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SHORT COMMUNICATION

Serum Hepcidin Levels in Multiple Myeloma

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SUMMARY

Background: **Multiple myeloma (MM) is a malignant disease with a 10% frequency among all haematology neoplasms. It is characterized by clone proliferation of plasmatic cells in bone marrow, monoclonal gammopathy, and anemia, hypercalcemia, and kidney failure and bone lesions. IL-6 is an inflammatory cytokine, potential growth factor for myeloma cells, as elevated serum levels are connected with poor disease prognosis. IL-6 modulates many gene transcriptions, encoding synthesis of acute phase proteins, including C-reactive protein (CRP) and hepcidin.**

Methods: **We evaluated 21 patients newly diagnosed with multiple myeloma at the Department of Haematology at "Aleksandrovska" Hospital; average age 51 ± 5.2. Their results were compared to 21 age matched controls. Included patients were enrolled before treatment onset. In the included groups, we measured CBC, serum iron and total iron binding capacity, ferritin, soluble transferrin receptors, IL-6, and hepcidin.**

Results: **We established elevated serum hepcidin levels in MM patients compared to the control group: 99.4 ± 10.5 µg/L to 19.9 ± 2.8 µg/L ($p < 0.001$). Serum IL-6 and CRP concentrations were elevated in MM cases compared to controls ($p < 0.005$). In patients with MM we found strong negative correlation between serum hepcidin and inflammation markers, IL-6 and CRP. IL-6 shows $r = -0.894$, and CRP - $r = -0.916$; $p < 0.001$. Soluble transferrin receptors correlate negatively to hepcidin: $r = -0.831$, $p < 0.001$. Transferrin concentration correlated highly positive to hepcidin: $r = 0.580$; $p < 0.001$.**

Conclusions: **Our results show an important role of hepcidin in the evaluation of iron homeostasis disorders. Quantification of the peptide's concentration shows increased concentration in multiple myeloma patients. Treatment of anemia associated with multiple myeloma is still a serious challenge for clinicians.**

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KEY WORDS

multiple myeloma, hepcidin, iron, interleukin-6, anemia

LIST OF ABBREVIATIONS

ACD - anemia of chronic disease
BMP - bone morphogenic protein
BRES - BMP-response elements
CBC - complete blood count
CLIA - chemiluminescent immunoassay
CRP - C-reactive protein

ELISA - enzyme-linked immunosorbent assay
IL-6 - interleukin-6
MM - multiple myeloma
mRNA - messenger ribonucleic acid
STAT 3 - signal transducer and activator of transcription 3
sTfR - soluble transferrin receptors
TIBC - total iron binding capacity
TRSF - transferrin

INTRODUCTION

Hepcidin is known as a regulatory protein that controls iron absorption in the duodenum [1]. Its liver synthesis is in response to a different signal, including iron levels in the human organism. Hepcidin acts by connection and initialization of degradation of ferroportin - the iron exporter from the cells. Ferroportin is found in duodenal enterocytes, hepatocytes, macrophages, and placenta [2]. Cellular uptake of iron in various forms is also subject to regulation, but it seems that ferroportin regulation is the predominant way that iron transport is controled in the organism.

Pathology induction of hepcidin secretion from inflammation causes hypoferremia, iron restriction for erythropoiesis, and finally leads to anemia. The axis of interleukin-6 - hepcidin is important for hypoferremia in inflammatory diseases [3]. It was found that in chronic inflammation IL-6-independent routes can also induce hepcidin mRNA [4].

Hepcidin expression is regulated primarily at the transcriptional level. Two groups of cytokines are known to be key regulators of hepcidin: IL-6 and bone morphogenic protein (BMP). BMPs and IL-6 affect the promoter of human hepcidin through BMP-response elements (BRES) and signal transducer and activator of transcription 3 (STAT3) [5,6]. They are secreted in multiple myeloma. This leads to the assumption that they are also involved in the pathogenesis of myeloma-related anemia [7,8].

Multiple Myelomas are severe haematological system diseases, accompanied by antibody deficiency caused by monoclonal expansion of the malignant cell clone. This leads to inflammation and infection processes. The anemia of the disease is multifactorial. Surely the hepcidin - ferroportin mechanism can play an important role in an accompanying ACD but an aplastic anemia caused by suppression of normal erythropoiesis plays the major role. Dependent on the grade of disease bone marrow infiltration with malignant plasma cells increases. Causal treatment of this anemia form is only possible by chemotherapy/stem cell transplantation.

Multiple myeloma (MM) is a malignant disease with a 10% frequency among all haematology neoplasms [9]. It is characterized by clone proliferation of plasmatic cells in bone marrow, monoclonal gammopathy, and anemia, hypercalcemia, and kidney failure and bone lesions [10]. IL-6 is an inflammatory cytokine, potential growth factor for myeloma cells, as elevated serum levels are connected with poor disease prognosis. IL-6 modulates many gene transcriptions, encoding synthesis of acute phase proteins, including C-reactive protein (CRP) and hepcidin.

