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The correlation of antioxidant levels of breast cancer

A case controlled study

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Abstract

Many free radicles are implicated to activate a number of oncogenic signaling, cause damage to deoxyribonucleic acid and tumor suppressor genes, or promote expression of proto-oncogenes. Reduced level of antioxidants and increases oxidative stress markers are associated with the development of various types of cancer.

This prospective study included 60 women who were grouped into equal groups. Patients group included 30 breast cancer women and control group consisting of 30 apparently healthy women. Both groups were compared regarding the serum levels of antioxidants biomarkers (vitamin C, ceruloplasmin, glutathione) and oxidative stress biomarkers, malondialdehyde (MDA), peroxynitrite, and gamma-glutamyl transferase.

In regard to the antioxidant biomarkers, there was a significant difference between the patients and the controls regarding the levels of serum ceruloplasmin and glutathione, (*P* values .000) for each while vitamin C showed no significant correlation (*P* value .053), while regarding oxidative stress biomarkers, the correlation was significant for both peroxynitrite and MDA (*P* value .000 and .001) respectively, and not significant for gamma-glutamyl transferase (*P* value 1.00).

Reduced level both ceruloplasmin and glutathione is seen in patients with breast cancer while vitamin C is not associated. Elevated levels of both peroxynitrite and MDA is seen in patients with breast cancer which may be used as serum markers for the early detection of breast cancer.

Abbreviations: DNA = deoxyribonucleic acid, GGT = gamma-glutamyl transferase, MDA = malondialdehyde.

Keywords: breast cancer, ceruloplasmin, gamma-glutamyl transferase, glutathione, malondialdehye, peroxynitrite, vitamin C

1. Introduction

Breast cancer is the most common type of cancer that affect women during their lifetime. Many causes have been implicated in the development of cancer in general and breast cancer specifically which can be categorized into modifiable and non-modifiable causes, modifiable causes are described as factors that can be modified or changed such as the body weight, reproductive factors, the use of hormone replacement therapy, diet, and some environmental factors, while the non-modifiable factors include sex, genetic mutations, menstrual history, and family history of breast cancer. The theories that explain cancer development are still under investigation and many theories are currently present explaining the role of the oxidative stress and the antioxidant biomarkers in the development of cancer at the molecular basis.[1–3]

The contribution of the free radicles in the pathogenesis of many types of cancerous and non-cancerous diseases is investigated widely. The pathogenesis of disease occurrence due to oxidative stress can be explained by one or both of the following mechanisms, the first one is that there is decrease in the antioxidant defense mechanism which is in turn due to genetic defect resulting in the reduction of the production of the antioxidant enzymes, the second mechanism is that there is an increased production of the free radicals due to excessive exposure to toxins or the presence of higher concentration of various types of them.[1]

Antioxidants are substances that are able to inhibit oxidation, this mechanism include molecules that can prevent the formation of the reactive oxygen radicles or are able to capture them and conjugate with them preventing their interaction with their cellular targets. Antioxidants include antioxidant enzymes, proteins molecules, many chelating agents like ceruloplasmin, and non-enzymatic substances like glutathione.[2]

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Glutathione conjugate with various xenobiotics in the body then they are metabolized, high exposure to xenobiotics will result in depletion of the glutathione. Inadequate levels of these antioxidants may be due to decrease in the dietary supplementation or very high levels of the oxidative stress.[1]

Malondialdehye is a normal ketoaldehyde product that is synthesized by peroxidative metabolism of unsaturated fats and as one of the byproducts of arachidonate metabolism. Excessive amounts of malondialdehye is formed due to tissue injury, which can bind with the free amino groups of proteins to form malondialdehye-modified protein complex, this complex is an immunogenic. Elevated levels of malondialdehye have been detected in the serum of patients with different types of cancer.[2,4]

Gamma-glutamyl transferase (GGT) is a glycoprotein (oncofetal protein) that is found normally in the serum in low concentrations. High serum levels GGT have been linked to high level of oxidative stress and increased risk for cancer development.[5,6]

