



THE IMPACT OF SEX AND AGE ON SERUM PROHEPCIDIN CONCENTRATION IN HEALTHY ADULTS

Joanna Jasiniewska¹, Ewa Zekanowska¹, Grazyna Dymek², Marzenna Gruszka²

1) Department of Pathophysiology, 2) Department of Laboratory Medicine, Collegium Medicum, University of Nicolaus Copernicus, Bydgoszcz, Poland

Correspondence

Department of Pathophysiology, Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland; M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland; phone: +48 52 585 35 95; email: zhemostazy@cm.umk.pl

Abstract

Introduction: Within the last 8 years, it has become evident that hepcidin has a diagnostic and therapeutic potential. Therefore, it is a great need to establish the reference interval for hepcidin and its precursor. The aim of this study was to assess the impact of age and sex on serum prohepcidin concentration in healthy adults. **Material and methods:** 100 healthy volunteers were enrolled during the 18 months of the study - 56 males and 44 females, mean age 34.8±10.1 yrs. Serum prohepcidin, ferritin, soluble transferrin receptor (sTfR) and plasma erythropoietin were examined by enzyme-linked immunosorbent assay (ELISA) kits. Serum iron and unsaturated iron binding capacity were determined on ARCHITECT ci8200 (Abbott Diagnostics) according to the manufacturer's instructions.

Results: Serum prohepcidin concentrations ranged from 74.9 ng/ml to 300.4 ng/ml in healthy adults of both sexes. However, prohepcidin levels were significantly higher in males (median value 145.7 ng/ml) than in females (median 127.3 ng/ml) (p=0.0016). Serum prohepcidin was not associated with age in the group of healthy adults.

Conclusions: Serum prohepcidin concentrations were found to be related to sex. Significantly lower prohepcidin levels were observed in females compared with males.

Key words: iron metabolism, prohepcidin, reference interval

1. INTRODUCTION

Hepcidin is a circulating 25-amino acid disulfide bonds-rich peptide hormone. It is primarily, but not exclusively, secreted by hepatocytes [1]. Recent studies indicate lower hepcidin gene expression in the heart, kidney, pancreas, adipose tissue, as well as pathogen-activated neutrophils and macrophages [2, 3, 4]. Hepcidin is synthesized as a 84-amino acid pre-pro-peptide, that undergoes two enzymatic cleavages. However, only mature 25-amino acid hepcidin has assigned biological function [5, 6]. The small size of hepcidin-25 (approximately 2 kDa) and its highly cross-linked structure is the main problem in developing a reliable method for quantification of serum, plasma and urine hepcidin. For this reason the majority of clinical research, measure a high molecular weight precursor- prohepcidin by ELISA kits [7, 8, 9, 10].

Hepcidin acts by binding to ferroportin, a cellular iron exporter, inducing its internalization and subsequent proteolytic degradation in the cytoplasm. Thus, overall, hepcidin inhibits iron absorption in the small intestine, iron release from the reticuloendothelial macrophages and iron transport across the placenta [11]. There are many different factors

which can regulate hepcidin expression, including iron excess, tissue hypoxia, erythropoietin and cytokines [12, 13]. This seems to occur at the level of gene transcription via different, closely coordinated signaling pathways [14, 15].

Since the discovery of hepcidin, experimental and clinical studies have been carried out to determine the relationships between hepcidin (or prohepcidin) and iron-related disorders, i.e. haemochromatosis, thalassaemia intermedia, renal anaemia and anaemia of chronic disease [10,16, 17, 18]. However, clinical research pertaining to healthy individuals are unique. As a consequence, a scientific knowledge of hepcidin's physiology and pharmacokinetics hasn't increased significantly over the past few years. Little information exists regarding hepcidin and prohepcidin levels in infants, men during transient hypoxia and women of reproductive age, including those who are pregnant [19, 20, 21]. Moreover, the data available are based on small study groups. Within the last 8 years, it has become evident that hepcidin has a diagnostic and therapeutic potential. Therefore, it is a great need to establish the reference interval for this peptide.

The aim of this study was to assess the impact of sex and age on serum prohepcidin concentration in healthy adults.

2. MATERIAL AND METHODS

STUDY GROUP

A total of 100 healthy adult volunteers were recruited from blood donor candidates from the Regional Blood Transfusion Center in Bydgoszcz (Poland). During the 18 months of the study 56 males (aged 19-60 yrs) and 44 females (aged 19-50 yrs) were enrolled. Those who had taken any iron supplements or been vegetarian were excluded from this study. The local Bioethical Committee of Collegium Medicum, N.C. University in Bydgoszcz approved a study protocol and a written informed consent was obtained from the subjects.