MATERIALS AND METHODS

We evaluated 21 patients newly diagnosed with multiple myeloma at the Department of Haematology at "Aleksandrovska" Hospital; average age 51 ± 5.2. Their results were compared to 21 age matched controls. Included patients were enrolled before treatment onset. In the included groups we measured CBC, including reticulocyte count and haemoglobin in reticulocytes, using an ADVIA 2120 (by Siemens Healthcare). Serum iron and total iron binding capacity were evaluated by AAS (on a Perkin Elmer analyzer). Ferritin concentrations were measured by CLIA method on an Immulyte 2000. Soluble transferrin receptors were quantified by nephelometric method (on a ProBN Spec analyzer by Siemens Healthcare). IL-6 and hepcidin in serum were evaluated by ELISA method (provided by R & D Systems). All routine biochemical parameters were measured on Dimension RXL MAX (by Siemens Healthcare). All samples were analyzed after blood sampling. Serum for IL-6 and hepcidin was kept deep frozen during the process of recruiting patients.

Signed informed consent was obtained from all included patients and controls according to the Declaration of Helsinki (Directive 2001/20/EO). This study is part of Grant 2015, sponsored by the Medical University, Sofia, Bulgaria and was approved by its Ethics Committee.

For statistical evaluation of results, we used SPSS 13.0 (IBM). Correlations and significance were rated by Student's paired *t*-test and Pearson's correlation.

RESULTS

Table 1 presents the classification of the multiple myeloma patients.

Table 2 presents the parameters measured in included groups, expressed as mean value and SD.

We established elevated serum hepcidin levels in MM patients compared to control group: 99.4 ± 10.5 µg/L to 19.9 ± 2.8 µg/L ($p < 0.001$). Serum IL-6 and CRP concentrations were elevated in MM cases compared to controls ($p < 0.005$).

In patients with MM we found strong negative correlation between serum hepcidin and the inflammation markers, IL-6 and CRP. IL-6 shows $r = -0.894$, and CRP - $r = -0.916$; $p < 0.001$. Soluble transferrin receptors correlate negatively to hepcidin: $r = -0.831$, $p < 0.001$. Transferrin concentration correlated highly positive to hepcidin: $r = 0.580$; $p < 0.001$.

Serum hepcidin and IL-6 levels in measured groups are

Table 1. Demographic parameters and classification of MM patients.

Parameter	MM	Control group
Age	60.4 (44 - 81)	60.2 (43 - 80)
Gender (M)	6 (28.6%)	6 (28.6%)
DSS class.	I - 19 (90.5%) II - 2 (9.5%)	n.a.
ISS class.	I - 4 (19.0%) II - 14 (66.7%) III - 3 (14.3%)	n.a.

DSS - Durie-Salmon Staging classification, ISSc - International Staging System classification.

Table 2. Measured parameters in included groups (presented as mean value ± SD).

Parameter	MM patients		Control group	
	mean	SD	mean	SD
Hepcidin (µg/L)	99.4	10.5	19.9	2.8
Hb (g/L)	91.4	9.4	139.7	8.8
IL-6 (pg/mL)	3.5	0.9	1.1	0.2
CRP (mg/L)	9.1	1.0	1.9	0.3
Iron (µmol/L)	11.4	0.6	21.7	4.1
TIBC (µmol/L)	59.9	9.4	61.4	2.3
Ferritin (ng/mL)	392.6	20.1	189.6	11.4
sTfR (mg/L)	3.6	0.3	1.9	0.4
TRSF (g/L)	3.9	0.7	1.8	0.5
β2-microgl. (mg/L)	11.01	2.7	0.9	0.1
Albumin (g/L)[µ]	46.6	9.4	34.6	7.9
IgG κ (%)	10 (47.6)		n.a.	
IgG λ (%)	6 (28.6)		n.a.	
Ig M κ (%)	1 (4.8)		n.a.	
IgM λ (%)	1 (4.8)		n.a.	
IgA κ (%)	2 (9.5)		n.a.	
IgA λ (%)	1 (4.8)		n.a.	

SD - standard deviation, Hb - haemoglobin, IL-6 - interleukin-6, CRP - C-reactive protein, TIBC - total iron binding capacity, sTfR - soluble transferrin receptors, TRSF - transferrin, β2-microgl - β2-microglobulin.
[µ] - albumin concentration based on protein electrophoresis.

shown in Figure 1 and Figure 2.

DISCUSSION

In approximately 97% with MM anemia occurs during the disease, and 70% have anemia when MM is diagnosed [11]. Usually anemia is determined by multiple factors and evolves as anemia of chronic diseases (ACD), i.e., caused by inflammatory cytokines. Pathogenesis of ACD is still insufficiently understood, but it was found that high IL-6 levels stimulate liver secretion of hepcidin, that then blocks iron exemption from duodenal enterocytes and macrophages. As a result, iron deficiency occurs [4]. Anemia is resistant to iron supplementation and erythropoiesis stimulating agents, and is usually normocytic, normochromic, with low reticulocyte levels and normal or elevated serum ferritin concentrations [12].

