Determination the Erythrocyte glutathione peroxidase activity and Serum selenium level in patients with breast cancer

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Abstract
Selenium is a key component of a number of functional selenoproteins required for normal health. The best known of these are the antioxidant glutathione peroxidase enzymes, which remove hydrogen peroxide and damaging lipid and phospholipids hydro peroxides generated in vivo by free radicals and other oxygen derived species. Antioxidant has been suggested to play a role in some physiological conditions and in many disease processes, including carcinogenesis. The aim of the study was to investigate the erythrocyte glutathione peroxidase (GSH-Px) and serum selenium level in patients with breast cancer. The study included thirty female breast cancer patients and twenty normal healthy individuals of age- and sex match who served as controls. In all investigated breast cancer patients significantly lower serum (Se) level, and significantly higher (GSH-Px) activities were found, as compared with the values of healthy controls.
Results from this prospective study suggest the increased of erythrocyte(GSH-Px) activity may be a result of a protective mechanism that develops in breast cancer patients against free radical damage, and low serum Se levels is associated with increased breast cancer risk.

Introduction:

Breast cancer is a malignant (cancerous) growth that begins in the tissues of the breast. Cancer is a disease in which abnormal cells growth in an uncontrolled way. Breast cancer is 100 times more common among women than men [1]. Glutathione peroxidase (GSH-Px) (EC 1.11.1.9) is a selenium-dependent enzyme, which decomposes H_2O_2 and various hydro- and lipid peroxides [2]. The classical form of (GSH-Px) is cellular and dispersed throughout the cytoplasm but (GSH-Px) activity is also found in mitochondria [3].
Mills (1957, 1959) first demonstrated that this enzyme was present in mammalian red cells and that it protected hemoglobin form oxidative break down by hydrogen peroxide H_2O_2. The presence and importance of glutathione peroxidase in human and cells has subsequently been clearly shown [4,5]. The antioxidant enzyme glutathione peroxidase is part of the enzymatic defense preventing oxidative damage to (DNA), proteins and lipids, by detoxifying hydrogen- and lipid peroxides[6]. The activity of glutathione peroxidase has been measured in the blood compartment and shown to be lower in women with breast cancer in the per-diagnostic stage compared with healthy controls [7-10].
Selenium is an essential nutrient for humans being necessary for activity of Glutathione peroxidase (GSH-Px) [11]. Several studies suggest that low levels of selenium (measured in the blood or in tissues), may be a risk factor for developing cancer [12-17]. Population studies suggest that people with cancer are more likely to have low selenium levels than healthy matched individuals, but in most cases it is not clear if the low selenium levels are a cause or nearly a consequence of disease [13,14]. It remains unclear if selenium is beneficial in the treatment of any type of cancer.
The aim of the study was to investigate the selenium status and define the relationship between (GSH-Px) activities in patients with breast cancer and controls.

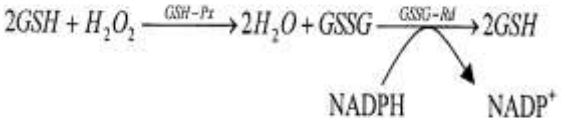
Materials and methods:

1-Subjects: the samples collected from thirty female breast cancer patients from Al-Ilwia hospital- center for early diagnosis of breast cancer. None of those patients was on a special diet, or taking any antioxidant (vitamin E, C, etc) or treated with antioxidant drugs (such as allopeurinol) or on any special treatment other than their regular chemotherapeutic schedule or had a recent history of blood transfusion.
Table (1): show the host information for women with breast cancer where the number of samples, age of patients, menopausal status: wherever nineteen patients were premenopausal and eleven postmenopausal, the family history whether having one or more first degree relatives (mother, sister or daughter) diagnosed with breast cancer approximately doubles risk wherever only twelve patients had breast cancer in their families, the marital status and whether they had previous history of abortion or not.
All the collected samples were to women in advanced stage of the disease, the samples collected (six weeks after excision of breast mass). Twenty normal healthy controls of comparable age and sex were considered as normal control.
2-Blood sampling: blood samples (5ml) transferred into plain tubes containing (acid-citrate-dextrose) (ACD) as anticoagulant. Tubes were mixed and placed immediately in crushed ice, then assayed within (1-2) hrs. of blood collection. Blood samples were centrifuged at (5000) rpm for (10) min., then plasma and buffy coat were removed by aspiration. Erythrocytes were washed three times with phosphate buffered saline (PBS) pH= 7.4 (0.02 M phosphate; 0.123M NaCl). The packed cell volume (PCV) after the final wash was used for the assay of (GSH-Px). Plasma was stored at (-20˚C) and used for the determination of selenium level.

