Medico Research Chronicles

Original Research Article

ALTERATION OF GLUTATHIONE DEPENDENT ENZYMES IN PRE AND POST OPERATIVE BREAST CARCINOMA

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Submitted on: October 2014
Accepted on: October 2014
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Abstract:
Ubiquitous free radical (Reactive Oxygen Species) production occurs continuously in our body from metabolic pathways and electron transport chain. Cigarette smoke, automobile-exhaust, industrial effluents, etc., are polluting the environment with the free radicals. Glutathione is the most abundant low molecular mass molecule that provides reducing equivalents to protect the cells against free radicals (oxidative stress). The activity of glutathione dependent enzymes (Glutathione reductase, glutathione peroxidase and glutathione – S – transferase) were estimated in 50 histopathologically proven breast cancer patients before and after surgery and compared with age matched control. The activity of Glutathione related enzymes were found to be significantly increased in breast cancer patients when compared to control and mild decrease after surgery indicating prognosis after treatment.

Key Words: Free radicals, Oxidative stress, Glutathione (GSH), Glutathione Peroxidase (GPx), Glutathione –S- transferase (GST), Glutathione Reductase

Introduction:

Breast cancer is the most common malignant tumor in our country. Recently, people suffering from and dying of breast cancer are in the ascending trend. Though early diagnosed, mortality due to metastasis of breast cancer still continues. Antioxidant enzyme represents an important defense mechanism against free radicals (oxidative stress). ROS are involved in initiation and promotion of carcinogenesis and also involved in inactivation or loss of certain tumor suppressor genes (1, 2). Glutathione act against free radical damage by participating in a variety of metabolic processes, transport and detoxification. Under pathological conditions, enzymes activate glutathione level by regulating the content of oxidized and reduced form that is influenced by antioxidant (3). Glutathione, Glutathione reductase, Glutathione peroxidase (GPx) and Glutathione – S – transferase (GST) are the main components of the glutathione-dependent anti-peroxide system. Glutathione is substrate for GPx and GST during the detoxification of hydrogen peroxide - part of basic antioxidant system in humans (3, 4).

Glutathione peroxidase constitutes a family of enzymes, which are capable of

reducing variety of organic and inorganic hydroperoxides to the corresponding hydroxy compounds utilizing GSH. There are several tissues specific GPx's that exhibit tissue specific function. GPx enzyme was discovered as a factor preventing lipid peroxidation and found to be involved in the protection of bio membranes against oxidative stress. The isoenzymes of GPx play a vital role in regulating the delicate regional redox balance and involved in cell-signaling and thereby evoke several cellular responses like programmed cell death, proliferation & cytokine production (5). Glutathione – S – transferases are three enzyme families – cytosolic, mitochondrial and microsomal – that detoxify noxious electrophilic-xenobiotics such as chemical carcinogens, environmental pollutants and antitumor agents. Moreover, they protect against reactive compounds produced *in-vivo* during oxidative stress by inactivating unsaturated aldehydes, quinones, epoxides and hydroperoxides (6). Glutathione–S–transferase exerts its protective effects by catalyzing the conjugation of GSH with the products of free radicals and thus represents a second line of defense against the highly toxic spectrum of substances produced by ROS. Both GPx and GST can eventually lower the level of total intracellular GSH (7). In the presence of oxidative stress, GSH concentration decreases but, GSSG (highly cytotoxic) increases because of reduction of peroxides or as a result of free radical scavenging (8). Glutathione disulphide (oxidized glutathione) can also be reduced back to GSH by Glutathione reductase utilizing NADPH as a reductant. Glutathione reductase - a flavo enzyme, when exposed to agents that leads to increased oxidative stress, leads to increase in its mRNA content. Further, experimental data have shown the importance of Glutathione reductase activity in GSH metabolism, demonstrating that the enzymatic activity is regulated in response to stress so, mutations affecting the gene of this enzyme would have deleterious consequences (9). All these scientific facts prompted us to study the alterations in the level of Glutathione dependent enzymes (Glutathione Peroxidase, Glutathione-S-transferase & Glutathione reductase) in pre and post operative breast cancer patients.

Methodology:

Fifty (50) clinical cases required for our study were selected from Aringar Anna Cancer Institute, Kancheepuram. Clinically & histopathologically proven breast cancer patients, who were not on hormone replacement therapy, not on oral contraceptives and non-smokers, were included for our study. None of the clinical subjects included in our study had secondary disorders. Age-matched control (50 N: Number) was included in the study. Informed consent was obtained from all the participants. Human ethical committee clearance for this study was obtained (vide Ref.No.262/E1/08) from the Director, Govt. Arignar Anna Memorial Cancer Hospital, Regional Cancer center, Karapettai, Kancheepuram.