Peroxynitrite is a potent oxidant and nitrosative molecule that has the ability to react with various nucleic acids, protein molecules, lipoproteins, cardiolipins, and many other molecules. It can modify the ribonucleic acid which is immunogenic and its autoantibodies can be detected in serum of breast cancer patients and other types of cancer like colorectal cancer. It causes destruction of the typical epitopes on ribonucleic acid and mediates defect in cell signaling and cancer cell progression.[7]

Free radicles are implicated to be the causative agent for the development of cancer by many mechanisms such as deoxyribonucleic acid based damages, damaging tumor suppressor genes, and promoting the expression of the proto-oncogenes. Elevated level of malondialdehyde (MDA) and reduced level of antioxidants such as glutathione peroxidase, vitamin C, and some other vitamins like vitamin E in the serum or body tissues have been shown to be associated with the development of various types of cancer.[8–11]

Deoxyribonucleic acid damage and genomic instability is associated with the acquisition of new genetic mutations which are associated with cell transformation and survival of the malignant cells. They also induce defects in the cellular signal transformation and the progression and the resistance to treatment.[2]

2. Patients and methods

This is a prospective study which included 60 individual women who were grouped into equal age matched groups. The first group (patients group) consists of 30 women with breast cancer regardless of the type and clinical stage, and the second groups (controls) consist of 30 women who are apparently normal with no know clinical problem and no history of chronic drugs intake.

The analyses were carried out in the laboratory of the Department of Chemistry, College of Science/University of Duhok.

Breast cancer patients with history of neo-adjuvant chemotherapy or those who refused to be included in this study were excluded. Male patients with breast cancer also were excluded.

An informed written consent was obtained from each participant commenced before the study and the recruited one were approved by the Ethics Committee at the Research Registration Department, University of Duhok.

2.1. Collection and processing of blood samples

Venous blood samples were drawn from forearm veins of the patients 1 hour before breast surgery and then placed into gel tubes, the serum were separated by centrifugation at 3500 rpm for 5 minutes, and then it was stored at –20 °C until ready for the assay of biochemical parameters. Similarly blood samples were drawn from controls and processed.

2.2. Measurements

The levels of antioxidant biomarkers (vitamin C, ceruloplasmin, glutathione) and oxidative stress biomarkers, (MDA, and peroxnitrite) were estimated by using manual methods. Vitamin C in serum was determined photometrically with 2,4-dinitro phenylhydrazine method. The levels of ceruloplasmin was determines using a colorimetric method, which described by Menden et al in 1977. Serum glutathione was determined by a modified procedure utilizing Ellman reagent. The level of serum lipid peroxidation MDA was also determined colorimetrically by the method described by Guidet and Shah in 1989. Finally, peroxnitrite ($ONOO^-$) levels were estimated by the method of Vanuffelen et al described in 1998. The levels of other biochemical parameters (GGT) as marker of oxidative stress was analyzed using the commercial available kits (Roche Diagnostics, Mannheim, Germany); and the analyses were carried out according to the manufacturer's instructions using COBAS 311 Autoanalyzer and Fortress Diagnostic respectively.[12–14]

Data analyses were done using the Statistical Package for the Social Sciences, version 25, IBM, USA (Armonk, NY: IBM Corp.).

3. Results

The mean level of the serum glutathione among our patients was 6.247 (SD: 1.35688), and it was normal in all patients, the mean level of serum vitamin C in our patients was 5.4788 (SD: 2.8798) and it was normal in 21 patients (70%). The mean level of the serum ceruloplasmin in our patients was 27.03 (SD: 6.531) and it was normal in 28 patients (93.3%). Table 1.

Estimation of the levels of the oxidative stress biomarkers in our patients showed that the mean serum level of the MDA was 5.3129 (SD: 1.15844) and it was above 4 μmol/L in 29 patients (96.7%), the serum peroxy nitrite level was elevated in 29 patients (96.7%), with a mean level of 5.212 μmol/L (SD: 1.15844). The serum level of the GGT was normal in 29 patients (96.7%), with a mean level of 30.43 μmol/L (SD: 10.792). Table 2.

