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SERUM FERRITIN IN HEALTHY WOMEN AND BREAST CANCER PATIENTS

SERUMSKI FERITIN U ZDRAVIH ŽENA I OBOLELIH OD RAKA DOJKE

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Summary: Serum tumor markers are important tools in managing patients with breast cancer. Currently used CA 15-3 and CEA have found their clinical application particularly in the follow-up of patients with advanced disease. Ferritin belongs to a group of other molecules of potential interest to clinicians whose concentration is also altered in sera of patients with breast tumors. In this study the serum ferritin concentration was estimated in the sera of breast cancer patients before initial surgical treatment or those with advanced disease, and compared to healthy women as control. Ferritin level was measured by an immunoradiometric assay. The aim was to asses whether the serum ferritin concentration was altered in breast cancer and whether it could be related to progression of the disease. In healthy women, a statistically significant difference ($p<0.05$) in ferritin concentration was observed between premenopausal and postmenopausal women. In both breast cancer groups ferritin levels were higher than in healthy premenopausal women (both $p<0.05$). In patients with advanced disease, ferritin was further elevated ($p<0.05$) compared to preoperative levels in the patient group undergoing initial surgical treatment. These results indicate that an elevated ferritin concentration in the serum of younger women could serve as an additional parameter in breast cancer diagnosis and staging.

Keywords: breast cancer, tumor marker, iron, ferritin

Kratak sadržaj: Serumski tumorski markeri su značajno analitičko sredstvo u proceni kancera dojke. Trenutno najznačajniji markeri raka dojke, CA 15-3 antigen i CEA, su posebno značajni u praćenju pacijenata sa uznapredovalom bolešću. Feritin pripada grupi drugih molekula koji mogu biti potencijalno interesantni u praksi i čija je koncentracija takođe promenjena u serumu pacijenata sa tumorima dojke. U ovoj studiji serumska koncentracija feritina je određena u grupi pacijenata sa kancerom dojke pre hirurške intervencije i grupi pacijenata sa uznapredovalom bolešću i upoređena je sa vrednostima izmerenim u populaciji zdravih žena. Koncentracija feritina određena je pomoću imunoradiometrijskog testa. Cilj je bio da se utvrdi da li se serumska koncentracija feritina menja u prisustvu kancera dojke i da li postoji veza sa napredovanjem bolesti. U populaciji zdravih žena određena je statistički značajna razlika ($p<0,05$) u koncentraciji serumskog feritina između pre- i postmenopauzalnih žena. Kod obe grupe pacijenata sa kancerom dojke serumski nivo feritina je bio viši u odnosu na nivo dobijen kod zdravih žena pre menopauze (u oba slučaja $p<0,05$). U pacijenata sa uznapredovalom bolešću, koncentracija serumskog feritina je bila viša ($p<0,05$) u odnosu na preoperativne vrednosti izmerene kod pacijenata koji su podvrgnuti hirurškoj intervenciji. Ovi rezulatati ukazuju na to da bi merenje koncentracije feritina u serumu mlađih žena moglo da posluži kao dodatni parametar u dijagnozi i proceni stadijuma kancera dojke.

Ključne reči: kancer dojke, tumorski marker, gvožđe, feritin

Introduction

Breast cancer is the most frequent malignant disease and the main cause of cancer mortality among women worldwide (1). In Serbia, cancer is second to cardiovascular diseases as a cause of death. For women in Serbia, breast cancer is the most frequent malignancy and the main cause of cancer related mortality (2).

Serum tumor markers are an important analytical and diagnostic tool in managing cancer patients. Currently known tumor markers are not reliable enough for early diagnosis of breast cancer. The most important serum tumor markers in patients with breast cancer are mucin antigen CA 15-3 and carcinoembryonic antigen (CEA). Their main application is

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in the follow-up of patients with metastatic disease, in combination with imaging techniques and physical examination (3). The large number of patients suffering from breast cancer has put this malignant disease in the focus of interest for many clinicians and investigators. Efforts are directed to identify new markers of breast cancer, which might be used for early detection, staging and prognosis, and prediction of therapy response. Ferritin is one of the proteins whose concentration may be altered due to breast cancer presence (4).

Ferritin is a large macromolecule (450 kDa) which is synthesized in the liver, spleen, myocardium, placenta and other tissues and plays a major role in iron storage. It consists of 24 subunits which form protein shell (apoferritin) around an insoluble core of stored iron. There are two types of subunits, the basic L and acidic H type. Different isoferritins have different proportions of these two subunits (5). Ferritin is a sensitive indicator of iron deficiency, thus the main clinical application of serum ferritin measurement is in differential diagnosis of anaemia. Ferritin concentration may increase in case of iron overload (haemochromatosis or haemosiderosis), infection or inflammation, neurodegenerative disorders, malignancies and destruction of liver tissue (6).

Iron is an essential element, but its excess may be harmful. Iron overload is connected with increased risk for some malignant diseases, among them breast cancer. Iron is necessary for cell proliferation, and iron metabolism is influenced by estrogen hormones. Interactions between iron and estrogen may synergistically promote breast cancer (7). Iron overload 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). In conditions with elevated iron, increased ferritin concentrations may have a protective role, preventing oxidative stress caused by excess iron (9, 10).