to Pagil and Valantine method [18], with some modifications from Hopkins and TudHop [19] and Pleban *et al*. [20]. The recycling procedure for the determination of (GSH-Px) activity depends on the oxidation of (GSH) to (GSSG) by the enzyme in the presence of (NADPH) and exogenous glutathione reductase which regenerates (GSH) for (GSSG) [20].

3-Chemicals: the chemicals and reagents used in this study were of anular grade unless otherwise specified and were obtained from BDH chemicals Ltd., England; Sigma, chemicals USA; Fluka A.G., Germany.

Enzyme assay: Erythrocyte Glutathione peroxidase (GSH-Px) activity (GSH-Px) was assayed according



kit (Randox) procedure no. 540-UV 1996, the (Hb) level were measured in the patients and control individuals to determine the activity of (GSH-Px). (Hb) and enzyme (GSH-Px) measurements of the matched sets were performed on the same day.

The results were analyzed by student's (t-test) to find out level of significance. P value ≤ 0.05 is considered as statistically significant.

The rate of enzyme activity was monitored by following the decrease in absorbance at 340 nm as a function of (NADPH) exhaustion [20].

Selenium level: serum selenium level was measured using atomic absorption spectrometry in a procedure based on the methods described by Lewis *et al*. [21] and, Pasched and Kimberly [22] with some modification from Gunther *et al*. [23].

Hemoglobin concentration: hemoglobin concentration (Hb) was followed using hemoglobin

Table (1): Clarify the host information of the patients with breast cancer.

No. of samples	Age	Marital status	family history (found/not found)	Abortion (found/not found)	Menopausal status(pre or post)
1	30	Marred	Not Found	Not Found	Pre
2	39	Not Marred	Found	Not Found	Pre
3	42	Not Marred	Not Found	Not Found	Pre
4	40	Not Marred	Not Found	Not Found	Pre
5	55	Marred	Found	Not Found	Post
6	32	Marred	Found	Found	Pre
7	43	Not Marred	Not Found	Not Found	Pre
8	44	Not Marred	Not Found	Not Found	Pre
9	52	Marred	Not Found	Not Found	Post
10	48	Marred	Not Found	Not Found	Post
11	51	Marred	Not Found	Not Found	Post
12	39	Marred	Not Found	Found	Pre
13	42	Marred	Found	Not Found	Pre
14	35	Marred	Found	Not Found	Pre
15	59	Marred	Not Found	Not Found	Post
16	40	Not Marred	Found	Not Found	Pre
17	57	Not Marred	Found	Not Found	Post
18	40	Marred	Found	Not Found	Pre
19	43	Not Marred	Not Found	Not Found	Pre
20	41	Marred	Not Found	Not Found	Pre
21	62	Marred	Not Found	Not Found	Post
22	44	Not Marred	Not Found	Not Found	Pre
23	39	Marred	Not Found	Found	Pre
24	53	Not Marred	Found	Not Found	Post
25	41	Not Marred	Found	Not Found	Pre
26	62	Marred	Not Found	Not Found	Post
27	42	Marred	Not Found	Not Found	Pre
28	55	Marred	Found	Not Found	Post
29	44	Marred	Found	Not Found	Pre
30	59	Marred	Not Found	Not Found	Post

increased in (group 2) as compared with healthy controls (group 1) ($P < 0.00001$).
Table (3) demonstrates the mean ± SD of serum selenium level expressed as µg/L of normal healthy controls (group 1) and breast cancer patients (group 2). Serum selenium level was significantly decreased in (group 2) as compared with (group 1) ($P < 0.0001$).

