixty age matched (>31–60 years) normal healthy females who volunteered were also included in the present study. The patients were recruited from the wards and OPDs of the Department of Surgery, Guru Nanak Dev Hospital, Amritsar after obtaining a written informed consent. These subjects were divided into two groups:

Group 1: This group consisted of age matched healthy females

Group 2: This group consisted of females suffering from breast carcinoma. These female patients were further graded into different clinical stages of breast cancer i.e. stage 1, 2, 3 and 4

Blood sample were taken from female patients preoperatively and after 1 week postoperatively. All the blood samples of patients and healthy females were subjected to analysis. The blood was collected by venupuncture and was allowed to stand for 15 min for clot formation. Then the clotted sample was centrifuged for 10 mins at 3,000 rpm for serum separation and then analysis for serum SOD and ADA activity was done. Sample for reduced glutathione analysis was collected in heparin vial. Serum ADA activity was determined by colorimetric method of Guisti [19], serum SOD activity was determined by modified method of Nandi and Chatterjee [20] while reduced glutathione levels were determined by the method of Beutler et al. [21]. The respective values of all the three parameters were calculated from the standard curve. Statistical analysis was done between pre operative and post operative patients of carcinoma breast and control subjects by using students *t* test.

Results

Preoperative female patients had significantly low SOD activity ($p < 0.001$) in comparison to normal females (Table 1). Activity improved after mastectomy indicating lowering of oxidative stress. Similar results were observed in case of GSH levels (Table 1).

Serum ADA activity was significantly high ($p < 0.001$) in females patients preoperatively as compared to normal females (Table 1). However, after tumour excision, ADA activity decreased. This shows the decline in inflammatory response because of reduced oxidative stress.

Table 2 shows the variations in serum ADA, SOD and GSH levels in different clinical stages of breast carcinoma. Serum ADA activity increased with the progression of breast cancer as evident from significantly increased ($p < 0.01$) ADA activity in females with clinical stage 4 of this disease. On the other hand, SOD activity and GSH levels were low ($p < 0.01$) in females having clinical stage 4 of breast cancer. This trend is indicative of increased oxidative stress and consequently increased inflammation with the progression of the disease.

Table 1 Serum SOD, GSH levels and serum ADA activity in females of carcinoma breast in comparison to normal females

Parameters → Subjects ↓	Serum SOD activity (U/L)		Plasma GSH levels (mg %)		Serum ADA activity (U/L)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Normal females ($n = 60$)	10.0–15.5	13.1 ± 2.3	22.2–39.2	29.2 ± 3.0	25.0–84.5	49.7 ± 15.5
Females of carcinoma breast ($n = 60$)						
Preoperative cases	4.4–14.5	9.4 ± 2.6*	10.3–20.0	15.0 ± 2.9*	36.6–125	70.0 ± 24.5*
Post-operative cases	8.8–20.0	11.8 ± 2.5	16.8–32.0	23.0 ± 3.8	20–113	55.0 ± 21.7

* $p < 0.001$: significant difference in serum SOD, plasma GSH and serum ADA activity in normal females and preoperative female patients

Table 2 Increased oxidative stress and inflammation with the progression of CA breast in female patients

Parameters Clinical stages of breast cancer	Serum SOD activity (U/ L)	Plasma GSH levels (mg %)	Serum ADA activity (U/L)
Stage 1 range	10.24–14.56	18.26–20.08	25.82–84.23
Mean $\pm$ SD	12.05 $\pm$ 1.81	19.3 $\pm$ 0.93	48.52 $\pm$ 18.5
Stage 2 range	9.93–14.23	12.23–18.56	38.8–92.86
Mean $\pm$ SD	8.74 $\pm$ 1.84	16.23 $\pm$ 2.37	62.82 $\pm$ 20.7
Stage 3 range	8.87–9.24	13.89–20.48	36.62–105.26
Mean $\pm$ SD	7.45 $\pm$ 1.08	15.18 $\pm$ 3.26	70.37 $\pm$ 18.26
Stage 4 range	4.40–6.64	10.24–12.45	59.78–125.2
Mean $\pm$ SD	5.6 $\pm$ 1.10*	11.32 $\pm$ 1.03*	94.86 $\pm$ 2.92*

* $p < 0.01$: significant decrease in serum SOD activity and plasma GSH levels from stage 1–4

* $p < 0.01$: significant increase in serum ADA activity from stage 1–4

Discussion

Breast cancer is a multifactorial disease process associated with increased oxidative stress and inflammation. In the present study, role of oxidative stress has been investigated in relation to ADA in breast cancer patients.

