STM JOURNALS

Research & Reviews: A Journal of Life Sciences
ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print)
Volume 8, Issue 3
www.stmjournals.com

Glutathione peroxidase (GPx) in Breast Carcinoma

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Abstract

Cancer is one of the significant problem from health perspective, every year many females are diagnosed with breast cancer out of which many die because of this disease worldwide. Oxidative stress in human body is a result of imbalance between free radical production and antioxidant defense which may result in carcinogenesis. Primary defense Primary defenses rely on the direct scavenging and inactivation of the reactive oxygen species (ROS) which includes Superoxide dismutase, Catalase, Glutathione peroxidase. In this study Glutathione peroxidase was studied in breast carcinoma and normal healthy controls. For this heparanised blood samples were collected from 60 breast carcinoma and 30 normal healthy control cases. All the data presented in the result of this study was analyzed on the SPSS statistical software version 12.0 and P – values less than 0.01 were considered highly significant. There was significant increase in Glutathione peroxidase in normal healthy controls as compared to pre and post operative cases of breast carcinoma, which proves that primary defense in normal healthy controls is more powerful than pre and post operative cases which help them fight against oxidative stress.

Keywords: *Breast Cancer, Glutathione peroxidase, Oxidative stress*

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INTRODUCTION

Breast cancer, a disease of multifactorial origin have been seen more frequently in women of older age, family history with breast cancer, affluent society, late age at first child birth, early menarche, late age of menopause, nulliparity and exogenous and endogenous hormone exposure [1]. Of the various diseases that affect the breast, cancer represents a significant problem from clinical and public health perspectives. Every year about 9, 00, 000 women are diagnosed with breast cancer and nearly 3,76,000 die of the disease worldwide [2]. Breast cancer is the leading cancer in the affluent population and is second to the cervical cancer which is the most prevalent cancer in the most developing and rural areas of the world. Breast cancer, a disease of multifactorial origin have been seen more frequently in women of older age, family history with breast cancer, affluent society, late age at first child birth early menarche, late age of menopause, nulliparity and exogenous and endogenous hormone exposure [1].

Oxidative stress results when the balance between ROS production and antioxidant defense is lost. The biological consequences are mutations, sister chromatid exchanges, chromosomal aberrations, base modifications, strand breaks, DNA protein cross link, cytotoxity, carcinogenesis and cellular degeneration leading to aging [3,4]. Outcomes are pre malignant lesions, experimental evidence reveals that ROS are involved in the initiation, promotion and progression of carcinogenesis and inactivation or loss of certain tumor suppressor genes [5, 6]. Most of the pathophysiological effects of oxidative stress are mediated by hydroxyl radical OH^{-} [7] Hydroxyl radical production involves the presence of superoxide, hydroxide peroxide and either iron or copper ions via the Fenton and Haber – Weiss reactions. It is also end product of interactions between nitric oxide (NO) and superoxide (O_2^{-}) peroxynitrite the intermediate in this reaction.

Mammalian cells can induce the production of oxidative stress [8,9]. The antioxidants that are a major cell defense against oxidative stress inflicted damage include both low molecular weight free radical scavenger and complex enzymes systems. The former includes exogenous and endogenous antioxidant molecules, such as tocopherol, ascorbate,

β- carotene, uric acid and Glutathione (GSH). The enzymatic defense system involves primary and secondary protection against lipid peroxidation and other oxidative stress [10]. Primary defenses rely on the direct scavenging and inactivation of the reactive oxygen species (ROS) before lipid peroxidation take place, while secondary defense involve excision and repair of the damage. Enzymes involved in primary protection are: 1) Manganese – superoxide dismutase (MnSOD) and copper / Zinc superoxide dismutase (CuZnSOD), which convert superoxide (O_2^-) to Hydrogen peroxide; 2) Catalase (CAT), which scavenges hydrogen peroxide at relatively high concentrations; and 3) Cytosolic glutathione peroxides (GPx1), which scavenges H_2O_2 at relatively low concentrations. Enzymes involved in secondary protection include non – Seleno glutathione S- Transferees (GST) and Seleno – dependent glutathione peroxides (GPxs), which detoxify fatty acid hydroproxides, phospholipids hydroperoxides and cholesterol hydroperoxides. The glutathione peroxides fairly is compromised of four members: 1. Cytosolic glutathione peroxides (GPx1), which is ubiquitously distributed in the cytosol [11]; 2. gastrointestinal tract [12]; Plasma glutathione peroxides (GPx3) [13], which is directed to the extra cellular compartments and expressed in various tissues that are in contact with the body fluids and 4. Phospholipids hydroperoxide protein [14].

