Detection of Serum Ferritin in Women with Breast Cancer

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Abstract

Breast cancer is one of the most common cancers in females. In Iraq there are noticeable elevation in incidence rates and prevalence of advanced stages of breast cancer. Ferritin is intracellular iron storage protein abundant in circulation and its main application in differential diagnosis of anemia.
The level of serum ferritin was found raised in various cancers including breast cancer. The aim of this study was to assess whether the serum ferritin concentration would be altered in Iraqi women with breast cancer and it could be related to progression of disease.

Sixty eight females participated in this study .The mean age of these females was 53.25± 9.52 .The level of serum ferritin was measured in 24 Iraqi women of early stage of breast cancer (stage I and II) and 24 Iraqi women of advanced stage (III and IV). These levels were compared with 20 healthy females as controls. Serum ferritin was estimated by using enzyme linked immune sorbent assay method. This serum ferritin was found to be raised in all breast cancer patients ($p<0.05$) as compared to controls. The rise in ferritin level was significant in advanced stage ($p<0.05$) as compared to early stage. Thus the estimation of ferritin may aid in diagnosis, assessment of severity and monitoring of Iraqi women with breast cancer.

Key words: - Ferritin, Breast cancer, Iraq.

قياس مستوى الفرتيين في المصل في النساء المصابات بسرطان الثدي

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الخلاصة

يعتبر سرطان الثدي واحد من اهم السرطانات الشائعة لدى الاناث في العراق هناك زيادة واضحة في نسبة حدوث المرض ووصوله الى مراحل متطورة .الفرتيين هو البروتين المسؤول عن خزن الحديد موجود داخل الخلايا وايضا في الدورة الدموية.
اهم تطبيقاته هو للتشخيص التميزي لانواع فقر الدم. مستوى الفرتيين الموجود في مصل الدم وجد انه يرتفع في حالات السرطان ومن ضمنها سرطان الثدي. الهدف من هذه الدراسة هو لتقييم فيما اذا كان مستوى الفرتيين في مصل الدم يتغير في النساء العراقيات المصابات بسرطان الثدي وهل ان مستواه في الدم له علاقة بتقدم الحالة المرضية (مراحل المرض).

ثمانية وستون امراة شاركت في هذه الدراسة ومعدل اعمارهم ٥٣,٢٥ ±٩,٥٢ في هذه الدراسة تم قياس مستوى الفرتيين في مصل الدم لـ ٢٤ امرأة عراقية مصابة بسرطان الثدي في المراحل الابتدائية (مراحل ١ و ٢) وايضا تم قياسه في ٢٤ امرأة عراقية مصابة بسرطان الثدي في المراحل المتقدمة (مراحل ٣ و ٤) وتم مقارنة النتائج مع الاصحاء باخذ عينات دم من ٢٠ امرأة عراقية غير مصابة بالمرض.تم فياس مستوى الفرتيين في مصل الدم باستعمال طريقة فحص المناعي المرتبط بالانزيم .لقد وجد ان مستوى الفرتيين مرتفع في النساء المصابات بسرطان الثدي مقارنة من النساء الاصحاء وايضا وجد ان مستوى الفرتين في النساء المصابات بسرطان الثدي في المراحل المتقدمة اعلى من مثيلاتهم في المراحل الابتدائية من هذه النتائج نتوقع انه من الممكن الاستفادة من قياس مستوى الفرتيين. لمتابعة النساء المصابات بسرطان الثدي ومعرفة مدى تقدم الحالة وتطورها.

الكلمات المفتاحية :- الفرتيين، سرطان الثدي، العراق.

