

Salivary and Serum Glutathione Enzymes with their Cofactors Roles in Breast Cancer

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Abstract— Background: Reactive oxygen species (ROS) show a key role in the expansion of tumors. The enzymatic and non-enzymatic antioxidant guarding systems of the cell avoid oxidant harm to various biomolecules by neutralizing produce of free radicals. Glutathione (GSH) and glutathione dependent enzymes [glutathione reductase (GR) glutathione-s-transferase (GST) and glutathione peroxidase (GPx) are the crucial bases of cellular response to oxidative stress. Glutathione acts an essential part in detoxification of carcinogens. **Objectives:** To assess the role of antioxidant defense an accomplished by glutathione dependent enzymes with their cofactors and comparative investigation activities of the glutathione-dependent enzymes in the blood serum and saliva in the patients with breast cancer. **Materials and Methods:** Serum and salivary GSH and glutathione dependent enzymes such as GPx, GST, and GR levels were assessed in 40 healthy controls and 48 females with breast cancer utilizing a spectrophotometer. Serum and salivary trace elements (zinc and selenium) were estimated by utilizing the atom absorption photometry. **Results:** Serum and saliva analysis displayed significantly diminished ($P < 0.0001$) of GSH, GST, GR and GPx activities in breast cancer patients compared to control group. The mean levels of serum and salivary trace elements (zinc and selenium) were significantly reduced (< 0.001) in breast cancer patients as compared to levels of healthy controls. **Conclusion:** Serum and salivary antioxidant scheme (GSH, GST, GR, GPx, Zn and Se) were affected (reduced) in breast cancer patients and this may be deliberated as a proper indicator of the antioxidant status in the body.

Keywords: breast cancer, glutathione dependent enzymes, trace elements, saliva.

1. Introduction

Breast carcinoma is individual public malignancies in females. It is curable, if it is distinguished¹. The exact etiology of breast cancer is still anonymous, whereas Kumaraguruparan et al., have revealed the action of reactive oxygen species (ROS) in the pathogenesis of carcinoma². ROS plays like a double edge sword. The slight amount of ROS are indispensable for suitable biochemical progressions, however unnecessary production or inefficient subtraction pointers to oxidative stress that may be the reason of oxidative destruction to biomolecules subsequent in lipid peroxidation, mutagenesis, and carcinogenesis³. A body has urbanized an efficient way to tackle with free radicals by several cellular shield mechanisms concerning of enzymatic and non-enzymatic⁴ antioxidant shield systems of the cell prevent oxidant mediated breakdown to different biomolecules by deactivating free radicals⁵. However, these antioxidants may possibly stop apoptosis in carcinogenic cells induced by oxidative stress⁶. Glutathione (GSH) and glutathione dependent enzymes [glutathione peroxidase (GPx) and glutathione-s-transferase (GST)] are the main determinants of cellular reply to oxidative stress⁷. GSH successfully scavenges free radicals and other ROS directly and indirectly via enzymatic metabolism⁸. Furthermore, GSH is intricate in other biological functions plus its character in maintaining body homeostasis and keeping cells against toxicants, DNA harmful agents and carcinomas source are either exogenous or endogenous⁹. GSTs catalyze the conjugation of GSH to electrophilic sorts producing from hydrophilic sorts that are further more excreted, which act a vital part in drug metabolism¹⁰. GST has lately been implicated in shielding against apoptosis. GPx is a significant enzyme in charge for detoxification of cellular H_2O_2 utilizing GSH; it appears in two forms selenium

dependent and selenium independent. The selenium-dependent GPx detoxify H_2O_2 and transformed to lipid hydroperoxides to non-hazardous alcohols, whereas the selenium-independent enzyme is in authority for metabolizing lipid peroxides solitary¹¹. Micronutrients perform a vigorous part in continuously occurring regenerative processes, organisation with ongoing oxidative stress in the body tissues, and supporting sufficient immunity against pathogens¹². Trace elements are chemical micronutrients which are needed by a tiny concentration but show a vibrant part in keeping the integrity of uncountable physiological and reactive processes. The low levels of one of the trace elements can be apparent as a blend of numerous clinical manifestations, also every trace element is correlated to numerous enzyme systems. Selenium is notorious to possess immunomodulating and anti-proliferative characterize and can affect immune reply by varying the expression of cytokines through their receptors or building immune cells extra resistant to oxidative stress¹³. Selenium constituent forms are one of the domineering antioxidant protection systems of the body¹⁴. Zinc tasks in biology are too many. It is mandatory for the catalytic activity of many numbers of enzymes¹⁵. It roles a significant character in immune function, preserve adequate levels of GSH in the blood, and etc.¹⁶. Saliva is a complex secretion whose substances exert a well-documented part in health and disease and its diagnostic usage is spreading¹⁷. Besides, saliva comprises various antioxidants,¹⁸ containing GSH and its precursors¹⁹. Genetic and biochemical evidence has demonstrated that GSH and glutathione-dependent enzymes act a principal character in the cellular shielding against toxic environmental agents²⁰.