Figure 1 shows the differences between the levels of the antioxidant biomarkers, that is, vitamin c, ceruloplasmin, and glutathione between the patients and the control groups.

Figure 2 shows the differences between the levels of the oxidative stress biomarkers, that is, GGT, peroxynitrite, and MDA between the patients and the control groups.

In regard to the antioxidant biomarkers, there was a significant difference between the patients and the controls regarding the levels of serum ceruloplasmin and glutathione, (P values <.000) for each of them while vitamin C showed no significant correlation (P value .053). Table 3.

While for the oxidative stress biomarkers, the correlation was significant between the patients and the controls regarding the serum levels of both peroxy nitrite and MDA (P value .000 and

Table 1

Showing the levels of the antioxidant biomarkers among the patient group.

Antioxidant biomarkers	Levels	Frequency	Percentage
Serum glutathione, μmol/L. M±SD Range: 0.88–8.45		6.2470	1.35688
Serum glutathione	Normal	30	100.0
Serum vitamin C, μmol/L. M±SD Range: 0.92–8.24		5.4788	2.87980
Serum vitamin C	Normal	21	70.0
	Low	9	30.0
Serum ceruloplasmin, μmol/L M±SD Range: 14–46		27.03	6.531
Serum ceruloplasmin	Normal	28	93.3
	Low	2	6.7

.001) respectively, and was not significant for GGT (*P* value 1.00). Table 4.

4. Discussion

Oxidative stress is one of the major etiologies for cancer initiation and its progression. MDA is the final product of lipid peroxidation which is present in the serum at normal concentrations between 0 and 2 μmol/100 mL, this normally bind to xenobiotics which enhance their metabolism. Ceruloplasmin which is formed mainly in the liver and to a lesser extent extrahepatically is normally present in the serum at concentrations between 80 and 120 IU, it also has a major role in the binding of free radicles and enhancing their scavenging and metabolism.[1,2]

Serum vitamin C level was normal in 90% of the control group and 70% of the breast cancer group, although the difference between both groups showed no significant correlation (*P* value .053), but the patient group showed that 30% of them showed low level of vitamin C compared with 10% from the control group. Many epidemiological studies confirmed the protective effect of vitamin C in lowering the risk of cancer development and also associated with better survival rates among cancer survivors. Vitamin C cause up regulation of tumor necrosis factor-related apoptosis-inducing ligand, low levels of vitamin C are associated with reduction of this pathway and increase risk for cancer and suggesting the potential role normal serum vitamin C in the prevention of breast cancer.[15–18]

Ceruloplasmin is one of the acute phase proteins and is synthesized mainly in the liver and is found to have a role in the development of cancers including breast cancer, this role is attributed to its effects on the angiogenesis and neovascularization. It has been shown in breast cancer cell lines in some studies. In our study the levels of the ceruloplasmin was within normal levels in 93.3% of patients with breast cancer while was low in the all individuals of the control group, although it was not elevated in the patient group but the difference was very significant between both groups (*P* value .000). This signifies that the measurement of the serum ceruloplasmin is valuable in the diagnosis of breast cancer, in some studies it's level is correlated with the clinical stage of the tumor, thus it may be used with other clinical and imaging studies in the diagnosis of breast cancer.[19–21]

Glutathione is found to be an important marker in patients with breast malignancy that is not dependent of hormone receptor status and the clinical stage of the tumor, it may indicate disseminated disease. In our study serum glutathione was normal in all patients with breast cancer and was elevated in 83.3% of the control group. This finding is the contrary to most of the published papers which show the reverse, this may be attributed to the small sample size. Long term follow up and estimation of the glutathione may be done to predict the prognosis, the recurrence, and the responsiveness to chemotherapy, although this is still under investigation.[22,23]

GGT levels are elevated in the majority of malignant and non-malignant liver disorders, however it is level have been linked to other types of cancers such as breast cancer. In a Swedish cohort study involving 545,460 persons, they found that there is an association between GGT and different types of cancer, and high association with breast cancer particularly. Our results suggest no significant correlation between breast cancer and GGT, its level was normal in 100% of the control group and 96.7% of the breast cancer group and the *P* value for this correlation was 1:00.[5,6]

The level of the serum peroxynitrite was elevated in 96.7% of the patient group and in 6.7% of the control group, the association was very significant with a very clear deference between both groups (*P* value .000). Many studies also concluded that increased levels of peroxynitrite are found with patients with breast cancer. Some theories are now present about the possible use of antibodies against peroxynitrite which may help in the diagnosis of early breast cancer.[24]

Table 2

Showing the levels of the oxidative stress biomarkers among the patient group.