LABORATORY MEASUREMENTS

Blood samples were drawn from an antecubital vein between 8 and 10 a.m. with minimal venous stasis and centrifuged. Serum and plasma were stored at -80°C until analysis.

Serum prohepcidin concentration was measured by ELISA using commercially available kit (DRG International, Marburg, Germany). Serum iron-, unsaturated iron binding capacity- (UIBC) and total iron binding capacity (TIBC) concentrations were measured on Architect ci8200 System (Abbott Laboratories, IL, USA) according to the manufacturer's instructions. The percentage of transferrin saturation (TfS) levels was determined by the ratio of serum iron levels to TIBC. Serum ferritin was measured using DRG Ferritin kit (EIA-1872, DRG Intl, Inc., USA) and soluble transferrin receptor (sTfR) concentrations were examined by an sTfR ELISA (BioVendor Laboratory Medicine, Inc., Czech Republic). Plasma erythropoietin (EPO) was determined by the EPO ELISA kit (Roche Diagnostics GmbH, Mannheim, Germany).

STATISTICAL ANALYSIS

All statistics were calculated using Statistica 8.0. computer software. Data are reported as mean \pm 1SD for parametric data and median (Q1-lower quartile; Q3-upper quartile) for nonparametric data. Differences were evaluated by the independent-sample t test or the Mann-Whitney U test as appropriate. The correlation of serum prohepcidin with iron indices was examined using the Spearman rank correlation coefficient. A probability of < 0.05 was considered statistically significant.

3. RESULTS

The results of iron metabolism parameters of healthy individuals are summarized in Table 1.

Table 1. Iron metabolism parameters in males and females

Parameter	HEALTHY ADULTS				P
	MALE (N=56)		FEMALE (N=44)		
	Me X±SD	Q ₁ /Q ₃ Min-max	Me X±SD	Q ₁ /Q ₃ Min-max	
Iron (µg/dl)	88,00	65,00/109,00	89,00	59,00/108,00	NS
Ferritin (ng/ml)	15,3	9,6/19,07	8,1	4,00/12,4	0,0006
sTfR (µg/ml)	1,02	0,72/1,41	1,25	0,78/1,78	NS
UIBC (µg/dl)	212,3±64,90	142,00-328,00	241,9±60,9	109,00-353,00	0,0278
TIBC (µg/dl)	309,7±43,7	231,00-456,00	329,5±46,2	236,00-442,00	0,0375
EPO (mIU/ml)	4,60	2,88/6,82	4,35	2,61/5,51	NS
TfS (%)	26,4	19,6/34,97	25,7	17,40/34,7	NS

Serum ferritin levels were significantly higher in males (Me=15.3 ng/ml) than in females (Me=8.1 ng/ml) (p=0.0006). To our surprise, depletion of body iron stores, defined as serum ferritin below 10 ng/ml, was observed in 20% of male and 44% of female healthy volunteers. Serum UIBC and TIBC levels also showed sex-related differences. We found significantly higher UIBC and TIBC values in females than in males. However, the results remained within the reference interval for each gender.

No significant differences in serum iron, sTfR, EPO and transferrin saturation between males and females were detected.

Prohepcidin levels were significantly higher in males (median 145.7ng/ml; (123,3-213,7) than in females (median 127.3 ng/ml; 107,4-158,6) (Fig. 1).