Superoxide dismutase is an important antioxidant enzyme which decomposes the harmful superoxide anions into hydrogen peroxide thus protects the body from the action of free radicals. In the present study, significantly low SOD activity has been observed in female patients suffering from carcinoma breast both pre as well as post operative in comparison to healthy females. These results are in agreement with those reported by other workers [7], who also observed significantly low SOD activity in breast cancer patients as compared to normal subjects. Activity was relatively more depressed in pre operative female patients. Reduced SOD activity might be responsible for excessive accumulation of superoxide anions leading to increased free radical mediated injury. Increased free radical production has been shown to be responsible for chromosomal damage leading to mutagenicity, cell proliferation and carcinogenesis. SOD activity showed marked improvement after mastectomy indicating the lowering of oxidative stress.

Another potent antioxidant molecule is reduced glutathione. It acts as reductant which converts hydrogen peroxide into water and reduces lipid peroxidation products into their corresponding alcohols and thus mediates protective action. In the present study, significantly low GSH levels were observed in female patients of carcinoma breast as compared to normal females. Other workers also reported similar findings [11, 12]. Similar to SOD the GSH levels also seemed to improve in female patients after surgery in comparison to the pre operative levels. The

increase in SOD activity and GSH levels post-operatively was indicative of restoration of antioxidant defence system in these patients

An important aspect of cancer cells is their penetration beyond original tumour which consequently produces various clinical symptoms. There are normally 4 clinical stages of breast cancer that have been highlighted in the literature that is clinical stage 1 to 4. We observed significantly decreased SOD activity and GSH levels in patients belonging to clinical stage 4 as compared to those having stages 1, 2 or 3 of breast cancer. Antioxidant status was highly depressed in advanced stages of breast cancer as compared to initial stage. Marked oxidative stress in stage 4 of breast cancer indicated advancement of the disease, hence checking oxidative stress at initial stage could be helpful for controlling the progression of the disease.

Prevalence of oxidative stress gives rise to inflammation. Adenosine is an endogenous purine nucleoside generated from ATP [18]. This is an important signalling molecule that exerts major anti-inflammatory action. Tumours have high concentration of adenosine which could inhibit the function of tumour infiltrating lymphoid cells [14]. This could be taken as a compensatory mechanism of the body against the tumorous conditions. ADA enzyme scavenges adenosine by degrading it into inosine which gets finally converted into uric acid [15]. Significantly increased activity of ADA was observed in female patients as compared to healthy females. Increased ADA activity in breast cancer patients has also been reported by others workers. Walia et al. (1995) reported increased ADA activity in breast cancer patients as compared to age matched normal subjects. They concluded that ADA is a better probable parameter for detection of breast cancer [17]. In another study, higher values of ADA and 5' NT were observed in breast cancer patients and a positive correlation was reported between ADA activity and 5' NT in those patients [18]. Increased ADA activity in patients is indication of decreased availability of adenosine which is a protective molecule in case of tumorous condition. ADA activity seemed to improve after mastectomy indicating relatively less inflammation and better clinical condition after surgery.

Inflammation progresses with the advancement of the disease as increased ADA activity was observed in patients with clinical stage 4 as compared to those having stage 1, 2 or 3 of breast cancer. Berrie [18] showed maximum activity of ADA at stage 3 of breast cancer, however during their study probably they did not come across any patient at stage 4 of carcinoma breast. Physiologically, serum ADA activity tends to increase with advancing age, hence it is suggested that necessary life style modifications should be adopted such as regular exercise, consumption of antioxidant rich and fat free diet to combat the oxidative stress and hence risk of breast cancer with aging.