Results:

Table (2) represents the sample size (n), mean ± SD and significance of (GSH-Px) activity expressed U/gHb in erythrocyte of normal healthy controls (group 1) and breast cancer patients (group 2). Erythrocyte (GSH-Px) activity was significantly

Table (2): Biostatistical calculations and student t-test of erythrocyte (GSH-Px) activity for normal healthy controls (group 1) and breast cancer patients (group 2).

Erythrocyte (GSH-Px) activity U/gHb	**Normal healthy control (group 1)**	**Breast cancer patients (group 2)**
Sample size (n)	20	30
Mean ± SD	32.5 ±2.1	47.52 ± 5.14
Probability		< 0.00001*

*normal healthy control (group 1) versus breast cancer patients (group 2)

Table (3): Biostatistical calculations and student t-test of serum selenium level for normal healthy controls (group 1) and breast cancer patients (group 2).

Serum selenium level µg/l	Normal healthy control (group 1)	Breast cancer patients (group 2)
Sample size (n)	20	30
Mean ± SD	106 ± 26.28	75 ± 20.37
Probability		< 0.0001*

*normal healthy control (group 1) versus breast cancer patients (group 2)

stage compared with healthy controls was found by Abiaka *et al*.[7], Kumar *et al*.[8], Kumaraguruparan *et al*.[9] and pawlowicz *et al*.[10].
In our study our results have shown significant increased in erythrocyte (GSH-Px) activity and decreased serum selenium level in the women with advanced stage of breast cancer as compared with that of control (table 2,3) respectively. These results are in agreement with Banu Sancak *et al*.[30].
The cause of the difference in the results of Abiaka *et al*.[7], Kumar *et al*.[8], Kumaraguruparan *et al*.[9] and pawlowicz *et al*.[10] and the results in this study is that those researchers collect their samples from the women in the pre-diagnostic stage whereas the sample in the present study collected from the women in advanced stage (six weeks after excision of breast mass).
The increasing of erythrocyte (GSH-Px) activity in patients with breast cancer could possibly be an adaptive response forced by erythrocytes of breast cancer patients to compensate with the increased free radicals in the extracellular component. A defective antioxidant capacity, as judged by decreased serum selenium levels in breast cancer patients. This defect may lead to the accumulation of ROS, especially ($O^{\cdot-}_{2)}$ in the plasma. ($O^{\cdot-}_{2)}$ can easily be converted to H_2O_2 by metal ions present in plasma, and H_2O_2 can

Discussion:

Glutathione peroxidase (GSH-Px) was assumed that the enzyme protects hemoglobin oxidative denaturation by H_2O_2 [24]. In 1967, Little and O'Brien described a (GSH-Px) activity in rat liver which reduced not only H_2O_2 but also a variety of hydroperoxides including hydroperoxides of unsaturated fatty acids. The enzyme has a high specificity to GSH as substrate [25] and it requires selenium as cofactor [26]. Selenium deficiency demonstrated to be a risk factor for cancer [27]. Studies on animals have shown that selenium is protectant against cancer [28]. Selenium has roles that support immune function and, through specific cellular pathways, may play a preventive role in both the initiation and promotion of specific cancer [29].
In most studies, plasma, serum, or erythrocyte levels of selenium were found to be significantly lower in breast cancer cases compared to healthy control [12-17]. These studies showed a positive benefit of selenium on breast cancer patient's reduction of biomarkers.
(GSH-Px) activity was slightly lower in breast cancer cases than in controls, but this did not reach statistical significance. A number of case-control studies have measured erythrocyte antioxidant enzyme activities in breast cancer. Significantly lower activities of (GSH-Px) in women with breast cancer in the per-diagnostic

selenium is associated with increased breast cancer risk and increased (GSH-Px) activity in breast cancer may be a result of a protective mechanism that develops in breast cancer patients against free radical damage.

penetrate through erythrocyte and neutralized by (GSH-Px).