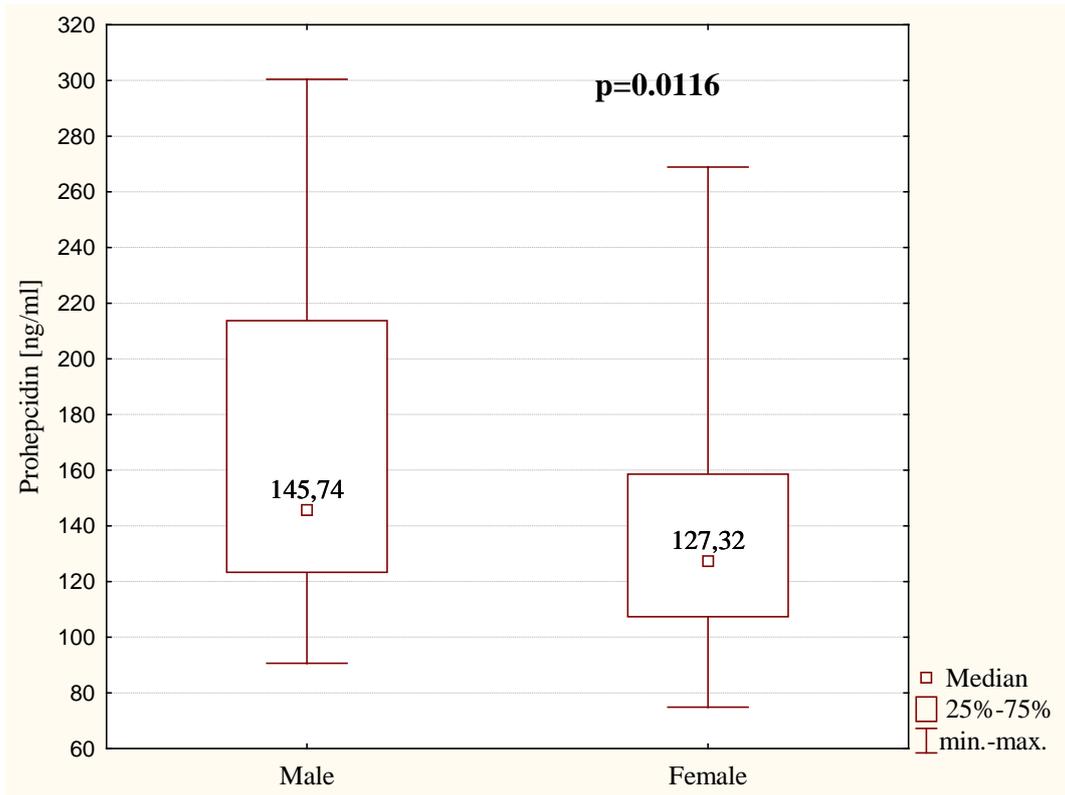


Figure 1. Serum prohepcidin concentrations in males and females

Serum prohepcidin levels were not associated with age in both sexes ($r=-0.2064$, $p=0,1270$; $r=-0.0196$, $p=0,8996$, respectively for males and females). Linear regression analyses on serum prohepcidin concentrations with iron metabolism parameters revealed statistically significant negative correlations between prohepcidin and ferritin in males ($r=-0.52$; $p<0.01$) (Fig. 2) as well as females ($r=-0.44$; $p<0.05$) (Fig. 3). No other correlations in the study group were found.

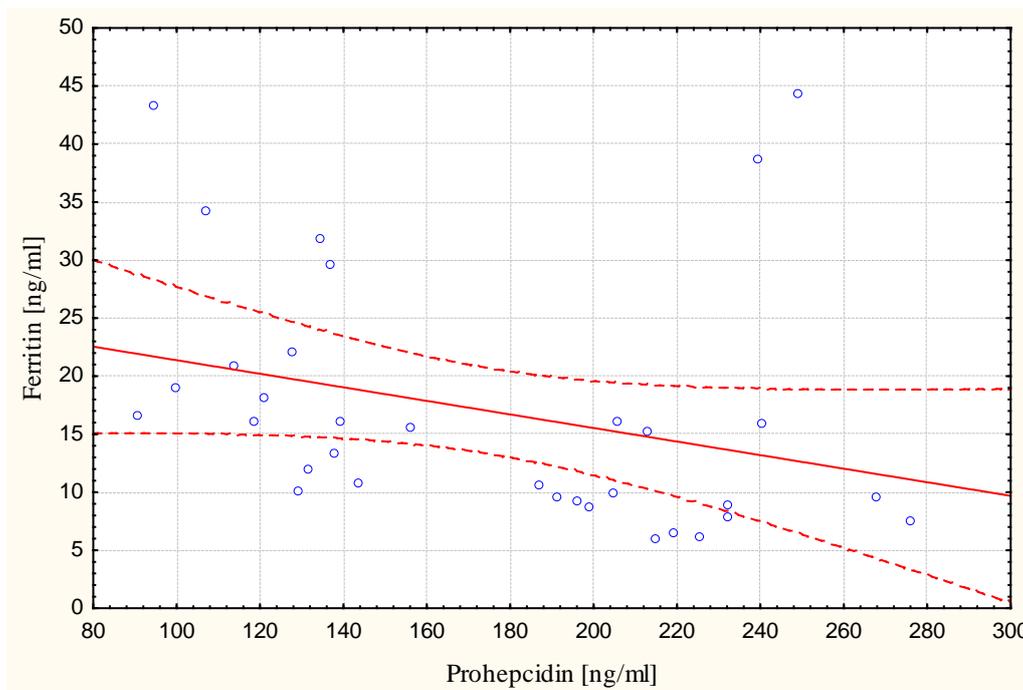


Figure 2. Scatter plot of correlation between prohepcidin levels and ferritin concentrations in the group of males (Spearman rang correlation coefficient $r=-0.52$; $p=0.0021$)

