

SERUM FERRITIN CONCENTRATION IN SOLID TUMOR PATIENTS

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Summary

Ferritin is a positive acute phase reactant. It is produced and secreted by various cells such as hepatocytes, macrophages and cancer cells. This was a retrospective study which included 46 patients with solid tumors who were tested for ferritin level in plasma. The measured value of the ferritin concentration in plasma of patients was in the range from 5.98 to 850.78 µg/L, and the average measured value was 174.62 µg/L. For the control group, we also included 46 subjects. The measured value of ferritin in the control group was in the range from 5.94 to 351.54 µg/L, and the average measured value was 63.17 µg/L. There is a statistically significant difference between the two groups. Our data shows that the highest level of ferritin was measured in patient with breast cancer. By many studies done until now it is shown that iron metabolism is dysregulated in cancer, and that changes occur in both intracellular and extracellular ferritin.

KEYWORDS: *ferritin, cancer, H-subunit, iron*

KONCENTRACIJA FERITINA U SERUMU KOD BOLESNIKA SA SOLIDINIM TUMORIMA

Sažetak

Feritin je reaktant akutne faze. Proizvode ga i luče različite stanice, jetrene, makrofagi i neoplastične stanice. U ovoj retrospektivnoj studiji obrađeni su podaci 46 bolesnika sa solidnim tumorima kojima je određena koncentracija feritina u seruu. Koncentracija feritina kod bolesnika kretala se u rasponu od 5,98 do 850,78 g / L, a prosječna izmjerena vrijednost je 174,62 g / L. Kontrolan skupina je 46 zdravih osoba. Izmjerena vrijednost feritina u kontrolnoj skupini kretala se u rasponu 5,94 do 351,54 g / L, a prosječna izmjerena vrijednost je 63.17 g / L. Postoji statistički značajna razlika između dvije skupine. Naši podaci pokazuju da je najviša razina feritina izmjerena kod bolesnice s rakom dojke. Mnoge studije učinjene do sada se pokazale su da postoji poremećaj metabolizma željeza kod malignih stanja, te da dolazi do promjena i unutarstaničnog i izvanstaničnog feritina.

KLJUČNE RIJEČI: *ferritin, tumor, H-podjedinica, željezo*

INTRODUCTION

Ferritin is a positive acute phase reactant, exhibiting increased levels in blood during the acute phase response. Ferritin is produced and secreted by various cells such as hepatocytes, macrophages and cancer cells. Expression of ferritin and its subunits is regulated by the amount of metabolically

available iron, the presence of oxidative stress and both pro- and anti-inflammatory cytokines. Ferritin as an acute phase reactant is well known for its intracellular iron sequestration and storage abilities during immune activation (1).

The major functions of intracellular ferritin are the sequestration, storage and release of cellular iron. Body iron can be present as either the

highly toxic Fe²⁺-ions or as harmless, insoluble Fe³⁺-ions. Iron is mainly absorbed in the duodenum and upper jejunum. A transporter protein called divalent metal transporter 1 (DMT1) facilitates transfer of iron across the intestinal epithelial cells. DMT1 also facilitates uptake of other trace metals, both good (manganese, copper, cobalt, zinc) and bad (cadmium, lead). Iron within the enterocyte is released via ferroportin into the bloodstream. Iron is bound by the transport glycoprotein named transferrin. Both DMT-1 and ferroportin are found in a wide variety of cells involved in iron transport, such as macrophages. Most absorbed iron is utilized in bone marrow for erythropoiesis. About 10 to 20% of absorbed iron goes into a storage pool in cells of the mononuclear phagocyte system, particularly fixed macrophages, which is also being recycled into erythropoiesis. There is a balance of storage and use. Iron is initially stored as a protein-iron complex ferritin. Ferritin can be incorporated by phagolysosomes to form hemosiderin granules (2). Ferritin detoxifies Fe²⁺-ions by converting them to the insoluble Fe³⁺-ions. Ferritin can accumulate up to 4500 iron atoms as a ferrihydrite mineral in a hollow protein shell and releases these iron atoms when there is an increase in the cell's need for bio-available iron. The ferritin protein consists of 24 protein subunits of two types: the H-subunit and the L-subunit (3,4). The ferritin protein shell can exist as heteropolymers of various combinations of these two types of subunits – a phenomenon that gives rise to the existence of iso-ferritins (5). H-subunit rich ferritins have been shown to accumulate and release iron faster than do L-subunit rich ferritins (6, 5, 7 and 8). Cells with a high content of H-subunit rich ferritins include erythroid cells, heart cells, pancreatic cells, kidney cells, lymphocytes and monocytes (9, 10), whereas the L-subunit rich ferritins are found predominantly in liver and spleen – organs associated with long-term iron storage (11, 10). High plasma ferritin levels have been reported for various types of cancers, irrespective of the amount of total body iron (12). In patients with solid tumors, such as pancreatic carcinoma, lung cancer and hepatoma, there is a particularly high incidence of elevated plasma ferritin, and in patients with breast cancer, with metastasis, ferritin plasma concentrations are commonly elevated (10).