The conclusion is selenium may play an important role in the pathophysiologic processes of breast cancer patients and the decreasing levels of serum

References:

prostate cancer risk, J. Natl. Cancer Inst., 96(9)(2004): 696-703.

14- Mark, s. D., Qiao, Y. L., Dawsey, S.M., *et al*., Prospective study of serum selenium levels and incident esophageal and gastric cancers, J. Natl. Cancer Inst. 92(21)(2000): 1753- 1763.

15- Postovsky, S., Arush, M.W., Diamond, E., *et al*., The prevalence of low selenium levels in newly diagnosed pediatric cancer patients, Pediatr. Hematol. Oncol. 20(4)(2003): 273-280.

16- Yoshizawa, K., Willett W. C., Morris, S. J. *et al*., Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer, J. Natl. cancer Inst. 90(16) (1998): 1219-1224.

17- Yu, S., Chu, Y., Gong, X., *et al*., Regional variation of cancer mortality incidence and its relation to selenium levels in china, Biological Trace Element research.7(1985): 21-29.

18- Paglia, D.E. and Vanlentin, W.N., Studies on the quantitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70(1967): 158.

19- Hopkins, J. and Tudhope, G.R., Glutathione peroxidase in human red cells in health. Br. J. Haemat.25 (1973): 563.

20- Pleban, P.A., Munyani, A. and Beachum, J., Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes, Clin. Chem.28 (1982): 311-316.

21- Lewis, S.A., Hardison, N.W. and Veillon, C., Comparison of isotope dilution mass spectrometry and graphite furnace atomic absorption spectrometry with zeeman background correction for determination of plasma selenium, Anal. Chem.58(1986): 1272-1275.

22- Paschal, D.C., Kimberly, M.M., Automated direct determination of selenium in serum by electrothermal atomic absorption spectroscopy, Atomic spectrose.7(1986): 75-78.

23- Gunther, E. W., Lewis, B. L. and Koncikowsk, S. M., Laboratory procedures used for the third national health and nutrition examination survey (NHANESIII), 1988-1994, Atlanta, GA, U.S. Dept. of health and human services, 1996.

24- Mills, G. C., Hemoglobin catabolism: I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown, J. Biol. Chem.229(1957): 189-197.

25- Flohe , L., Günzler, W.A., Jung, G., Schaich, E. and Schneider, F., Glutathione peroxidase II, Substrates pezifität and Hemmbar keit durch substratanaloge, Z. Physiol. Chem. 325(1971):159-169.

1- Ambrosone, C. B., Oxidants and antioxidants in breast cancer, Antioxid. Redox Signal, 2 (2000): 903-917.

2- Kinnula, V. L., Crapo, J. D. and Raivio, K. O., Generation and disposal of reactive oxygen metabolites in the lung, Lab. Invest. 73(1995): 3-19.

3- Buettner, G. R., Antioxidant enzymes and functions, naturally occurring antioxidants, Oxygen 98, Washington DC, USA. 1(1998): 1-20.

4- Cohen, C. and Hochstein, D., Glutathione peroxidase: The primary agent for the elimination of hydrogen peroxide in erythrocytes, Biochemistry. 2(1963): 1420.

5- Hill, A. S., Haut A., Cartwright, G. E. and Wintrobe, M. M., The role of monhemoglobin proteins and reduced glutathione in the protection of hemoglobin from oxidation *in vitro*, J. Clin. Inve 43(1964): 17.

6- Haren, G. R., Olsen, A., Tjonneland, A., Dragested, L. o., Nexo, B. A., Wallin, H., Overvad, K., Nielsen, O.R. and Vogel, V., Associations between GPX1 Pro 198 Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study, carcinogenesis 27(4) (2006): 820-825.

7- Abiaka, C., Al-Awadi, F., Al-Sayer, H., Gulshan, S., Behbehani, A. and Forghally, M., activities of erythrocyte antioxidant enzymes in cancer patients. J. Clin. Lab. Anal. 16(2002): 167-171.

