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FERRITIC AS A POTENT MARKER OF BREST CANCER

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ABSTRACT
Background and objectives: Iron is one of the major component of hemoglobin, is required for transport of oxygen, ferritin is a protein for storage iron, and transferring is a protein for iron transport, all these it may be change in breast cancer.
The aim of present study was to measure the serum ferritin, iron, total iron binding capacity, transferring, and C-reactive protein concentration in breast tumors.
Material and method: A prospective study was carried out from April 2013 to August 2014 by clinical biochemistry department in College of Pharmacy-University of Sulaimani on (45) healthy female individuals, (group 1) and (50) females with breast tumor (group 2).
Results: The mean value of serum iron, transferring, total iron binding capacity were significantly lower in females with breast tumors (group 2), than that of healthy female individuals, (group 1), while serum ferritin was significantly higher in females with breast tumors (group2), than that of healthy individuals (group 1).
Conclusion: Based on findings of the present study it can be concluded that breast tumors can cause deficient of all iron profile except ferritin will be increase in female breast cancers.
Key words- Serum iron, S.Ferritin, S. Total iron binding, S. Transferrin, S.C-Reactive protein, breast tumors.

I. INTRODUCTION

Breast cancer is the most common malignancy in women. Successful treatment of breast cancer relies on a better understanding of the molecular mechanisms involved in breast cancer initiation and progression[1]. Ferritin is the primary intracellular iron storage protein and it also abundant in circulation. In breast cancer patients, ferritin is detected at higher levels in both serum and tumor lysates, and its increase correlates with poor clinical outcome [2].

Ferritin has been traditionally considered a cytoplasm iron storage protein. However, several studies over the last two decades have reported the nuclear localization of ferritin, specifically H-ferritin, in developing neurons, hepatocytes, corneal epithelial cells and some cancer cells. This ferritin beyond iron storage, such as a role component, DNA protection form iron - induced oxidative damage, and transcription at regulation [3]. The role of iron in breast cancer could potentially benefit patients by decreasing recurrence and incidence and increasing overall survival[4].

Iron is an essential element and critical component of molecules involved in energy production, cell cycle and intermediate metabolism. However, the same characteristic chemistry that makes it so biologically versatile may lead to iron - associated toxicity as consequence of increased oxidative stress. Iron has been consistently linked to carcinogenesis[5]. Iron is abundant universally. During the evolutionary processes humans have selected iron as a cancer as a carrier of oxygen inside the body. However, iron works as a double - edged sword, and its excess as a risk for cancer, presumably via generation of relative oxygen species[6].

Iron metabolism is influenced by a stronger may synergistically promote breast cancer. Iron overland is more often seen in the modern world, due to increased dietary intake (meat meals) or iron supplements, and is considered as one of the risk factors for development of breast cancer[7,8]. Transferrin is a glycoprotein and is the chief iron transport protein in mammalian blood, the more aggressive the tumor, the higher the transferrin receptor levels and the greater the proliferative index[9].

Serum C-reactive protein (CRP), a sensitive marker of systemic inflammation, has been reported to be associated with the risk of a number of cancers including breast cancer. However, the results are in consistent[10].

II. MATERIALS AND METHODS

A. Subjects

This study was conducted over a period of sixteen months, from April 2013 to August 2014, and the subjects include (45) healthy female volunteers (group1), aged between 20-60 years, and (50) females with breast tumor (group 2), aged between 20-60 years. All the cases in both groups (1&2), are non smoking and non drinking alcohol. The diagnosis was based on clinical examination in Hiwa hospital.

B. Serum Sampling

Four to six mls. of venous blood was withdrawn from each individual using disposable syringes. The samples were immediately centrifuged for [10] min at 3000 rpm, the serum obtained was removed and kept at -20c· till analysis.

1. Estimation of Serum ferritin

Principle
The assay principle combines a one-step enzyme immunoassay sandwich method a final fluorescent detection. The solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predispensed in the sealed reagent strips.
All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into a fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of the antigens present in the sample.
At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory and then printed out[11].

2. Estimation of Serum iron

Principle

After dissociation of iron transferring bound in acid medium, ascorbic acid reduces Fe^{+3} iron into Fe^{+2} iron. Fe^{+2} irons then form a colored complex with 3-(2-pyridyl) -5, -6-difuryl-1, -2, -4-triazine-disulfonate (Ferene). The absorbance thus measured at 600 nm is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent the copper interference [12].