In this preliminary study, the ferritin concentration was determined in sera of healthy individuals and patients diagnosed with breast cancer. The aim was to investigate potential relations between ferritin serum levels in breast cancer patients and the progression of the disease compared to healthy subjects.

Materials and Methods

Samples

Serum samples were collected from healthy women (n=31, age range 26–69) and patients with breast cancer (n=57, age range 28–74). All samples were collected according to the local ethical principles.

Control group was formed from volunteers from our laboratory personnel (premenopausal group, n=15, age range 26–48) and our retired personnel (postmenopausal group, n=16, age range 61–69), who applied for regular annual control testing in the Laboratory of the Institute for the Application of Nuclear Energy (INEP), during the period June–November 2008. According to the red blood cell count and hemoglobin concentration, none of these persons had evident anemia. None of the subjects included suffered from chronic disease or abused alcohol.

Diagnosis of breast cancer was established at the Institute of Oncology and Radiology of Serbia, Belgrade, Serbia, during 2007, independently of this investigation. Breast cancer patients were clustered in two groups. The first group (n=36, age range 28–63) consisted of patients directed to surgery. The second group (n=21, age range 36–74) consisted of patients whose disease advanced after initial surgical treatment.

Blood samples from control subjects and patients with advanced disease were taken in the morning between 8 and 9 a.m., after 12 h of fasting. In patients from the first patient group, blood samples were drawn immediately prior to surgery. Sera were separated within 1 h from venipuncture, and samples were aliquoted and stored at –20 °C until assayed.

Ferritin determination

Ferritin concentration in sera was determined using the immunoradiometric assay IRMA Ferritin (INEP, Belgrade-Zemun, Serbia). Radioactivity was measured on a gamma-counter (WIZARD, LKB). All measurements were made in duplicate. Ferritin concentration was expressed in μg/L.

Statistical analysis

Statistical analysis was done using the statistical software program MedCalc (version 11.2.1.0. http://www.medcalc.be). Chi-square test was used for testing the distribution of obtained data. Obtained values for ferritin concentrations were not normally distributed and data were presented as median (Me) with central 95% range (between 2.5^{th} and 97.5^{th} percentiles). The differences between groups were tested using the Kruskal-Wallis test, followed by post-hoc analysis. The statistical significance was set at $p < 0.05$.

Results

Ferritin concentrations in the sera from healthy subjects and patients with breast cancer are summarized in *Table I*. In general, a wide distribution of ferritin levels was observed in all groups.

In the control group, the determined median was 38 (range 9–207). Statistically significant diffe-

Table I Ferritin concentrations in healthy and women with breast cancer.

Group	Ferritin (μg/L)
Healthy premenopausal (n=15)	18 (9–41)
Healthy postmenopausal (n=16)	72 (11–166)*
Breast cancer preoperative (n=36)	66 (12–246)*
Breast cancer advanced disease (n=22)	141 (18–719)*

Ferritin concentration is presented as median with central 95% range.
* Statistically significant difference compared to healthy premenopausal women ($p < 0.05$)

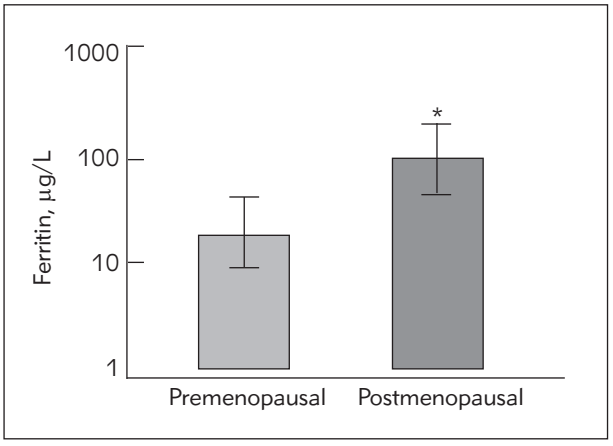

Figure 1 Differences in ferritin levels in the control group in respect to menopausal status (median with central 95% range).
* Statistically significant higher ferritin concentration in healthy postmenopausal women compared to healthy premenopausal women ($p < 0.05$)

rence ($p < 0.05$) was observed between ferritin levels in premenopausal (Me = 18, range 9–41) and postmenopausal women (Me = 72, range 11–201). (*Figure 1*). Comparing the ferritin levels in breast cancer patients, there was no difference between pre- and postmenopausal women, hence the preoperative and advanced patient groups were further analyzed regardless of menopausal status.

Significantly higher ferritin levels were measured in breast cancer patients before surgery, as well as in those with advanced disease, than in healthy premenopausal women (both $p < 0.05$). On the other hand, there was no difference in ferritin levels between healthy postmenopausal women and preoperative patients. The highest ferritin concentrations were found among patients with advanced disease (Me = 140, range 18–719). These values were significantly higher compared to healthy persons (both pre- and postmenopausal), as well as breast cancer patients in early disease stages ($p < 0.05$ in all cases, *Figure 2*).