2. Materials and methods

The revision comprised of 48 cases of clinically diagnosed women with breast cancer (newly diagnosed /untreated/advanced stage) and 40 healthy volunteers. The mean age of the breast cancer patients was 62.03 ± 12.4 years while the mean age of the control group was 61 ± 12.1 years. The blood samples, after overnight fasting, were collected. Five milliliters of blood was collected in sterile vials containing anticoagulant (EDTA) for the preparation of plasma. The serum was prepared by collecting 5 ml of the blood without anticoagulant. Serum and plasma were formed by centrifugation. Biochemical assessments were carried out immediately. Unstimulated saliva was claimed from the similar patient's after the diagnosis, under resting situations between 10.0-11.0 AM. Patients were asked for saliva by spitting into a wide test tube (10 ml), and then it was centrifuged. Then biochemical assessments were done. Each blood sample and saliva were examined the following assays: serum and saliva GSH, GST, GR, and plasma with saliva GPx activity were estimated by utilizing the marketable Randox-Glut Red kit (Randox Laboratories, Crumlin, Antrim, UK) according to the procedure provided by the manufacturer using BioTek Instruments, Milan, Italy. The level of GSH was calculated by using the standard curve of GSH. While the trace elements levels (Zn and Se) were assessed in the blood (serum and plasma) samples and saliva by using 1275 A A Varian, atomic absorption spectrophotometer. The program of SPSS Version 15.0 was run for all statistical analyses.

3. Results

Demonstrations the results of GSH, GPx, GST and GR in blood (serum and plasma) samples of control and breast cancer patients are in Figure (1). The results reflect a significant decline ($P < 0.0001$) in the activities of enzymes of breast cancer patients in comparison to the healthy groups, while Figure (2) display the mean values of salivary GSH, GPx, GST and GR levels concentrations, and revealed a significant diminution ($P < 0.0001$) in breast cancer patients in comparison to the healthy collections. The results presented in Figure (3) expression the mean values of serum Se and Zn in control and breast cancer patient groups. The results imitated a significant decrease ($P < 0.001$) in sera samples of breast cancer groups in comparison to the control. Salivary Se and Zn concentration results

are presented in Figure (4). The results illustrate a significant decrease ($P < 0.001$) in salivary Se and Zn in breast cancer patients groups in evaluation to the control groups.

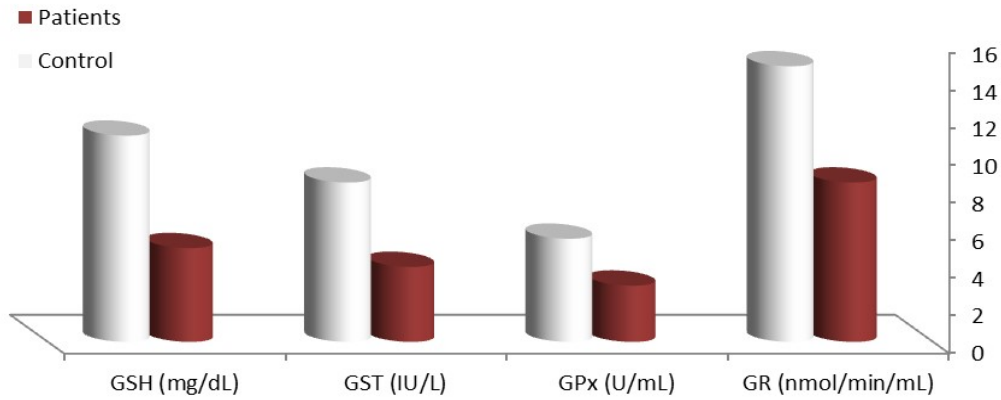


Figure (1): Mean values of blood GSH, GPx, GST, and GR in control and breast cancer patients groups