In our study we wanted to confirm the null hypothesis that there is no significant difference between the levels of serum ferritin in patients with cancer and healthy controls, and no statistically significant differences in the level of ferritin among the groups tested in relation to sex.

PATIENTS AND METHODS

This was a retrospective study which included 46 patients with solid tumors who were tested for ferritin level in plasma within regularly pre-operative testing in University Hospital for Tumors from 2008 to 2010. The study included patients with normal CBC, with no signs of anemia.

Blood was taken in EDTA specimen tube. Test was made with Vidas Ferritin quantitative test, using ELFA technique (enzyme linked fluorescent assay). The intensity of the fluorescence is proportional to the concentration of ferritin present in plasma. Solid phase is coated with mouse monoclonal anti-ferritin immunoglobulins. Manufacturer reference value of serum ferritin is shown in table 1.

Table 1.

REFERENCE VALUE RECOMMENDED BY MANUFACTURE

Men 18-30 years	18,7-323µg/L
>31 years	16,4 - 294µg/L
Normal menstruating women	6,9 – 282,5 µg/L
Menopausal women	14,0 – 233,1 µg/L

All the results were processed using descriptive statistical methods. To test the statistical significance of the difference between the mean values of ferritin measured in plasma we used t-test. Statistical analysis was performed with SPSS 17.0 software.

RESULTS

The study included 46 patients with histologically proven solid tumor, of whom 78%(n=36) were female and 22% (n=10) were male. The mean age of patients was 58±13 years. Patients in this study were diagnosed and differed according to the type of cancer.

With breast cancer were 54% patients, 10% with colon cancer and 8.7% with liver cancer. Oth-

Table 2.
DISTRIBUTION OF TYPE OF CANCER AMONG PATIENTS GROUP

Cancer	Frequency	Percent
cervix	1	2,2
breast	25	54,3
liver	4	8,7
colon	5	10,9
pancreas	1	2,2
lungs	1	2,2
prostate	2	4,3
rectum	3	6,5
testicles	2	4,3
stomac	1	2,2
gallbladder	1	2,2
Total	46	100,0

er cancers are much less represented, in 2 - 6.5% of cases (Table 2).

In the group of female patients and high values of plasma ferritin, the most frequent were the patients with breast cancer. (n=25).

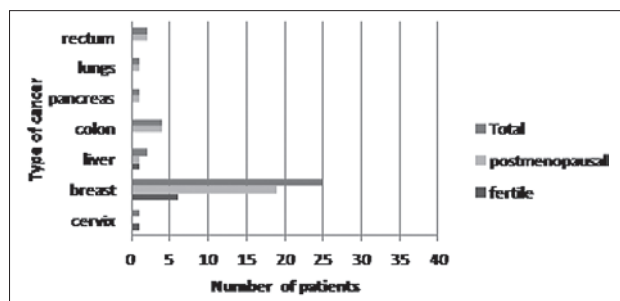


Figure 1. Type of cancer among female patients.

Prevalence of cancer in men is different; some are more frequent than the other such as prostate and liver cancer.

The measured value of the ferritin concentration in plasma of patients was in the range from 5.98 to 850.78 µg/L, and the average measured value was 174.62 µg/L. The average measured value in the group of patients was within the reference value of ferritin in plasma regardless of whether they are men or women.(Table 3).

Value of ferritin found in our patients sample was in 76% cases within the expected reference values (Table 4) even if we took into account the age and sex of the patients (Figure 2).

Table 3.
MEASURED VALUES IN THE GROUP OF PATIENTS

Value of ferritin measured in cancer patients	
average measured value	174,62
minimum measured value	5,98
maximum measured value	850,78

Table 4.
VALUE OF SERUM FERRITIN IN CANCER PATIENTS ACCORDING TO REFERENCE VALUE

Value of serum ferritin in cancer patients		
	Frequency (N)	Percent (%)
below the reference value	1	2,2
elevated level	10	21,7
within reference value	35	76,1
Total	46	100,0

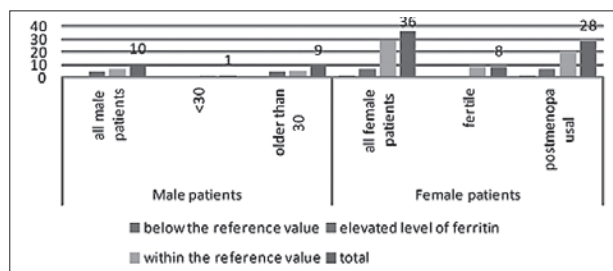


Figure 2. Deviation of ferritin level among tested patients according to gender and age.