To protect against toxic effects of ROS and to modulate physiological effects of ROS, the cell developed an intricately regulated antioxidant defense system. This system is very complex being composed of small molecular weight antioxidant compounds like Vitamins E, C, A and so forth. Primary defense system by superoxide dismutase (SOD), catalase and Glutathione peroxides (GPx) constitute a mutually supportive team of defense against ROS.Glutathione peroxides (GPx) belongs to a family of selenoprotein and plays an important role in defence mechanisms against oxidative damage by catalyzing the reduction of variety of hydroperoxides, using glutathione as the reducing substrate. The cellular GPx, extracellullar GPx, (together known as GPx) and the phospholipids hydroperoxide Ph-GPx are the more commonly known isozymes of Gpx. Each contains a seleno cysteine in its catalytic center. A non –seleno, cysteine dependant GPx has been recently identified.

Cellular GPx is the most characterized form, can react with hydrogen peroxide and organic peroxide like DNA hydroperoxides but not lipid hydroperoxide .GPx as well as bcl 2 , a protoncogene that block apoptotic cell death in multiple contexts can prevent apoptosis caused by oxidative stress .GPx can also be inactivated by NO (15-Michio, 1995).Mitochondrial and cytosolic GPx protects against peroxynitrite – mediated oxidations, functioning as peroxynitrite reductases.Protective action of GPx may be its capacity to destroy clastogenic lipid hydroperoxides [15,16]. GPx activity is highly variable in tumour cells [17,18,19]. A cellular imbalance among antioxidant enzymes and or ROS is considered to contribute to a pro-oxidant environment in cancer [3].These antioxidant enzymes act in a synergistic way to ensure global cell protection. Changes in cellular redox sate related to the altered cystolic and mitochondrial GPx activity will affect cell growth. Growth stimulation may occur when cells are protected from excessive oxidant toxicity but only when a sufficient oxidant signal remains to activate the necessary growth pathways.PH – GPx overexpression carries a poor prognosis in patients with breast cancer, results suggest that PH – GPx expression may be nedded to control the redox status in breast cancer tissues. There is increase in GPx activity after chemotherapy compared to the baseline levels [17] on the other hand increase in GPx level is reported in breast cancer cases compared to the normal healthy controls [18].

Analysis of Results

All the data presented in the result of this study was analyzed on the SPSS statistical software version 12.0. For comparing cancer and normal Non parametric “Wilcoxon Mann Whitney” test was applied because data do not follow normal distribution. For correlating between pre and post operative cases in cancer group Non parametric “Wilcoxon signed rank ”sum test was applied. For c- fos and c- jun as a categorical variable Chi – square test was applied between cancer and normal cases. For finding correlations between c- fos and c – jun with oxidative stress kruskal wallis test was used.

P – values less than 0.05 were considered significant.
P – values less than 0.01 were considered highly significant.
P – values more than 0.05 were considered non – significant.

Determination of Glutathione peroxidase (GPx)

Glutathione peroxidase was determined in heparanised blood Pagalia and Valentine, 1967 [19]. GPX catalyzes the oxidation of glutathione by cumene hydroperooxide. In the presence of glutathione reductase and NADPH the oxidized glutathione was immediately converted to the reduced form with concomitant oxidation of NADPH to NADP. The decrease in oxidation was measured at 340 nm.

The activity of Glutathione peroxidaes (GPx) in hepranised blood was determined according to the method of Pagalia and Valentine, 1967.

Principle

The oxidation of Glutathione (GSH) by cumene hydroperoxidase is catalyzed by Glutathione peroxides (GPx). In the presence of glutathione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance is measured at 340 nm.

$$GR2GSH + H_2O_2 \rightarrow 2\,H_2O + GSSG \rightarrow 2GSH$$

Reagents

The concentration of the reagents in the test tube are as follows: glutathione 4 mmol / L; glutathione reductase (GR) > 0.5 U/L; NADPH 0.28 mmol/L; Phosphate buffer 0.05 mol/l (ph -7.2); EDTA 4.3 mmol/L and cumene hydro peroxide 0.18 mmol/L; diluting reagent and Drabkins reagent. A precombination of glutathione, Glutathione reductase and NADPH were made to constitute a working reagent. Cumene Hydroperoxide 0.18 mmol/L; diluting reagent and Drabkins reagent. a precombination of Glutathione, glutathione reductase and NADPH were made to constitute a working reagent. Cumene hydroperoxide and the working reagent were prepared freshly before every test. The content of one vial of Drabkins reagent was diluted with 480 ml of redistilled water. It was stored in a brown bottle protected from sunlight.

Sample preparation and processing

The heparaized whole blood samples was brought to room temperature 0.05 ml heperanized whole blood was diluted with 1 ml of diluting reagent it was then incubated for 5 minutes and1 ml of double strength Drabkins reagent was added. This was mixed properly. 50 µl of the diluted sample / standard or blank was taken and 2.5 ml of reagent was added and mixed properly just prior of taking the absorbance. Three absorbances were noted using semi auto analyzer. The initial absorbance of sample and reagent blank was read after 1 minute and 2 minutes. Reagent blank value was subtracted from that of the sample / standard.Precaution was taken to assay the sample within 20 minutes of adding Drabkins Reagent.

Calculation

Glutathione peroxides concentration was calculated from the following formula;
GPx U/L of whole blood = 8412 x Δ A 340 nm / minutes x 41.