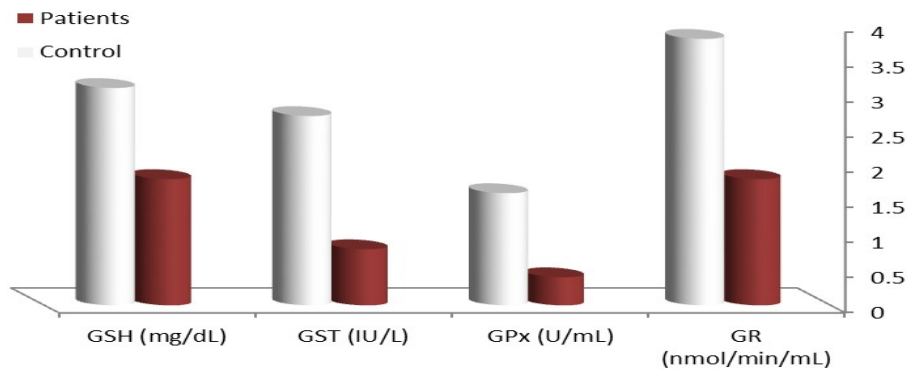


Figure (2): Mean values of salivary GSH, GPx, GST, and GR in control and breast cancer patients groups

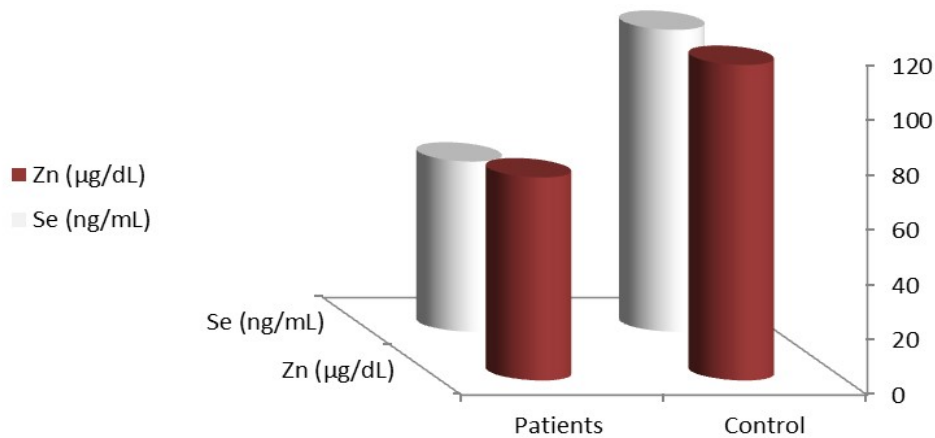


Figure (3): Mean values of serum Se and Zn in control and breast cancer patients groups

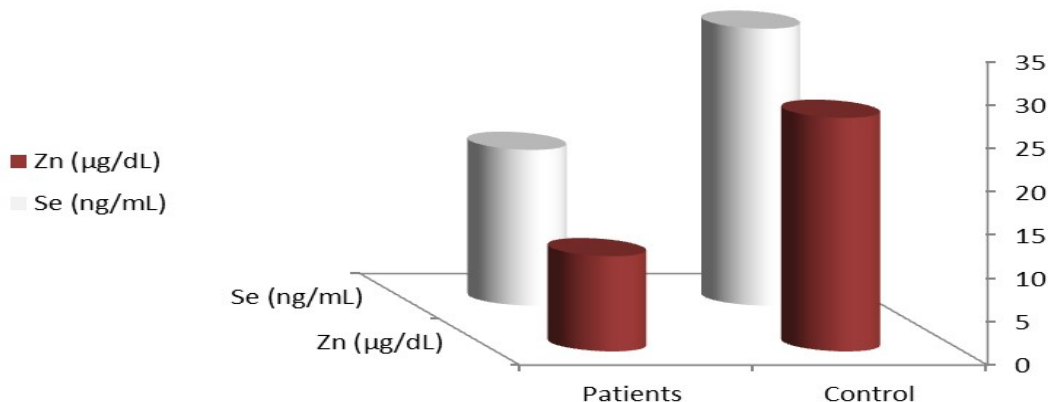


Figure (4): Mean values of salivary Se and Zn in control and breast cancer patients groups