Oxidative stress biomarkers	Levels	Frequency	Percentage
Serum malondialdehyde, μmol/L. M±SD Range: 3.24–8.33		5.3129	1.15844
Serum malondialdehyde	Normal	1	3.3
	Elevated	29	96.7
Serum peroxynitrite, μmol/L. M±SD Range: 4.44–93.18		5.2129	1.15844
Serum peroxy nitrite	Normal	1	3.3
	Elevated	29	96.7
Serum gamma-glutamyl transferase, μmol/L. M±SD Range: 6–46		30.43	10.792
Serum gamma-glutamyl transferase	Normal	29	96.7
	Low	1	3.3

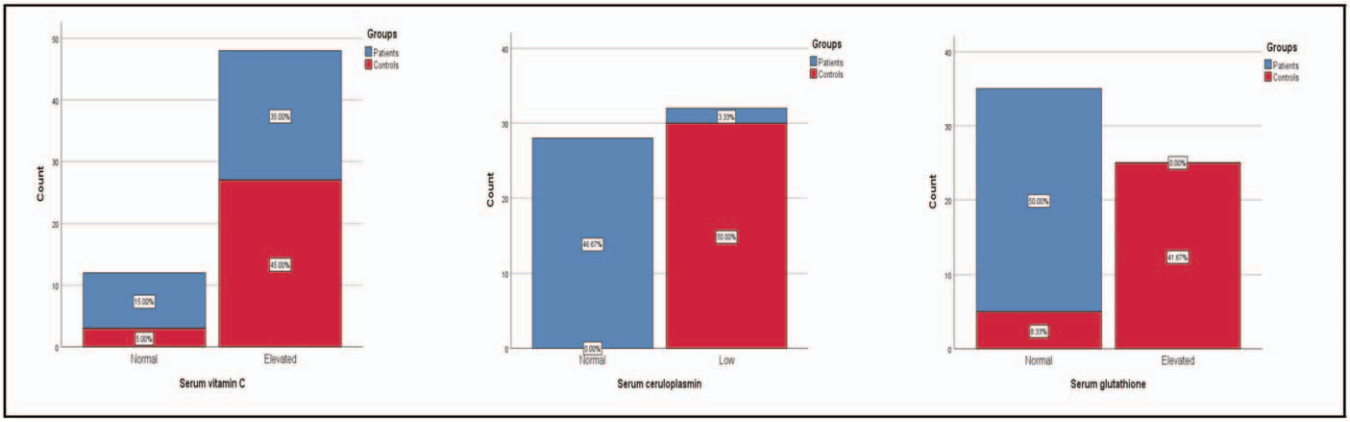

Figure 1. A stacked bar chart showing the antioxidant biomarkers between both groups.

