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Evaluation of Ceruloplasmin Oxidase Activity in Sera of Breast Cancer Individuals in Kurdistan Region/ Iraq

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Abstract

Ceruloplasmin is considered the main copper transport protein which is proposed to have a role in cancer. Ceruloplasmin is an acute phase reactant and antioxidant enzyme, has been found to be increased in sera of patients with several types of cancers including breast cancer.
The aim of present study was to determine of ceruloplasmin oxidase activity, specific activity, iron concentration in sera of patients with breast cancer and comparing with healthy group, and the ability of using enzyme as a tumor marker for breast cancer.
This study was performed from November 2018 to January 2019, blood samples were collected from breast cancer patients in Nanakeli Hospital in Erbil city. Study was included (65) female patients with breast cancer and (20) healthy donors as control group. Ceruloplasmin activity, specific activity, serum total protein concentration, serum total iron concentration levels were estimated for all samples by colorimetric methods.
The results shown that there was a significant increase in ceruloplasmin activity level for patients with breast cancer (227.8 U/L) compared with control group (101.1 U/L) while total protein level for breast cancer patients (6.592 g/dl) showed no significant change compared to control group(7.127 g/dL) likewise the result shown that there was a significant increase in specific activity of ceruloplasmin and total iron concentration level for patients with breast cancer (34.74 U/mg) (334.2 mg/dl) compared to control group (14.14 U/mg) (128.6 mg/dl)
The conclusion of this study was the concentration of ceruloplasmin activity, serum total iron concentration and specific activity of ceruloplasmin significantly increase in breast cancer patients while total protein level revealed no significant change.
Serum ceruloplasmin oxidase activity and specific activity were highly significant elevated in patient group when compared with control group. Also the total iron concentration results showed significant increasing ($P<0.0001$) between of patient with breast cancer and control group.

Key words: Ceruloplasmin Oxidase Activity, Breast Tumor, Iron.

1. Introduction

Breast cancer is one of the most common cancers among women around the world, accounting for about 570,000 deaths in 2015 [1]. More than 1,5 million women (25% of all individuals with cancer) were diagnosed with breast cancer worldwide each year [2] . Breast cancer is a metastatic cancer which can usually spread to distant organs such as the bone, liver, lung and brain, which are primarily responsible for its incurability [3]. Early diagnosis of the illness can lead to the best prognosis and increase the chance of survives [4]. Breast

cancer has two main types: non-invasive breast cancer and invasive breast cancer [5] .Ceruloplasmin (Cp) is an enzyme that may play a significant role in the progression of breast cancer [6]. The Cp is an acute-phase protein that is typically produced by hepatic cells [7] and is primarily secreted in the plasma [8], whereas Cp mRNA was found in cell lines of breast cancer patients [9]. It is comprised of angiogenesis and neovascularization thus it has a role in cancer [10]. Cp plays a significant role in iron homeostasis[8], consequently, it has a contribution to the antioxidant mechanism by preventing the formation of free radicals [11] or in oxidative damage mechanisms [12] Ceruloplasmin has two major functions: oxidase and ferroxidase activities [13], by its oxidase activity, play an important role in a variety of processes relevant to the copper metabolism [14] , some organic amines that produce in the body [15], and nitric oxide [16], on the other hand, iron is one of the sources of free radicals which release from Fenton/Haber-Weiss or autoxidation reactions, that lead to the formation of reactive oxygen species (ROS) and lipid peroxidation [17]. Cp acts as an antioxidant by oxidizing iron from its ferrous to ferric state, thus preventing oxidative damage induced by ferrous mediated free radical generation by the Fenton reaction [18]. By ferroxidase activity, Cp assists the export of iron from the cell because only ferric iron can be incorporated with transferrin in the bloodstream [19]; extracellular ferroxidases that included Cp are essential in ferroxidation, which is therefore required for the optimal export of iron, possibly through the production of ion gradients [20].

The present study aimed to find the relation between the oxidase activity of Cp and the specific activity of this enzyme with breast cancer. Also, the relation between the iron concentrations which can cause a high amount of free radicals in breast cancer and ceruloplasmin activity. In addition, the ability to use Cp as a tumor marker for the early diagnosis of breast cancer is possible.

2.Materials and Methods

2.1.Patients and sample age

This study is concerned with malignant breast cancer patients from Nanakeli Hospital-Erbil/Kurdistan region of Iraq from November 2018 to January 2019. The diagnosis of breast cancer was confirmed by 15-3 (CA 15-3) cancer antigen which is a protein formed by normal breast cells; which were carried in the laboratories of the hospital mentioned above.