Glutathione Peroxidase (GPx) in normal healthy controls and in pre and post operative cases of breast carcinoma.

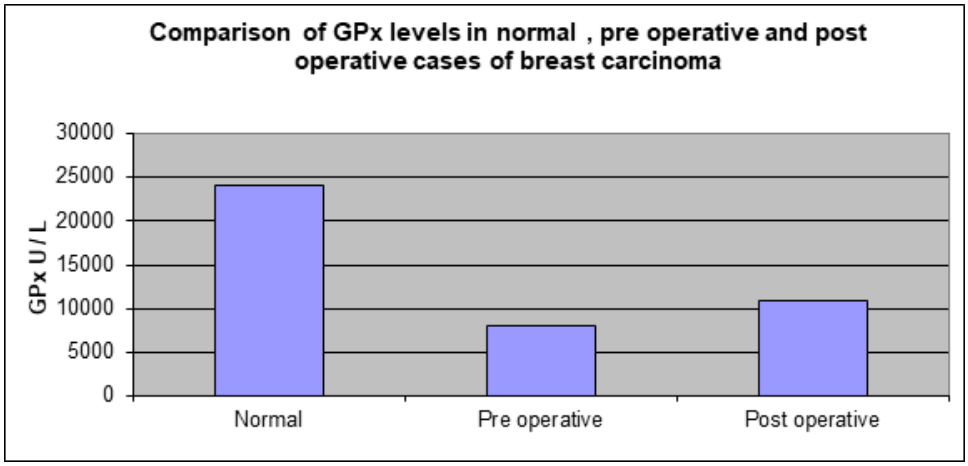
GPx level in pre operative cases of breast carcinoma (Table – 1) varied between (4483 - 12075 U / L) where as the level in normal cases ranged from (11381 - 161064 U / L). Table – 2 show percentage representation of pre operative cases and normal control with GPx levels.

Table 1: *Level of GPx in normal, pre and post operative cases of breast carcinoma.*

	Mean U / L	Standard deviation	Minimum– Maximum U / L	Range U / L
Normal cases (n = 30)	24031.07	37279.48	11381 -161064	149683
Pre Operative cases (n= 60)	7931.67	2198.626	4483 – 12075	7592
Post Operative cases (n=60)	10915.20	2903.645	1069 – 14900	13831

Table 2: *Percentage distribution of GPx in normal, pre and post operative cases of breast Carcinoma.*

GPx LEVELS	Normal controls, n= 30 (100 %)	Pre operative cases, n = 60 (100 %)	Post operative cases, N=60 (100 %)
4000 – 7000	50 %	50 %	5 %
7001 – 10001	23.3 %	23.3 %	35 %
10002 – 13002	26.6 %	26.6 %	35 %
13003 – 16003	0 %	0 %	25 %
16004 – 19004	0 %	0 %	0 %



Result and Discussion

Glutathione peroxidase which is one of the important enzyme of primary defense against oxidative stress is studied here in cases of breast carcinoma. Study was done in the department of Medical biochemistry, Lady Hardinge medical college, New Delhi. Heparinised blood was collected from 60 breast cancer patients and 30 normal healthy controls. Blood samples were collected from pre and post operative cases (15 days after chemotherapy) of breast carcinoma. There was significant increase in glutathione peroxidase in normal healthy controls as compared to pre and post operative cases of breast carcinoma. As seen in the study done on glutathione in breast cancer cases, there was significant increase in glutathione in pre operative cases compared to post operative cases and normal healthy controls. This proves presence of increased oxidative stress in preoperative cases of breast carcinoma, which was not compensated by glutathione peroxidase resulting in carcinogenesis. Steps should be taken to increase primary defense which includes glutathione peroxidase, so that glutathione in pre operative and post operative cases decreases comparatively compared to normal healthy controls.

There was significant increase in Glutathione peroxidase in normal healthy controls (24031.07 U/L) compared to pre (7931.67 U/L) and post operative cases of breast carcinoma (10915.20 U/L). Glutathione peroxidase varied between 11381-161064 U/L in normal healthy controls whereas range in pre operative (4483-12075 U/L) and post operative (1069-14900 U/ L) cases was on the lower side. The above findings show, that first line of defense which includes glutathione peroxidase are not present to counter increase in gluatathione in pre and post operative cases of breast carcinoma.

CONCLUSION

This study was done at the Department of Medical biochemistry, Lady Hardinge Medical College, New Delhi. According to the findings there was significant increase in glutathione peroxidase in normal healthy controls, which proves that presence of first line of defense help them fight against oxidative stress which may be the probable cause of initiation, promotion of carcinogenesis. Steps should be taken to increase presence of glutathione peroxidase in pre and post operative cases to counter increased oxidative stress, which may be done by making changes in glutathione peroxidase at genomic level.

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Cite this Article
Priyanka Verma. Glutathione peroxidase (GPx) in Breast Carcinoma. *Research & Reviews: A Journal of Life Sciences*. 2018; 8(3): 1–5p.