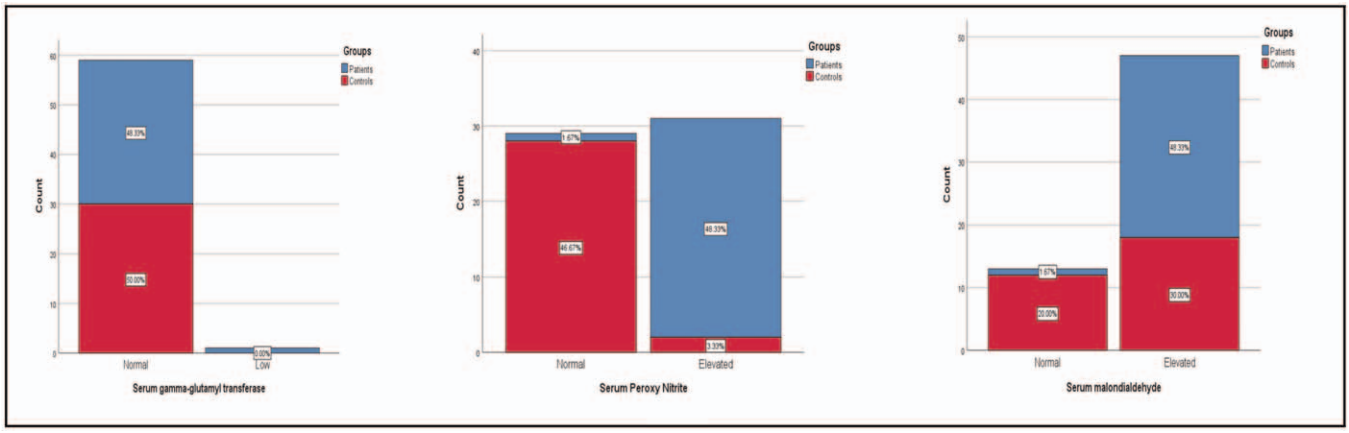

Figure 2. A stacked bar chart showing the oxidative stress biomarkers between both groups.

Table 3

Showing the correlation between both groups regarding the antioxidant biomarkers.

	Groups		
Antioxidant biomarkers	**Patients (n = 30)**	**Controls (n = 30)**	**Sig. (2-sided)**
Serum vitamin C			
Normal	21 (70.0%)	27 (90.0%)	0.053*
Low	9 (30.0%)	3 (10.0%)	
Serum ceruloplasmin			
Normal	28 (93.3%)	0 (0.0%)	0.000*
Low	2 (6.7%)	30 (100.0%)	
Serum glutathione			
Normal	30 (100.0%)	5 (16.7%)	0.000*
Elevated	0 (0.0%)	25 (83.3%)	

* Chi square test.

Lipid peroxidation is found to be associated with high risk for cancer development, MDA is formed by the peroxidative metabolism of unsaturated fats. In our study the level of the serum MDA was elevated in 96.7% of patients with breast cancer and was also elevated in 60% of the control group, the correlation was very significant with breast cancer 0.001. This provide an evidence for the relation between lipid peroxidation and breast cancer development.[25]

5. Conclusion

Reduced level both ceruloplasmin and glutathione is seen in patients with breast cancer while vitamin C is not associated.

Table 4

Showing the correlation between both groups regarding the oxidative stress biomarkers.

Oxidative stress biomarkers	Groups		Sig. (2-sided)
	Patients (n = 30)	Controls (n = 30)	
Serum peroxynitrite			
Normal	1 (3.3%)	28 (93.3%)	0.000*
Elevated	29 (96.7%)	2 (6.7%)	
Serum gamma-glutamyl transferase			
Normal	29 (96.7%)	30 (100.0%)	1.00†
Low	1 (3.3%)	0 (0.0%)	
Serum malondialdehyde			
Normal	1 (3.3%)	12 (40.0%)	0.001*
Elevated	29 (96.7%)	18 (60.0%)	

* Chi square test.
† Fischer exact test.

Elevated levels of both peroxynitrite and MDA is seen in patients with breast cancer which may be used as serum markers for the early detection of breast cancer.