8- Kumar, K., Thangaraju, M. and Sachdanandam P., Changes observed in antioxidant system in the blood of postmenopausal women with breast cancer, Biochem. Int. 25(1991): 371-380.

9- Kumaraguruparan, R., Subapriya, R., Kabalimoorthy, J. and Najini, S., Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast, Clin. Biochem, 35(2002): 275-279.

10- Pawlowicz, Z., Zachara, B. A., Trafikowska, U., Maciag, A., Marchaluk, E. and Nowicki, A., Blood selenium concentrations and glutathione peroxidase activities in patients with breast cancer and with advanced gastrointestinal cancer, J. Trace Elem. Electrolytes Health Dis. (1991): 275-277.

11- Ursini, F. and Bindoli, A., The role of selenium peroxidases in the protection against oxidative damage of membranes, Cem. Phys. Lipids. 44(1987): 255-276.

12- Garland, M., Morris, J. S., Stampfer, M. J., *et al*., Prospective study of toenail selenium levels and cancer among women, J. Natl. Cancer Inst. 87(7) (1995): 497-505.

13- Li, H., Stampfer, M. J., Giovannucci, E. L., *et al*., A prospective study of plasma selenium levels and

29- Combs, G. F. Jr., Impact of selenium and cancer prevention findings on the nutrition-health, paradigm, Nutr Cancer.40 (2001): 6-11.
30- Banu, S., Adem, V., Sakine, C., Ugur, C. and Nazan, G., Association between oxidative stress and selenium levels in patients with breast cancer at different clinical stages. The Journal of trace elements in experimental medicine.16 (2003): 87-94.
26- Flohe , L., Günzler, W. A. and Schoch, H.H., Glutathione peroxidase: A selenoenzyme, FEBS letters.32 (1973): 132-134.
27- Salonen, J. T. *et al*., Risk of cancer in relation to serum concentrations of selenium and vitamins A and E, Matched case control, Analysis of prospective data, Brit. Med. J. 290(1995): 417-420.
28- Burr, M. L. *et al*., Antioxidants and cancer, J. of human Nutr. And Dietetics, 7(1994): 404-416.

تقدير فعالية الانزيم المؤكسد للجلوتاثايون في كريات الدم الحمر ومستوى السلينيوم في مصل الدم لدى المرضى المصابين بسرطان الثدي

بسمة طالب جاسم

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الملخص:

السلينيوم هو عنصر رئيسي يحتاجه الشخص السليم لعدة وظائف وهو ضروري للأنزيم المؤكسد للجلوتاثايون الذي ينتزع بيروكسيد الهيدروجين من الدهون الضارة والدهون المفسفره التي تسير داخل الجسم بواسطة الجذور الحره وصنف الاوكسجين الفعال . مضادات الأكسدة تلعب دور مهم في الحالة الفسلجيه للجسم ومعظم الامراض بما فيها التسرطن. الهدف من هذه الدراسة هو فحص فعالية الأنزيم المؤكسد للجلوتاثايون (GSH-Px) في كريات الدم الحمر ومستوى السلينيوم (Se) في مصل الدم لدى المرضى المصابين بسرطان الثدي. تضمنت الدراسة(٣٠) مريضه مصابه بسرطان الثدي و(٢٠) شخص طبيعي غير مصاب بالمرض من نفس العمر والجنس لغرض المقارنة (كمجموعة تحكم سوية). جميع الفحوصات التي أجريت للمريضات المصابات بسرطان الثدي أظهرت أنخفاض ملحوض بمستوى السلينيوم في مصل الدم وأرتفاع ملحوظ لفعالية الأنزيم المؤكسد للجلوتاثايون مقارنة مع الاصحاء. النتائج المتوقعة لهذه الدراسة تقترح أن زيادة فعالية الأنزيم المؤكسد للجلوتاثايون هي نتيجة لحماية ألية عمل النمو للمريضات المصابات بسرطان الثدي ضد ضرر الجذور الحرة وأنخفاض مستوى السلينيوم في مصل الدم للمريضات يكون متحد مع زيادة خطر سرطان الثدي.