Introduction

Ferritin is the protein that stored iron [(1)]. This protein is synthesized in the liver, spleen, myocardium, placenta and other tissues. It is a large macromolecule (450kDa) which consists of 24 subunits that form protein shell (apoferritin) around an insoluble core of stored iron. There are 2 types of subunits, the basic L and the acidic H type [(2)]. Although the two subunits share approximately 55% of their amino sequences, in addition to their multi-helical three dimensional structures, they are functionally different [(3)]. The H subunit has ferroxidase activity and it is responsible for the oxidation of ferrous iron into ferric iron, whereas the L subunit contribute to the stable storage of iron in the ferritin core. The efficient storage of iron in ferritin requires cooperation of both [(4)]. Ferritin is an abundant protein in circulation which is known as serum ferritin in addition to its intracellular form. Serum ferritin was first detected in 1948 in animals experiencing hepatic cirrhosis or shock [(5)].These observation was later confirmed in humans with different types of liver disease [(6)]. Serum ferritin is a dependable indicator of the body's iron stores. Its level is significantly decreased in individuals suffering from iron deficiency anemia or undergoing phlebotomy.

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In contrast serum ferritin levels are increased in patients with iron over load (hemochromatosis or hemosidriosis), infection or inflammation, malignancies and damage of liver tissues [7, 8]. Ferritin has recently been implicated in the pathogenesis of disease including cancer. A number of mechanisms such as pro-oxidant and pro-inflammatory pathways are responsible for this [9]. Ferritin has long been disreputable for its association with breast cancer either as cause or result of the disease [10].

Breast cancer is the mainly frequent cancer among women, comprising about 23% of all females' cancer [11]. It is also the most important cause of cancer-related death worldwide, case fatality rates being highest in low resource countries [12]. In Iraq, breast cancer is the commonest type of female malignancy accounting for approximately one third of the registered female cancers according to the latest Iraqi cancer registry. This shows that the breast is the chief cancer site among the Iraqi women [13].

As projected by the world health organization, early detection and screening of the disease, especially when combined with adequate therapy, suggest the most immediate hope for decline in breast cancer mortality [14]. The tumor markers in breast cancer like CA 15-3 and CEA are generally useful in follow-up of patients with metastatic disease with other diagnostic technique like X-rays and CT scan. This disease is still in the need of more precise biomarkers which might help in early detection, assessment of severity and for prediction of therapy response [15].

Therefore this study was planned to estimate the level of serum ferritin in patients with breast cancer at different stages (early and advance). The aim was to inspect potential relations between the level of serum ferritin in Iraqi women with breast cancer and the progression of disease.

Material and Methods

The present study was conducted at the teaching hospital of oncology-Baghdad under specialized senior supervision for the period of November 2013- July 2014. Institutional ethics committee approval was obtained prior to initiation of the study.

An informed written approval was recorded from all the study subjects previous to their enrolment in the study. Sixty eight females were enrolled for this study. Out of these, 48 were patients with breast cancer (age range 33-69) and 20 were healthy females (age range 35-67).The patients were staged according to American Joint Committee of Cancer (AJCC) staging 2010 (TNM) [9].

The subjects were divided into three groups:

Group A: 20 healthy females with mean age 51.9 ± 9.71.

Group B: 24 patients with histopathologically proven breast cancer in early stage of disease with mean age 53.1± 10.25 (stage I and II, AJCC-TNM stage).

Group C: 24 patients with histopathologically proven breast cancer in advanced stage of disease with mean age 55.8 ± 8.80(stage III and IV, AJCC-TNM stage).

Inclusion criteria: healthy non anemic females were included in this study as control group .for patients group, subjects with untreated histopathologically proven breast cancer were chosen.

Exclusion criteria: for control group, subjects with fasting plasma glucose more than 120mg /dl and blood pressure more than130 /85mmHg were excluded from this study. For patients group, subjects with history of liver or kidney damage, acute inflammatory and infectious disease, anemia (Hb<10gm%), diabetes and those on medication like iron supplement, or thyroxin or having benign tumor or mass anywhere else in the body are excluded from the study as any of these factors may influence the serum ferritin levels. 5ml fasting blood sample was withdrawn from median cubital vein of each study participant with all necessary aseptic precautions. All samples were allowed to stand for 10 min to obtain serum. After centrifugation, non hemolysed sera were kept at -20 ^{0}C for subsequent analysis as early as possible.