Author contributions

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References

[1] Zob D, Vasilescu M, Gruia M, Anghel R. Breast cancer. Screening criteria. Chirurgia (Bucur) 2013;108:557–62.
[2] Bratt D, Kh J, Patel S, Zaveri M. Role of oxidative stress in breast cancer. Pharm Pharmaceu Sci 2016;5:366–79.
[3] Mohammed AA. Predictive factors affecting axillary lymph node involvement in patients with breast cancer in Duhok: cross-sectional study. Ann Med Surg (Lond) 2019;44:87–90.
[4] Akbulut H, Akbulut KG, Icli F, Büyükcelik A. Daily variations of plasma malondialdehyde levels in patients with early breast cancer. Cancer Detect Prev 2003;27:122–6.
[5] Chandrakanth K. Study of serum levels of gamma-glutamyl transferase, lactate dehydrogenase, malondialdehyde and vitamin-E in breast cancer. Int J Pharma Bio Sci 2011;2:489–98.
[6] Van Hemelrijck M, Jassem W, Walldius G, et al. Gamma-glutamyltransferase and risk of cancer in a cohort of 545,460 persons–the Swedish AMORIS study. Eur J Cancer 2011;47:2033–41.
[7] Tarannum S, Arif Z, Alam K. Binding of circulating autoantibodies in breast cancer to native and peroxynitrite-modified RNA. J Zhejiang Univ Sci B 2013;14:40–6.
[8] Malathi M, Vijay M, Shivashankara A. The role of oxidative stress and the effect of radiotherapy on the plasma oxidant-antioxidant status in head and neck cancer. J Clin Diagn Res 2011;5:249–51.
[9] Feng J-F, Lu L, Zeng P, et al. Serum total oxidant/antioxidant status and trace element levels in breast cancer patients. Int J Clin Oncol 2012;17:575–83.
[10] Mohammed AA. Quantitative assessment of Ki67 expression in correlation with various breast cancer characteristics and survival rate; cross sectional study. Ann Med Surg (Lond) 2019;48:129–34.
[11] Arif SH, Mohammed AA. Wide local excision and sentinel lymph node biopsy in early breast cancer in Duhok city. Ann Coll Med, Mosul 2020;42:19–26.
[12] Ashwood ER, Aldrich JE, Tietz NW. Tietz Fundamentals of Clinical Chemistry. 1996;Saunders,
[13] Menden EE, Boiano JM, Murthy L, Petering HG. Modification of a p-phenylenediamine oxidase method to permit non-automated ceruloplasmin determinations in batches of rat serum or plasma microsamples. Analyt Lett 1977;10:197–204.
[14] Muslih R, Al-Nimer O, Al-Zamely M. The level of Malondialdehyde after activation with H2O2 and CuSO4 and inhibited by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infection. J Chem 2002;5:148–9.
[15] Sant DW, Mustafi S, Gustafson CB, Chen J, Slingerland JM, Wang G. Vitamin C promotes apoptosis in breast cancer cells by increasing TRAIL expression. Sci Rep 2018;8:1–11.
[16] Harris HR, Orsini N, Wolk A. Vitamin C and survival among women with breast cancer: a meta-analysis. Eur J Cancer 2014;50:1223–31.
[17] Block G. Epidemiologic evidence regarding vitamin C and cancer. Am J Clin Nutr 1991;54:1310S–4S.
[18] Mohammed AA. Evaluation of mastalgia in patients presented to the breast clinic in Duhok city, Iraq: cross sectional study. Ann Med Surg (Lond) 2020;52:31–5.
[19] Özyilkan Ö, Ünlü E, Baltali E, et al. The value of ceruloplasmin levels in monitoring of patients with breast cancer. J Islamic Acad Sci 1991; 4:179–80.
[20] Varela AS, Saez JBL, Senra DQ. Serum ceruloplasmin as a diagnostic marker of cancer. Cancer Lett 1997;121:139–45.
[21] Raju KS, Alessandri G, Ziche M, Gullino PM. Ceruloplasmin, copper ions, and angiogenesis. J Natl Cancer Inst 1982;69:1183–8.
[22] Perry RR, Mazetta J, Levin M, Barranco SC. Glutathione levels and variability in breast tumors and normal tissue. Cancer 1993;72:783–7.
[23] Jardim BV, Moschetta MG, Leonel C, et al. Glutathione and glutathione peroxidase expression in breast cancer: an immunohistochemical and molecular study. Oncol Rep 2013;30:1119–28.
[24] Szabo C, Ohshima H. DNA damage induced by peroxynitrite: subsequent biological effects. Nitric Oxide 1997;1:373–85.
[25] Gönenç A, Özkan Y, Torun M, Şimşek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. J Clin Pharm Ther 2001;26:141–4.