4. Discussion

Antioxidants offer the first line of cellular safeguard against ROS, preserving the cell against the production of other deleterious metabolites, which play a fundamental character in human cancer development²¹. The oxidative stress is the circumstance of synthesis the reactive free radicals or the depletion of antioxidants in excess. GSH and GSH dependent enzymes straightly scavenge free radicals and shield cells from oxidative insults²². GSH deeds as both, a nucleophile and a reductant, therefore react with electrophilic or oxidizing species before the latter interact with more dangerous cellular constituents²³. GSH sometimes perform as a reactant for GPx and GST during the detoxification of ROS²⁴. So GSH plays a major part in cancer avoidance²⁵, and detoxifies carcinogens, also orders immune system. Richie et al have revealed declining in the levels of plasma GSH levels in oral sarcoma²⁶. Utilization of the GSH by tumor tissues or by extreme oxidation in circulation was the apparent reason for the lessening of GSH²⁷. As in this research we establish reduced serum and salivary GSH significantly ($P < 0.0001$) in breast cancer patients (Figure 1,2) due to the above explanations, so it could be due to an imbalance in the free radical and antioxidant homeostasis in the body fluids that occurs during carcinogenesis, which elevated level of free radicals leads further oxidation of GSH. GSH also acts in link with the detoxification enzymes GPx and GST. These enzymes safeguard cell from noxious substances²⁸. This antioxidant function of GSH along with its associated enzymes could offer protection in contradiction of oxidative stress mediated apoptosis²⁹. This study displayed dropped mean serum and salivary GST activity significantly ($P < 0.0001$) in breast cancer patients (Figure 1,2). Proteins of the GST family are a set of detoxification enzymes included in detoxifying a varied of hazardous matters, that they protect DNA against oxidative destruction, which possibly will cause mutations and in result favor carcinoma³⁰, or it could be theorized that GST being GSH depleting enzyme utilizes GSH as a substrate. GST being dependent on GSH becomes inactive as soon as GSH is depleted thus rendering cancer patients somewhat defenseless against oxy-radicals³¹. In this investigation, a significant reduction ($P < 0.0001$) in the serum and salivary GR activity has been observed from breast cancer patients (Figure 1,2). GR belongs to non-enzymatic shield system that transforms glutathione disulfide to the sulfhydryl form utilizing NADPH that is mandatory to deplete GSH. GR is a significant cellular antioxidant³². There is limited data available on serum GR levels in cancer patients. Redox state of GSH stabilized by the activity of depleting GPx and replenishing enzymes GR may possibly be converted by cancer³³. The results of our study indicated that the mean levels of plasma and salivary GPx were significantly

decreased ($P < 0.0001$) in breast cancer patients (Figure 1,2). GPx is played as a powerful antioxidant shield system in human body having selenium as cofactor, which altering H_2O_2 into no harmful molecular (oxygen and water). The breast cancer may perhaps alter the detoxification of H_2O_2 through the declining of GPx Activity. The excess production of H_2O_2 may well lead into improve consumption of GSH³⁴. There were significant decreased ($p < 0.001$) in serum and salivary for both Zn and Se levels in breast cancer group when compared to healthy control group (Figure 3,4). Trace elements such as zinc and selenium act as indispensable components of antioxidant enzymes. Se and Zn perform as cofactors for the accurate functioning of GSH which is mandatory to keep adequate amounts of GSH in the blood. Zinc shows an anti-carcinogenic part by stabilizing the configuration of DNA, RNA and ribosome. Zinc is similarly needed to the function of several transcription factors and proteins. Zinc guards cells from free-radical injury³⁵. Besides, it is mentioned that low levels of zinc can induce the pathogenesis of lung cancer³⁶. Lesser serum Zn concentrations in patients with ovarian and cervical cancers have been reported³⁷. The reason attributed for this decline in the values could be because the malignant cells perhaps want more zinc³⁸. Antioxidant enzymes GPx contain Se that catalyzes the reduction of H_2O_2 and organic hydroperoxide thus stopping from oxidative injury. Low levels of Se have been established to be linked with occurrence of malignant lesions of breast, ovary, etc. The role of Se is complex and can be accredited by constructing immune cells more strong to oxidative stress. Mates et al have been established that Se works as a cancer protective agent³⁹.

5. Conclusion

Serum and salivary antioxidant systems in the formula of GSH and glutathione dependent enzymes with their cofactors are affected (reduced significantly) in breast cancer patients and may be considered as an appropriate indicator of antioxidant status in the body, and this proved the possibility of utilizing the enzyme as a potent biochemical biomarker, whereas assessment of these markers lets us to identify subjects with high risk of developing breast cancer. Also varying the sample collection from blood to saliva as a non-invasive collection, that is easier and faster assessment method.

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Conflict of Interest

In this research, there are no conflicts.

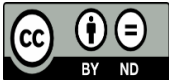
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