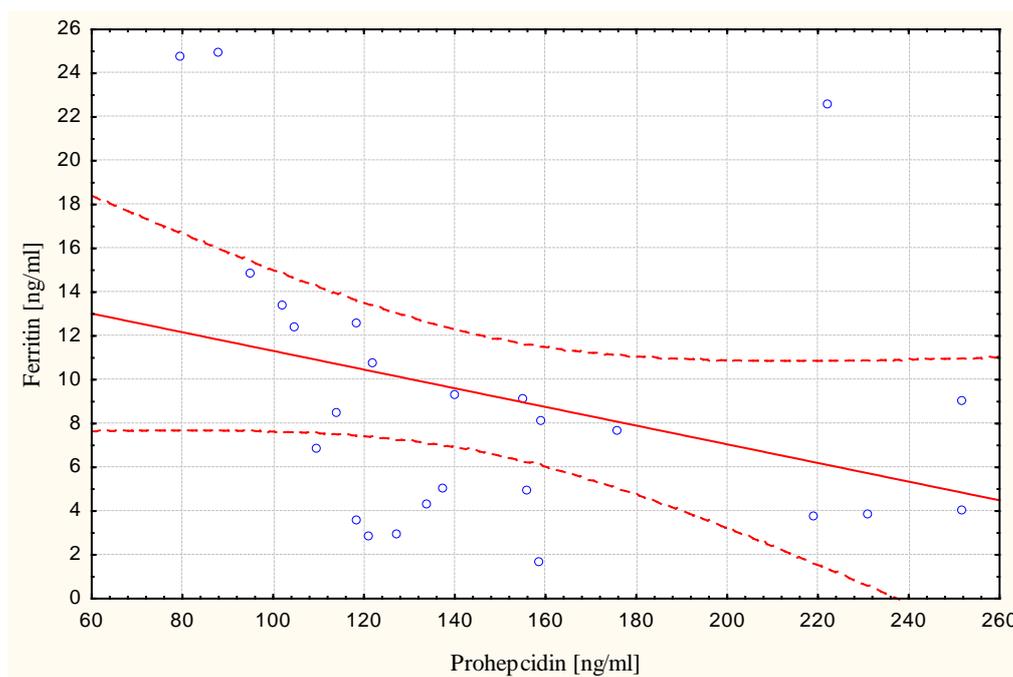


Figure 3. Scatter plot of correlation between prohepcidin levels and ferritin concentrations in the group of females (Spearman rang correlation coefficient $r=-0.44$; $p=0.0268$)

4. DISCUSSION

Hepcidin measurement might turn to be a useful tool in the differential diagnosis of iron deficiency or iron overload disorders in the near future [22, 23, 24, 25]. It is required to establish a reference interval for this hormone and to investigate important aspects of hepcidin biology i.e. diurnal variations, the half-life, age-, sex-, and race- related differences. We undertook the present study to examine the changes in serum prohepcidin concentration as a function of gender and age in healthy adults.

Our studies show that prohepcidin levels range from 74.9 ng/ml to 300.4 ng/ml in healthy adults of both sexes. Kulaksiz et. al. (2004) observed lower prohepcidin levels in the group of 26 healthy volunteers (51.6 – 153.4 ng/ml) [26]. On the contrary, significantly higher results were reported in studies on newborns, non-anemic infants and premenopausal women indicating that prohepcidin shows an extensive physiological variation in serum concentration in healthy subjects [6, 19, 27].

Differences in serum prohepcidin concentrations between healthy males and females were observed by Luukkonen et al. in 2006. They reported mean serum prohepcidin concentrations of 254 ng/ml and 227 ng/ml, respectively, for 16 males and 37 females [28]. However, the difference was not statistically significant because of the large between-subject variation. Our results confirm Luukkonen's observations. Serum prohepcidin levels were significantly higher in males than in females – healthy individuals (145.7 ng/ml vs 127.3 ng/ml). Lower prohepcidin concentration in women compared with men may be attributable in part to the high incidence of iron depletion observed in women (44%). It is known, that menstruating women are especially prone to develop iron deficiency because of ongoing blood loss, recent pregnancies, and inadequate dietary iron [29, 30]. We suggest therefore, that downregulation of serum prohepcidin is perhaps a physiological reaction of the organism to a low-grade iron deficiency observed in young females.