Ferritin was estimated by using enzyme linked immunosorbent assay method (ELISA).The Ferritin ELISA kit is based on the sandwich principle. This commercially available kit was supplied by Monobind Inc, USA (Accu-Bind). Serum sample were allowed to incubate in wells coated with specific anti-ferritin antibodies. This was further added with Horse-radish peroxidase conjugated anti ferritn antibodies. The amount of bound peroxidase is in direct proportion to the content of ferritin in the sample and the intensity of color formed is proportional to the quantity of ferritin in the samples. Later on stopping the reaction, the color intensity of final mixture was measured at 450nm wavelength with micro plate reader [10].

Statistical analysis and interpretation of data were done by using student's unpaired "t" test (for two group comparison) and ANOVA test (for comparison of three groups of variable sample size). All the data have been expressed as mean ± SEM [standard error of mean]. The levels of significance were calculated for all

three groups. Probability value "p" greater than 0.05 was considered as statistically non-significant alteration while P less than 0.05 was considered to be statistically significant. All statistical analyses were carried out using SPSS (version 17) software.

Results

The demographic characteristics of healthy women and patients with breast cancer in the current study were described in table (1).

Table (1): Demographic characteristics of healthy women and patients with breast cancer.

Age(years)	**less than 45**	**more than 45**	
Group A	5(25%)	15(%75)	
Group B	4 (16.6%)	20 (83%)	
Group C	3 (13%)	21 (87%)	
Marital status	**Married**	**Single**	**Widow/divorced**
Group A	18(90%)	2(10%)	
Group B	18 (75%)	4 (17%)	2 (8%)
Group C	20 (83%)	3(13%)	1 (4%)
Family history with breast cancer	**Positive**	**Negative**	
Group A		20(100%)	
Group B	15 (62.5%	9 (37.5%	
Group C	13 (54%)	11 (46%)	
BMI	**Normal**	**Over weight**	
Group A	7(35%)	13(65%)	
Group B	1(4%)	23 (96%)	
Group C	2(8%)	22(92%)	

BMI: body mass index describes relative weight for height. The healthy weight falls between BMI values of 18.5- 24.9. Overweight falls between 25-30 [16].

Table (2): Ferritin concentrations in healthy and women with breast cancer.

Parameter	**Group A Mean ±SEM**	**Group B Mean ±SEM**	**Group C Mean ±SEM**
Ferritin serum level (ng/ml)	44.1±6.19	196.6±27.68*	248.9±32.32*,**

* Significant difference from group A ($p<0.05$)
** Significant difference from group B ($p<0.05$)

In group A, the determined mean of serum ferritin was (44.1) while in group B the determined mean of serum ferritin was (196.6), and in group C, the determined mean of serum ferritin was (248.9). The levels of ferritin were found to be significantly raised in patients with breast cancer (group B and C) as compared to control (group A) as shown in table (2).Significant difference ($p<0.05$) was observed when compared group B and C to group A. statistically significant difference ($p<0.05$) was observed between group B and C.

Discussion

Cancer is the fourth ranked cause of death in the Eastern Mediterranean Region (EMR), after cardiovascular diseases, infectious /parasitic diseases and injuries according to world health organization (WHO) mortality estimates [17]. The major increase in cancer incidence among the WHO regions in the EMR, where breast cancer is recorded as the commonest type of female malignancy in almost all national cancer registers [18].

In Iraq in addition to being the most important cancer there are noticeable increase in incidence rates and prevalence of advanced stages at presentation associated with more aggressive tumor behaviors, resulting in larger fatality rates[14].