Blood samples were collected, centrifuged for 15 minutes at 3000 rpm and the serum was separated and stored at 4˚C. The activity of Glutathione peroxidase was estimated according to the method of Rotruck *et al* (10) with modification. A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of reduced glutathione. After specified period, the remaining (unutilized) glutathione in the assay system was measured as described by Anderson (11). Glutathione –S – transferase was assayed by the method of Habig *et al* (12) by following the increase in absorbance at 340 nm using 1 – chloro 2, 4 – dinitro benzene (CDNB) as the substrate. The activity of Glutathione reductase (13) was determined spectrophotometrically at 340nm.

Results and Discussion:

Table 1 shows the mean age and body weight distribution of both normal and

breast cancer patients. Table 2 shows the level of glutathione dependent enzymes in normal and breast cancer patients before and after surgery.

Table 1: Age and Body Weight (Mean ± SD, N=50) of Control & Patients with Breast Cancer.

S.No	Parameters	Control	Patient
1.	No. of persons	50	50
2.	Mean age	46 ± 4.3	44± 2.5
3.	Mean body weight	61.8 ± 5.5	62.5 ± 5.7

Table 2: Activity of Glutathione Dependent Enzymes in Patients with Breast Cancer, Before and After Surgery (Mean ± SD, N=50)

Parameters	**Control**	Patients with breast cancer: **Before surgery**	Patients with breast cancer: **After surgery**
Glutathione peroxidase (µmol/min)	**15.83 ± 0.21**	**26.19 ± 1.3***	**23.71 ± 0.12***
Glutathione-S-transferase (µmol/min)	**2.52 ± 0.91**	**8.54 ± 0.67***	**5.37 ± 0.03***
Glutathione reductase (µmol/min)	**0.46 ± 0.02**	**1.78 ± 0.04***	**0.91 ± 0.05***

Glutathione is a polyfunctional non – protein thiol plays a critical role in many biological processes, directly as cofactor in enzymatic reaction and indirectly as redox buffer in mammalian cells (14). Glutathione enzymes play an important role in metabolic detoxification of H2O2. Glutathione, an important substrate for GPx and GST, has been documented to have regulatory effects on cell proliferation (15).

GST plays an important role in the regulation of glutathione levels in cells. This enzyme catalyzes the formation of glutathione conjugates with cytotoxic agents and thereby protects the cells against various cytotoxic effects (16). High concentrations of GST may rapidly detoxify anticancer agents, thereby preventing their cytotoxic action. In the previous study, it was reported that Glutathione – S – transferase activity in malignant tumors of Uterus, Breast and Ovaries were higher than in normal (17). Our results lend credence to these report, that the levels of GST was found to be increased significantly in breast cancer patients before surgery but it was found to be decreased after surgery (mastectomy).

Glutathione reductase maintains the reduced thiols inside the cell and prevents high levels of oxidative stress by counteracting oxidation. In our study, the level of Glutathione reductase was significantly high in breast cancer patients, before surgery as compared to control. After surgery, this enzyme activity was slightly lowered indicating improvement in antioxidant-defense mechanism. GPx - an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and also in maintaining functional integrity of the cell membrane (18). It is suggested that the increase in GPx activity in breast cancer cell lines could be due to increased expression of genomic DNA (19). Thus, the elevation of GPx in breast cancer patient before and after

surgery may be markers of cell proliferation. Furthermore, GPx plays a key role in tumorigenesis by altering the lipoxygenase and cyclooxygenase pathways (20, 21). The rise in the activity of GPx could be due to its induction to counter the effect of increased oxidative stress. Skrydelwska *et al* have reported increased activities of superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase and concomitant decrease in GSH level in cancer tissue. They suggested that the activity of these antioxidant enzymes in cancer cells were increased in order to protect the cells against the oxidative stress/damage (22). Decrease in the GSH level could be due to its increased utilization by the antioxidant enzymes, which are challenging the increased oxidative stress under the influence of ROS. Since GSH depends on glutathione reductase for its regeneration, impaired activity of glutathione reductase might also be a reason for the depletion of GSH (23).

Conclusion:

Glutathione system plays a major role in peroxide inactivation in tumor cells. Impairment of functioning of antioxidant systems may influence cell proliferation and differentiation and promote malignant transformation of cells. In the present study, the activities of Glutathione dependent enzymes were found to be decreased after surgery indicating decrease in oxidative stress in patients after surgery. The results of our study suggest that the parameters of pre & post operative antioxidant status of the breast cancer patients could be reliable biomarkers for early diagnosis and prognosis. Nevertheless, further studies with larger sample size are required to substantiate our suggestion.

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