Eighty-five (85) females were involved in this study. 65 of the human breast cancer patients with age between 20-60 years as a patient group and 20 subjects as a control group with age between 22-56 years old, the distribution of patients age is shown in figure (1). Shows that there were 33% (n=28) of patients in age between (20-35) years & 54% (n=46) of patients in age between (36-55) years and 13% (n=11) of patients (up to 56) years.

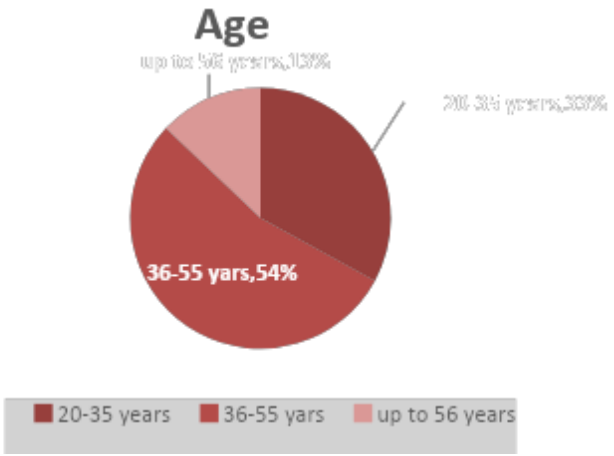

Figure 1. Distribution of patients' age including in this study

2.2. Sample collection

Blood samples were collected by taken 3 milliliters of venous blood from patients was put in a gel tube and left to clot then the serum was separated immediately from the cells by centrifugation at 3000 rpm for 10 minutes, stored until used. The sera which were obtained from blood sample should be un hemolyzed and non-jaundice to avoid any interference with the obtained results. Any case with chronic diseases or chemotherapy treatment were excluded.

2.3.Methods

Ceruloplasmin oxidase activity was performed by using the modified rice method and p-phenylene diamine -2HCL as a substrate [21]. Colorimetric method described by Gornall and et al.[22.23] was used to determine the concentration of total protein in the specimen. The specific enzyme activity is described as follow:

Specific activity (unit/g) = activity of enzyme (U/L) / Protein concentration (g/L)

The colorimetric method was used to estimate the total iron concentration in sera which depend on the dissociation of iron-transferrin bound in an acidic medium, ascorbic acid reduces ferric to ferrous and then to a colored complex of 3-(2pyridyl)-5,-6difuryl-1,-2,-4-triazine-disulfonate (ferene) [24].

2.4.Statistical Analysis

Statistical analysis for this study was done using the Graph Pad prism 7.1 software. The unpaired t-test descriptive statistics was used which is important to test for a statistical significance of the difference in original data.

3.Results and Discussion

3.1.Total protein concentration and total iron concentration

The level of serum total protein concentration was measured in all study groups sera. The results of statistical analysis showed no significant difference (p 0.0108) between the mean total protein concentration of the breast cancer patients (6.596 g / dl) and the mean total protein concentration of the control group (7.127 g / dl), as shown in **Table (1).**

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The level of serum total iron concentration was measured in all study groups. According to statistical analysis, the results show a significant increase (P<0.0001) in the patient group compared with the control group as shown in **Table (1).**

Table 1. The concentration of serum total protein and serum total iron in control and patient group.

Parameters	Mean±SD of Control group	Mean(g/dl) ±SD of Patient group	P value
Total Protein (g/dl)	7.127 ± 0.3527 (N=20)	6.592 ± 0.8926 (N=65)	0.0108
Serum total iron (µg/dL)	128.6 ± 46.02(N=20)	338.2 ± 137.7 (N=65)	(P<0.0001)

3.2. Oxidase activity and specific activity ceruloplasmin

The level of serum Cp activity was measured in sera of all study groups, and the statistical analysis of the findings showed a significant difference between the mean concentration of Cp in patients with breast cancer (227.8 U / l) and the mean activity of Cp in the control group (101.1 U / l) as shown in **Table (2).** Cp specific activity was calculated in all study groups. The results appeared a significant increase in enzyme-specific activity for the patient group compared with the control group as shown in **Table (2).**

Table 2: Oxidase activity and specific activity of ceruloplasmin in patient and control group

Parameters	Mean±SD of Control group	Mean±SD of Patient group	P-value
Cp oxidase activity (U/L)	101.1 ± 27.08	227.8 ± 54.99	<0.0001
Specific Activity of Cp (U/mg)	14.14 ± 3.665 N=20	34.74 ± 9.525 N=65	<0.0001

3.3.The relation between smoking and ceruloplasmin oxidase activity.

Figure (2) was shown a slight significant increase (<0.05) between smokers (Mean (U/L) 218.7 ±SD 56. 64) and nonsmoker (Mean (U/L) 229. 6 ±SD 55.) of the patient group.