In this study, breast cancer was diagnosed in 48 females. The percentage of patients with breast cancer increased with increasing age over 45. In table (1) we observed that 83% of the patients in group B are in age over 45 and 87% of the patients in group C are in age over 45. Approximately half of cancer cases in EMR occur before the age of 55 and the age standardized incidence rates of all cancer in this region is expected to double as risk factor exposure increases as revealed by WHO estimates [17].

In table (1) high numbers of patients with positive family history observed in this study, 63% of the patients in group B were with

positive family history and 54% of the patients in group C were with positive family history. The customary consanguineous marriages which are known to be common throughout this region could be the main cause [14].

Weight gain and being overweight are risk factor for breast cancer in women who have gone through the menopause [19]. In this study as we observed in table (1) 96% of the patients in group B were overweight and 92% of the patients in group C were overweight. It is believed that obesity is proinflammatory state as it results in release of inflammatory mediators that promote tumor growth [20].

One of the most vital nutritional elements required in physiological activities in the body is iron. Many processes like transport of oxygen, generation of energy and synthesis of DNA are dependent on Iron [21]. Despite the fact that iron is essential for growth and development of cells, it has been proven to be harmful when present in excess amount in the body. In recent times, several lines of evidence have established that the iron storage protein which is termed ferritin is a multi-functional protein that have possible role in proliferation, angiogenesis, immunosuppression and delivery of iron. In the context of cancer ferritin is detected at higher levels in the sera of many cancer patients [7].

In the current study as we observed in table (2) the levels of ferritin were found to be significantly raised in breast cancer patients (in both groups B and C) as compared to control (group A). This increase may either due to increase expression of a tumor derived protein which interferes with iron metabolism or due to nonspecific effect of malignancy on reticule-endothelial iron metabolism [22]. The rise in ferritin levels is reported to be linked with more advanced stage breast cancer [22, 23]. In our study, also the levels in group C were significantly higher as compared to group B ($p<0.05$).

Elevation of serum ferritin levels may be attributed to increased iron requirement by malignant cells for growth and for modulation of transferrin receptor. Transferrin is considered potential markers for identifying cells undergoing divisional activity and requiring the incorporation of additional iron [24]. In addition to increased synthesis by malignant cells, other causes of raised ferritin levels include presence of inflammation, hepatic necrosis due to metastasis and reduced hepatic clearance of ferritin [25].

Hypoxia often associated with solid tumors and it considers one of the factors that encourage production of ferritin [26].Ferritin receptor expression is post transcriptionally regulated by conserved mRNA sequence termed iron responsive element (IRE), to which a transacting protein called iron regulatory protein (IRP) is bound. Early studies demonstrated that hypoxia induces the specific and reversible expression of ferritin. This induction occurs primarily on post transcriptional level due to decreased mRNA binding capacity of IRP [27].

The tumor markers being presently used for breast cancer like CA15 -3 and CEA are not specific and are found raised in other disease too. The utility of these markers is more reliable if analyzed in conjunction with other markers [9].

Conclusion

It is concluded that ferritin may help in assessing the severity and monitoring of breast cancer patients. And it might prove more useful if combined with other biomarkers for breast cancer. Further prospective studies, of a large number of subjects, are required to confirm such a statement and to validate the usefulness of ferritin estimation in combination with other tumor markers

References

1. Marinova M and Vladimirova L. Atomic absorption assessment of mineral Iron quantity in ferritin. Bulg J phys. 2009, 36: 139-44.
2. Koort AM and Viljoen M. Ferritin and ferritin isoforms: structure, function relationships, synthesis, degradation and secretion. Arch physiol Biochem 2007; 113:55-64.
3. Lawson DM, Artymink pJ, Yewdall SJ, Smith JM. Solving the structure of human H ferritin by genetically engineering intermolecular crystal contacts. Nature 1991; 349 (6309): 541-544.
4. AL Khateeb AA, Han B, Connor JR. Ferritin stimulates breast cancer cell through an iron independent mechanism and is localized within tumor associated macrophages. Breast cancer Res treat 2013, 137: 733-744.
5. Abraham M and Ephraim S. Hepatorenal factors in cirrulatory homeostasis; the identification of the hepatic vasodepressor substance with ferritin. J. Biol Chem 1948; 176:771-787.
6. Reissmann KR and Dietrich MR. On the presence of ferritin in the peripheral blood of patients with hepatocellular disease. J. clin Invest, 1956; 33: 588-595.
7. Alkhateeb AA and Connor J.R. The significance of ferritin in cancer: Anti-oxidant, inflammation and tumorigenesis. Biochimica et Biophysica Acta 2013; 1836:245-254.