In conclusion, our study confirmed the role of oxidative stress in the pathogenesis of breast cancer. This oxidative stress gives rise to inflammatory conditions that worsen the diseased process. ADA seems to be a promising marker of inflammation in breast cancer thereby suggesting that it can be used as a diagnostic tool to detect the stage of breast cancer along with cytopathological studies. However, it is pertinent to mention here that in some patients where cytopathological examination cannot arrive at definite conclusion, the variations in serum ADA activity have been found to be the promising parameter for arriving at early diagnosis and following the prognosis of the disease as well.

References

1. Guyton KZ, Kensler TM. Oxidative mechanisms in carcinogenesis. *Br Med Bull*. 1993;49(3):523–44.
2. Mealiffe ME, Stokowski RP, Rhees BK, Prentice RL, Pettinger M, Hinds DA. Assessment of clinical validity of a breast cancer risk model combining genetic and clinical information. *J Natl Cancer Inst*. 2010;102:1618–27.
3. Benson JR, Jatoi I, Keisch M, Esteva FJ, Makris A, Jordan VC. Early breast cancer. *Lancet*. 2009;373(9673):1463–79.
4. Saslow D, Boetes C, Burke W, Harms S, Leach MO, Lehman CD, et al. American cancer society guidelines for breast screening with MRI as an adjunct to mammography. *CA Cancer J Clin*. 2007;57:75–89.
5. Guray M, Sahin AA. Benign breast diseases: C Breast disorders: introduction. *Merck manual of diagnosis and classification, diagnosis and management. Oncologist*. 2006;11:435–49.
6. Seth RK, Kharb S, Kharb DP. Serum biochemical markers in carcinoma breast. *Indian J Med Sci*. 2003;57(8):350–4.
7. Negahdar M, Abtahi H, Sadeghi MR, Javadi E, Aghvani T, Layegh H. Blood superoxide dismutase and catalase activities in women affected with breast cancer. *Iran J Public Health*. 2005;34:39–43.
8. Sharma P, Sharma DC, Mishra S. Studies of biochemical parameters in breast cancer with and without metastasis. *Indian J Clin Biochem*. 2004;19(1):71–5.
9. Perquin M, Oster T, Maul A, Froment N, Bagrel D. The glutathione related detoxification system is increased in human breast cancer in correlation with clinical and histopathological features. *J Can Res Clin Oncol*. 2001;127(6):368–74.
10. Aboughalia AH, Fouad IM. Glutathione and its metabolizing enzymes in patients with different benign and malignant disease. *Clin Biochem*. 2000;33(8):657–62.
11. Kumaraguruparan R, Subapriya R, Kabalimoorthy J. Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast. *Clin Biochem*. 2002;35(4):275–9.
12. Sieger S, Bose CP, Younes H, Younes E. Glutathione and GSH dependent enzymes in the tumours and non tumours mucosa of human colon and rectum. *J Cancer Res Clin Oncol*. 1984;107(288):340.
13. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. *J Lab Clin Med*. 1985;105(5):581–4.
14. Lokshin A, Tatiana R, Huang X, Zacharia LC, Jackson ED, Gorelik E. Adenosine-mediated inhibition of the cytotoxic activity and production by activated natural killer cells. *Cancer Res*. 2006;66:7758–65.
15. Eltzhig HK, Weissmüller T, Mager A, Eckle T. Nucleotide metabolism and cell cell interactions. *Methods Mol Biol*. 2006;341:73–87.
16. Aghaei M, Tehrani FK, Salami S, Atrib M. Adenosine deaminase activity in the serum and malignant tumors of breast cancer: the assessment of isoenzyme ADA1 and ADA2 activities. *Clin Biochem*. 2005;38(10):887–91.
17. Walia M, Mahajan M, Singh K. Serum adenosine deaminase in breast cancer patients. *Indian J Med Res*. 1995;101:247–9.
18. Berrie C. Blood serum enzyme activity profiles as indicators of prognosis and chemotherapy response in breast cancer. <http://www.docguide.com>. Accessed 18 Feb 2004.
19. Guisti G. Adenosine deaminase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*, 2nd ed. New York: Academic Press; 1974. p. 1092–9.
20. Nandi A, Chatterjee IB. Assay of superoxide dismutase activity in animal tissues. *J Biosci*. 1988;13:305–15.
21. Beutler E, Duron O, Kelly BM. Improved method of determination of blood glutathione. *J Lab Clin Med*. 1964;61:882–8.