3. Estimation of total iron binding capacity

Principle

Total iron binding capacity (T.I.B.C) is determined by addition of sufficient Fe^{+3} to saturate iron binding sites on apotransferrin. The excess Fe^{+3} are removed by adsorption with basic magnesium carbonate powder.
After centrifugation, bound iron remaining in supernatant is measured with direct method (Ferene) [13].

4. Estimation of transferrin

Principle

The transferring is a quantitative turbidimetric test for the measurement of transferring in human serum or plasma.
Anti-transferrin antibodies when mixed with samples containing transferring, from insoluble complexes. These complexes cause an absorbance change, dependent upon the transferring concentration of the patient sample, that can be quantified by comparison from a calibrator of know transferring concentration [14].

5. Estimation of C-Reactive protein (CRP)

Principle

Particle-enhanced immunoturbidimetric assay

- Sample and addition of Tris (hydroxymethyl)-aminomethane (TRIS) buffer: 16 mmol/L, pH 7.4; preservative
- Addition of Latex particles coated with anti-CRP mouse monoclonal antibodies: 0.1 %; glycine buffer: 50 mmol/L, pH 8.0; preservative and start of reaction:

Anti-CRP antibodies coupled to latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically [15].

III. SPASTICALLY ANALYSIS

All biochemical parameters were compared between groups using T-test. The statistical software SPSS Version 11(statistical package for social sciences) was used for this purpose. The value of $P<0.05$ was considered as significant[16]

IV. RESULTS

Table 1: provides the mean ± S.D. serum ferritin in both groups. The results obtained reveal that the mean ± S.D. serum ferritins in breast cancer were (672.3 ±570.8 ng/ml). These values exceed significantly ($P < 0.0000038$) than those obtained in normal group, mean ± SD (54.007 ± 26.15 ng/ml). On the other hand serum ferritin in patients thirteen folds more than those of normal subjects.

Table (1): The mean ± S.E.M. of S. ferritin (ng/ml) in normal and breast tumors groups.

Samples	Number	Mean ±S.D (ng/ml)
Healthy group Group (1)	45	54.007 ± 26.15
Breast cancer group Group (2)	50	672.3 ±570.8

Females with breast cancer V Healthy female individuals
Z= 476.29 P < 0.00000338

Table 2: Shows the mean ± S.D. serum iron in both groups. The results obtained reveal that the mean ± S.D. serum irons in breast cancer were (28.5 ± 9.48 ug/dl). These values lower significantly ($P < 0.001$) than those obtained in normal group, mean ± SD (91.84 ± 23.25 ug/dl).
On the other hand serum iron in patient's three folds lowers than those of normal subjects.

Table (2): The mean ± S.D. of S.Iron level in normal and breast tumors groups.

Samples	Number	Mean ±S.D
Healthy group Group (1)	45	91.84 ± 23.25 IU/
Breast cancer group Group (2)	50	28.5 ± 9.48

Females with breast cancer V Healthy female individuals
Z= -6.32 P < 0.001

Table 3: provides the mean ± S.D. of serum total iron binding capacity (T.I.B.C), in both groups. The results obtained reveal that the mean ± S.D. serum total iron binding capacity (T.I.B.C), in breast cancer were (259.09 ± 54.31 ug/dl). These values lower significantly ($P < 0.001$) than those obtained in normal group, mean ± SD (441.47 ± 47.45 ug/dl).

Table (3): The mean ± S.E.M. of S. total iron binding capacity (T.I.B.C) activity in normal and breast tumors groups.

Samples	Number	Mean ±S.D (ug/dl)
Healthy group Group (1)	45	441.47 ± 47.45
Breast cancer group Group (2)	50	259.09 ± 54.31

Females with breast cancer V Healthy female individuals
Z= -17.32 P < 0.0001

Table 4: Shows the mean ± S.D. of serum transferrin, in both groups. The results obtained reveal that the mean ± S.D. serum transferrin, in breast cancer was (153.89 ± 24.26 ug/dl). These

values lower significantly (P < 0.001) than those obtained in normal group, mean ± SD (283.4 ± 35.35 ug/dl).

Table (4): The mean ± S.E.M. of S.transferrin in normal and breast tumors groups.