For the control group, we also chose 46 subjects, of whom 74% were female (n = 34) and 26% were male (n = 12), average age of control group was 49 years. The measured value of ferritin in the control group is in the range from 5.94 to 351.54 µg/L, and the average measured value was 63.17 µg/L.(Table 5).

Table 5.
MEASURED FERRITIN VALUES IN CONTROL GROUP.

Value of ferritin measured in control group	
average measured value	63,17
minimum measured value	5,94
maximum measured value	351,54

Figure 3 shows graphical representation of measured values of ferritin concentrations in a group of patients and controls.(Fig.3).

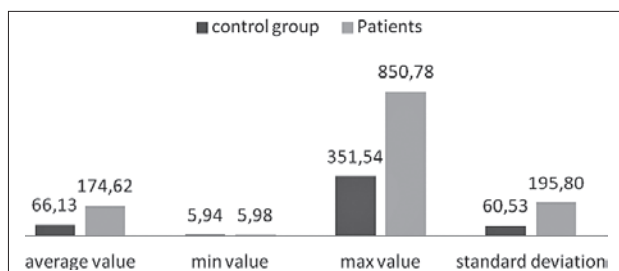


Figure 3. Comparison of measured values of ferritin between patient group and the control group

To test the statistical significance of the difference between the mean values measured in plasma ferritin of patients and control group, we used the t -test. As the determined value of $t > t = 3,55$ to 45 degrees of freedom, for the threshold of significance $p = 0.001$, $p = 0.05$ t -test was found to be statistically significant. We reject the null hypothesis and say that there is a statistically significant difference between the two groups, we accept the error $p < 0.05$ and p certainty $> 95\%$, where we argue that the patients measured ferritin levels were higher than in healthy patients. As it turned out (albeit very small) that there was statistically significant difference we tested the difference t test with respect to the sex of patients. As the t value was $1.38 < t = 3,55$ for 44 degrees of freedom, for the threshold of significance $p = 0.001$; t test was found to be statistically insignificant. We accept null hypothesis and say that there is no statistically significant differences in measured values of serum ferritin between groups of patients and control groups according to gender.

DISCUSSION

The aim of this study was to view if there is significant difference in ferritin level in plasma of cancer patients and healthy people. We gave a descriptive view of our data that we collected during a 3 years period, and showed that there was a statistically significant difference between tested groups. Our data show that the highest level of ferritin was measured in patient with breast cancer. It is of interest that ferritin levels in breast cancer are not only raised in the plasma, but also in breast tissue (13, 14). Although breast cancer is the most common in the population of female patients, also the most common in our sample ($n =$

25, Table 2), we concluded that it is not justified to perform further detailed analysis and determination of differences in ferritin levels measured in the plasma of the patients suffering from breast cancer and healthy subjects. Using the t test is justified if the sample size is less than 30, and if the measured units distributed under the rules of a normal distribution (15).

Many factors are suggested to contribute to the hyperferritinaemia associated with cancer, including inflammation, hepatic necrosis due to metastasis and chemotherapy, blood transfusions and a decrease in hepatic clearance of ferritin (16). Because of these reasons, we included only preoperative patients who did not receive any chemo or radiation therapy, or blood transfusion in time we collected our sample. In many instances the increased ferritin is shown to be H-subunit rich (17, 18, 19, 20, 16 and 21), and it has been suggested that the measurement of H-subunit rich ferritin may be of value in the diagnosis of malignancy (19). In a study involving breast cancer patients it has been shown that H-subunit mRNA was directly related to axillary lymph node status, the presence of metastatic disease and to the clinical stage (22).

The secretion of H-subunit rich ferritins in patients with cancer could be involved in the immunosuppression seen in these patients. Only the H-subunit rich ferritins were found to be elevated in melanoma patients, with the H-subunit to L-subunit ratio correlating with the levels of regulatory T-cells (23).

The main characteristic of cancer cell is rapid proliferation, and the iron in is necessary element in that process. Malignant cells, because of their higher requirement for iron, are very sensitive to iron depletion (24). By many studies done until now it is shown that iron metabolism is dysregulated in cancer, and that changes occur in both intracellular and extracellular ferritin (25, 26, 27, 28 and 29).

CONCLUSION

In our study we demonstrated that there is a statistically significant difference in measured serum ferritin between these two studied groups, but there is no statistically significant difference between serum ferritin in relation to gender.

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