8. Knovich MA. Ferritin for the clinician. Blood Rev. 2009; 23: 95-104.
9. Dhankar R .Evaluation of ferritin and Nitric oxide levels in breast cancer. American Journals of cancer science 2014; 3:1-6.
10. Norkhede HP. Breast cancer and serum ferritin-Menopausal status perspective: Menopause A fickle determinant. Int J Res Med Sci 2014; 2(1): 258-263.
11. Alwan NA, Mualla F.H. Promoting clinical breast examination as a screening tool for breast cancer in Iraq. Iraqi National Journal of Nursing specialties. 2014; 27(1): 76-82.
12. Anderson BO. Et al. Guideline implementation for breast health care in low income and middle-income countries: Overview of the Breast Health Global. Initiative Global Summit. 2007. Cancer 2008; 113(8): 2221-43.
13. AL-Hashimi MM. Breast cancer in Iraq, Incidence Trends from 2000-2009. Asian Pacific Journal of cancer prevention 2014; 15 (1): 281-286.
14. Alwan NA. Breast cancer: Demographic characteristics and clinical-pathological presentation of patients in Iraq. Eastern Mediterranean Health Journal 2010; 16(11): 1159-1164.
15. Gast MC. Clinical proteomics in breast cancer: a review. Breast cancer Res. Treat. 2009; 116: 17-29.
16. Whitney E and Rolfes SR. Understanding nutrition (10^{th} Ed.).Thomson Learning Inc., Wads Worth, 2005; p. 262-263.
17. Revised globle burden of disease (GBD), WHO 2002 estimate. Geneva, world Health organization, 2003.
18. WHO/EMRO Towards a strategy cancer control in the Eastern Mediterranean Region. 1^{st} ed. Cairo, world Health Organization Regional office for the Eastern Mediterranean 2010.
19. Revers GK, Pirie k, Beral V .Cancer incidence and mortality in relation to body mass index in million women study: cohort study BMJ 2007, 335 (7630); 1134.
20. Key TJ, Appleby PN, Reeves GK. Body mass index, serum sex hormones and breast cancer risk in menopausal women. J Nat Cancer Inst, 2003, 95: 1218-1226.
21. Jian J. Iron and menopause: Does increased iron affect the health of postmenopaused women. Antioxidant Redox Signal. 2009; 11(12): 2939-43.
22. Shpyleva SI, Tryndyak VP, Kovalchuk O.Role of ferritin alterations in human breast cancer cells. Breast cancer Res. Treat 2011, 126; 63-71.
23. Mishra S, sharma DC, shamra P. Studies of biochemical parameters in breast cancer with and without metastasis. Indian J clin biochem 2004, 19: 71-75.
24. Elliot RL, Elliot MC, Wang F. Carcinoma and the role of iron metabolism. A cytochemical tissue, culture and ultrasonic study. Ann Ny Acad Sci. 1993, 698: 159-166.
25. Toyokuni S. Iron and carcinogenesis: from fenton reaction to target genes. Redox Rep. 2002, 7: 189-197.
26. Torti FM. Regulation of ferritin genes and protein. Blood 2002; 99: 3505-10
27. .Qi Y, Jamindar TM, Dawson GJ .Hypoxia alters iron homeostasis and induces ferritin synthesis in oligodendrocytes. Neurochem 1995; 64: 2458-64.