Samples	Number	Mean ±S.D (ug/dl)
Healthy group Group (1)	45	283.4 ± 35.35 IU/
Breast cancer group Group (2)	50	153.89 ± 24.26

Females with breast cancer V Healthy female individuals
Z= -20.99 P < 0.0001

Table 5: provides the mean ± S.D. of serum C-reactive protein (CRP), in both groups. The results obtained reveal that the mean ± S.D. serum (CRP), in breast cancer was (5.42 ± 2.4 mg/dl). These values lower no significantly (P=0.895) than those obtained in normal group, mean ± SD (5.48 ± 2.4 mg/dl).

Table (5): The mean ± S.E.M. of S.C-reactive protein (CRP) in normal and breast tumors groups.

Samples	Number	Mean ±S.D (mg/dl)
Healthy group Group (1)	45	5.48 ± 2.4
Breast cancer group Group (2)	50	5.42 ± 2.4

Females with breast cancer V Healthy female individuals
Z= -0.132 P =0.89

V. DISCUSSION

Table 1: provides the mean ± S.D. serum ferritin in both groups. The results obtained reveal that the mean ± S.D. serum ferritins in breast cancer were (672.3 ±570.8 ng/ml). These values exceed significantly (P < 0.0000038) than those obtained in normal group, mean ± SD (54.007 ± 26.15 ng/ml). On the other hand serum ferritin in patients thirteen folds more than those of normal subjects. Serum ferritin is an acute phase reactant and thus may be elevated in a number of conditions, including infections, inflammation, malignancy, and liver disease.[17] Serum ferritin and iron both show acute phase responses to inflammation, so iron may fall and ferritin rise independent of the marrow iron store.[18] hyperferritinemia is commonly found in chronic inflammatory status or neoplasis.[19] Multiple factors, however, influence the iron status and ferritin level, which may complicate interpretation of data. Anemia is often present in breast cancer patients, and iron deficit may be hidden by high ferritin levels, elevated due to presence of cancer. In such cases, reticulocyte hemoglobin content and soluble transferring receptor may be used sensitive markers of iron deficiency[20].

Table 2: Shows the mean ± S.D. serum iron in both groups. The results obtained reveal that the mean ± S.D. serum irons in breast cancer were (28.5 ± 9.48 ug/dl). These values lower significantly (P < 0.001) than those obtained in normal group, mean ± SD (91.84 ± 23.25 ug/dl).

On the other hand serum irons in patient's three folds lower than those of normal subjects.

Understanding the role of iron imbalance in breast cancer could lead to adjuvant therapeutic treatments, and potentially benefit patients by decreasing recurrence and incidence and increasing overall survival[21].

Low S.transferrin may reflect iron deficiency, causing disequilibrium between iron stores, the circulation, and the bone marrow.[17] Toxicity of free, redox-reactive iron is largely based on Fenton and Harbef-Weiss chemistry, where catalytic amounts of iron are sufficient to yield hydroxyl radicals from superoxide and hydrogen peroxide[22]

Table 3: provides the mean ± S.D. of serum total iron binding capacity (T.I.B.C), in both groups. The results obtained reveal that the mean ± S.D. serum total iron binding capacity (T.I.B.C), in breast cancer were (259.09 ± 54.31 ug/dl). These values lower significantly (P < 0.001) than those obtained in normal group, mean ± SD (441.47 ± 47.45 ug/dl).
In previous studied similar results were obtained in decreasing the TIBC level in infection, inflammation, and neoplasia [24]. The decrease of TIBC may be caused by three factors, high iron requirements as a result of an enhanced erythropoiesis, an insufficient release of iron from the body iron stores, and insufficient iron absorption[25].

Table 4: Shows the mean ± S.D. of serum transferrin, in both groups. The results obtained reveal that the mean ± S.D. serum transferrin, in breast cancer was (153.89 ± 24.26 ug/dl). These values lower significantly (P < 0.001) than those obtained in normal group, mean ± SD (283.4 ± 35.35 ug/dl).

Transferring receptors on proliferating and malignant cells are well documented. Iron is an essential micronutrient cell growth that plays an important role in energy metabolism and DNA synthesis. Malignant cells requiring more iron modulate a transferring receptor. Iron-bound transferring interacts with the receptor, facilitating the transport of iron across the cell membrane. Transferring may be used as a carrier to target toxic therapy selectively to tumor tissue[23]

VI. CONCLUSION

This study attempted to establish the extent of serum iron. Ferritin, transferrin, total iron binding capacity and hs-CRP in breast tumors as compared with normal individuals (controls). Based on study results, it is concluded that the activity of serum ferritin is consistently higher in breast tumor. We can also conclude that there is a significant decrease of each; serum iron, transferring and total iron 23monitoring patients with breast tumors

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