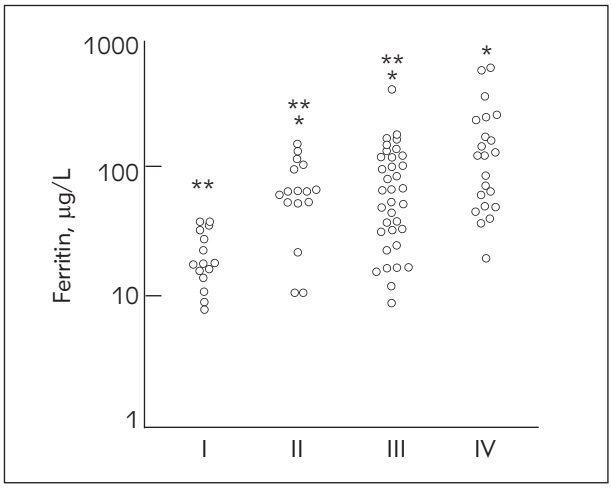

Figure 2 Distribution of ferritin levels in healthy women and patients with breast cancer (I control group premenopausal, II control group menopausal, III patients with breast cancer preoperative, IV patients with breast cancer advanced disease).
* Significantly higher ferritin levels, compared to healthy premenopausal women (I) were measured in healthy postmenopausal women (II), in breast cancer patients before surgery (III), and in patients with advanced disease (IV) (all $p < 0.05$).
** Ferritin concentrations among patients with advanced disease (IV) were significantly higher compared to healthy persons (both pre- (I) and postmenopausal (II)), as well as breast cancer patients in early disease stages (III) ($p < 0.05$).

Discussion

Ferritin is a protein highly conserved through evolution, suggesting its essential role. Iron is necessary for multiple vital functions, such as oxygen transport, electron transfer, as an enzymatic cofactor, cell division and proliferation, but excess iron may have adverse effects (5, 9). One of the possible mechanisms responsible for deleterious effects of iron is generation of free radicals. In these reactions estrogen might be a substrate (introduction of hydroxyl group and formation of catecholestrogen). Through generation of reactive oxygen species, which interact with and damage DNA, iron is included in the promotion of carcinogenesis (7, 11). In such conditions up-regulated ferritin expression might be a compensatory protective mechanism (9, 10).

The main regulator of ferritin synthesis is the iron level (12). In this study, the lowest ferritin concentrations were obtained in healthy premenopausal women. This finding is not unexpected, since in women of reproductive age the iron pool is relatively small due to physiological loss that is not compensated by adequate intake, and thus the ferritin levels are low. In postmenopausal women, decreased iron loss leads to increase in stored iron, and ferritin levels in elderly women rise and approach those seen in men. In the postmenopausal period, continually accumulated iron may be linked with age associated increased risk for breast cancer (7).

In breast cancer patients from this study no age/menopause difference in ferritin concentrations was observed. This might be due to the potential additional regulators involved in ferritin synthesis. Iron is the main, but not the only regulator of ferritin expression. Hypoxia, often present in neoplastic tissue, is also one of the factors that promote ferritin increase independently of the iron status (12, 13).

Published data suggest that in malignant diseases, not only is the ferritin concentration elevated, but isoferritin profile is also changed (12, 14). For instance, the H-type ferritin subunit is predominant in malignant tissue, but is almost very low in normal tissue. (7, 14, 15).

Increased ferritin content was demonstrated in malignant cells compared to normal tissue (16). In cell culture studies, ferritin expression was higher in cells with a more aggressive phenotype (17). It has been shown that ferritin concentration correlates with nodal status (18), and low expression of the ferritin light chain is connected with good prognosis in nodes negative patients (19). These results indicate that ferritin concentrations may be a prognostic indicator in some patients with breast cancer. In this study the highest levels of ferritin were obtained in the group of patients with advanced disease, which was in agreement with previous reports (20, 21).

Since none of the currently known tumor markers is reliable enough to reflect tumor presence or burden, there are attempts to improve diagnostic specifity and sensitivity by combining two or more markers, as in the combination of CA 15-3 and ferritin (21).

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 haemoglobin content and soluble transferrin receptor may be used as sensitive markers of iron deficiency (22).

Ferritin has been previously associated with breast cancer. Still, there is no consistent conclusion regarding its role or relevance in breast cancer (23). Iron overload may be present in neoplastic cells. There is a possibility that the determination of ferritin changes in the tissue would be a more effective parameter (20). Further analysis should be directed to identifying patients in whom tissue iron excess is present, and whether tissue ferritin levels correlate with serum concentration. Considering the alterations in ferritin structure in malignant disease, a test that is more specific for H type isoferritins might be interesting for patients with breast cancer. Our results showed that in younger women, additional determination of ferritin may be indicative of the development of a pathological process, as well as tumor burden in patients with advanced disease. Further prospective studies, of a large number of subjects, are required to confirm such a statement and to validate the usefulness of ferritin determination in the serum of breast cancer patients.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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