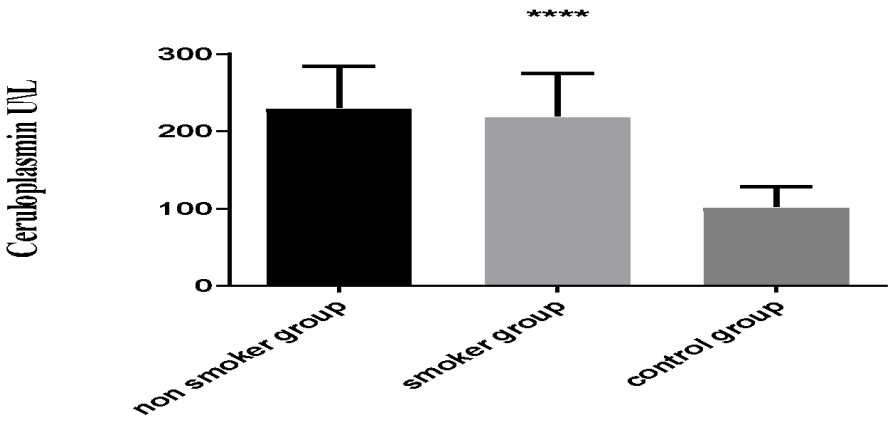

Figure 2. Ceruloplasmin activity in smoker and nonsmoker of the patient group.

In the last decade, researchers have confirmed a significant elevation in free radicals and their role in the micro-environmental tumor[25]. Antioxidants are the major preservation of the body against free radicals and other oxidants, being the substances that stop the attack and the production of radical species inside the cells [26]. The increase of iron concentrations after menopause could be an important etiological factor in the development of breast cancer [27]. The Cp is considered one of important antioxidant that prevents the formation of free radicals by inhibiting Fenton reaction, it can change Fe^{2+} to Fe^{3+} which represent a very important step to load Apo transferrin with iron [18], furthermore, it stimulates the reoxidation of copper I to copper II [28]. Iron imbalance is a distinct physiological phenomenon in women, which is probably to affect their health before, during, and after menopause [27]. There is a possibility that iron deficiency may contribute to a high recurrence of breast cancer in premenopausal women, while iron load may play a role in the incidence of breast cancer in postmenopausal women [28] High concentrations of iron, calculated as plasma iron, transferrin saturation and total iron-binding capacity (TIBC), have been correlated in human studies with elevated overall cancer risk [29] and increased risk of death from any form of cancer[30] . Ceruloplasmin plays a critical role in maintaining the iron level thus it has potential influences in the development of breast cancer [31]. Furthermore, plasma copper rises in the case of malignant tumors level [32], contributes to an increase in the concentration of ceruloplasmin [33] . There is also an increase in the rate of synthesis and secretion of this glycoprotein by the liver[32] in the tumor state. Tumor cells are capable of absorption from plasma so that they contain a relatively large amount of copper that affects pathogenic angiogenesis [34].
Ceruloplasmin was also reported to be associated with different types of cancers [35]. Increased glycoconjugates can be the product of an inflammatory reaction correlated with neoplasm since serum Cp is an acute phase reactant [36], it is also increased in patients of the uterine cervix [37]. Several studies were occurred on sera and solid tumor tissue to figure out the concentration of Cp and some analytes that correlated with this enzyme in cancer patients which emphasizes there is a relation between Cp and prostate, colon cancer patients [38] and with bile duct cancer [35]. The increase of Cp activity in this study has agreed with a study by Ozour et al which found increasing in Cp concentration in breast disease [39]. Further study confirmed that Cp concentration increased in patients with metastatic breast cancer [36] and patients with primary breast cancer [40]. In addition, the result in the current study agrees with research by Jing fan et al which studied the relationship between serum level of copper and Cp concentration in sera of patients with early breast cancer patients by using atomic absorption spectroscopy and immunoturbidimetry assay [41]. On the other hand, the result in the current study disagrees with Debek JT et al which found the Cp activity was significantly decreased in postmenopausal stage I and II breast cancer patients [42].

4.Conclusions

According to the results of this study, serum Cp oxidase activity and specific activity were highly significantly elevated in-patient group when compared with the control group. Likewise, the results showed a significant increase ($P<0.0001$) between the total iron concentration means of a patient with breast cancer and the control group. Protein concentration was increased but insignificantly (p 0.0108) in the patient group when compared with control group.

We propose that ceruloplasmin can use as a tumor marker to diagnose or the follow-up patients with breast cancer. Further study can occur for ceruloplasmin in malignant and benign groups like determination of molecular weight and finding the isomers or forms of this enzyme to find the ability to use this enzyme as a tumor marker. And determine other activities of ceruloplasmin. Finally measure other parameters such as copper, transferrin that related to ceruloplasmin.

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