On the other hand, liver hepcidin synthesis is regulated

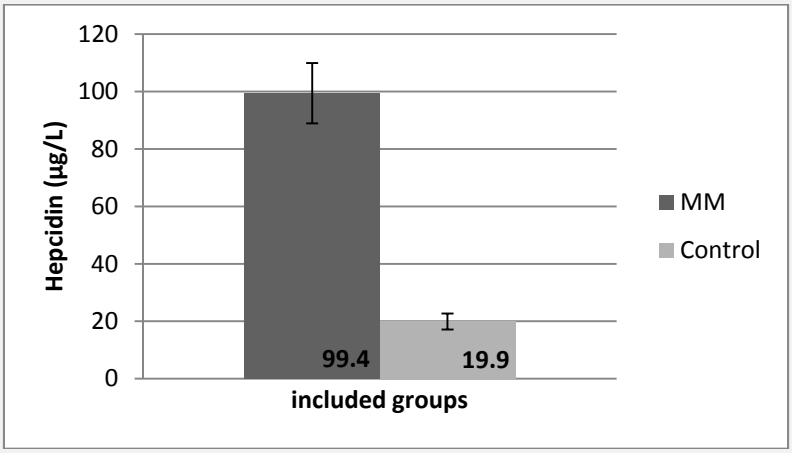

Figure 1. Hepcidin concentration in serum (in µg/L) in multiple myeloma patients and control group.

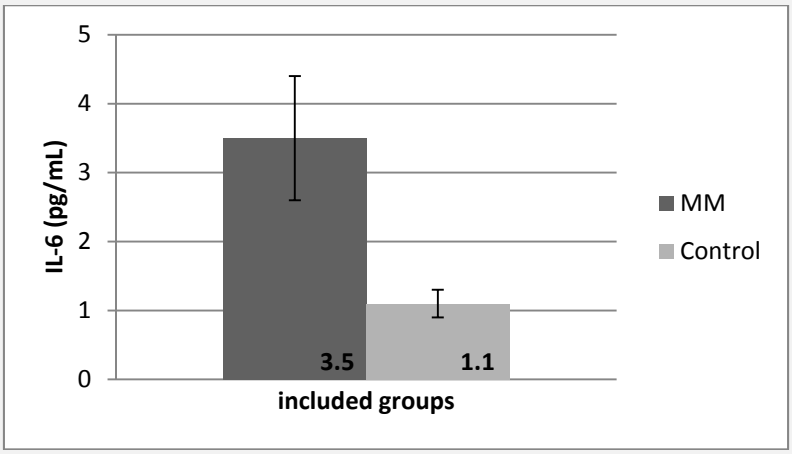

Figure 2. Interleukin-6 concentration in included groups (in pg/mL).

by erythropoiesis activity. In this case elevated hepcidin concentrations might present, independently from IL-6 regulation.

In our previous studies, we found increased serum hepcidin and IL-6 concentrations in patients with ischemic stroke. In the pathogenesis of stroke we found a correlation between destabilisation of atherosclerotic plaques and increased stroke incidents.

Treatment of anemia associated with multiple myeloma is still a serious challenge for clinicians. Frequent blood-transfusions are associated with risk for transmissive infections, iron overload, as well as the progressive increase in serum autoantibody. The administration of recombinant erythropoietin on the other hand is expensive, with insufficient therapeutic response at high levels of hepcidin. This implies seeking new opportunities for correction of anemia, especially in high hepcidin levels to improve the quality of life of affected patients. It seems that hepcidin evaluation is important in different conditions that involve disorders of iron homeostatsis. Elevation of peptide's concentration is seen in rheumatoid arthritis, ischemic stroke, multiple myeloma, in-

testinal bowel diseases, patients with chronic kidney diseases, even in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. In recent years, the focus of scientific interest is focused on application of new therapeutic agents with effect on the axis hepcidin-ferroportin: they could inhibit pathways for hepcidin synthesis or stimulate exports by ferroportin. Similar therapeutic strategies would lead to better management of the violations in the distribution of iron and evolving subsequently into anemia of inflammation.

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Declaration of Interest:
The authors declare that they have no conflicts with any organization or institute during preparation of materials in short communication called "Serum hepcidin levels in multiple myeloma" that is given to Clinical Laboratory GmbH. All patients included in the trial have signed an informed consent according to respective requirements from The Code of Ethics of the World Medical Association (Declaration of Helsinki).
This article has been prepared after one year collection of samples from patients diagnosed with multiple myeloma from the Department of Haematology at "Aleksandrovska" hospital. During this period, no pharmaceutical or other company was involved in the trial. All authors disclose that they have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.
There is no potential conflict of interest related to individual authors' commitments. All authors are responsible for disclosing all financial and personal relationships that might bias their work. All authors state that no potential conflicts exist.

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Ethics:
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