Our study group consisted of 100 healthy individuals between 18 to 60 years of age. Nevertheless, analysis revealed no significant correlations between prohepcidin and age in both male and female subjects. Instead, prohepcidin levels in serum of healthy adults of both sexes were negatively correlated with ferritin. The well-established negative

relation between prohepcidin and ferritin, observed in the present study, could be explained by the physiological feedback loop where increased hepcidin levels through degradation of ferroportin leads to systemic iron deficiency (low ferritin concentration). In contrast to our results, some clinical studies indicated positive correlations between prohepcidin and ferritin [31, 32, 33, 34]. Hepcidin is well known acute phase protein. Its synthesis is induced by proinflammatory cytokines such as IL-1, IL-6 or TNF- α . Therefore, during inflammation both hepcidin and ferritin increase significantly and are thus positively correlated.

The results of our study should be taken into consideration while establishing reference intervals for serum prohepcidin. Further studies are required to examine serum hepcidin levels and their mutual relations with prohepcidin.

5. CONCLUSIONS

Serum prohepcidin concentrations in healthy adults were found to be related to sex. Significantly lower prohepcidin values were observed in females compared with males.

References

1. Park C.H., Valore E.V., Waring A.J., Ganz T.: Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 2001; 276: 7806-10
2. Liu X.B., Nguyen N.B., Marquess K.D., Yang F., Haile D.J.: Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. *Blood Cells Mol Dis* 2005; 35: 47-56
3. Bekri S., Gual P., Anty R., Luciani N., Dahman M., Ramesh B., Iannelli A., Staccini-Myx A., Casanova D., Ben Amor I., Saint-Paul M.C., Huet P.M., Sadoul J.L., Gugenheim J., Srai S.K., Tran A., Le Marchand-Brustel Y.: Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; 131: 788-96
4. Peyssonnaud C., Zinkernagel A.S., Datta V., Lauth X., Johnson R.S., Nizet V.: TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. *Blood* 2006; 107: 3727-32
5. Kato A., Tsuji T., Luo J., Sakao Y., Yasuda H., Hishida A.: Association of prohepcidin and hepcidin-25 with erythropoietin response and ferritin in hemodialysis patients. *Am J Nephrol* 2008; 28: 115-21
6. Hadley K.B., Johnson L.K., Hunt J.R.: Iron absorption by healthy women is not associated with either serum or urinary prohepcidin. *Am J Clin Nutr* 2006; 84: 150-5
7. Taes Y.E., Wuyts B., Boelaert J.R., De Vriese A.S., Delanghe J.R.: Prohepcidin accumulates in renal insufficiency. *Clin Chem Lab Med* 2004; 42: 387-9
8. Roe M.A., Spinks C., Heath A.L., Harvey L.J., Foxall R., Wimperis J., Wolf C., Fairweather-Tait S.J.: Serum prohepcidin concentration: no association with iron absorption in healthy men; and no relationship with iron status in men carrying HFE mutations, hereditary haemochromatosis patients undergoing phlebotomy treatment, or pregnant women. *Br J Nutr* 2007; 97: 544-9
9. Nagashima M., Kudo M., Chung H., Ishikawa E., Hagiwara S., Nakatani T., Dote K.: Regulatory failure of serum prohepcidin levels in patients with hepatitis C. *Hepatology* 2006; 36: 288-93
10. Christiansen H., Saile B., Hermann R.M., Rave-Fränk M., Hille A., Schmidberger H., Hess C.F., Ramadori G.: Increase of hepcidin plasma and urine levels is associated with acute proctitis and changes in hemoglobin levels in primary radiotherapy for prostate cancer. *J Cancer Res Clin Oncol* 2007; 133: 297-304
11. Nemeth E., Tuttle M.S., Powelson J., Vaughn M.B., Donovan A., Ward D.M., Ganz T., Kaplan J.: Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; 306: 2090-3
12. Nicolas G., Chauvet C., Viatte L., Danan J.L., Bigard X., Devaux I., Beaumont C., Kahn A., Vaulont S.: The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002; 110: 1037-44
13. Lee P., Peng H., Gelbart T., Wang L., Beutler E.: Regulation of hepcidin transcription by interleukin- 1 and interleukin- 6. *Proc Natl Acad Sci USA* 2005; 102: 1906-10
14. Wrighting D.M., Andrews N.C.: Interleukin-6 induces hepcidin expression through STAT3. *Blood* 2006; 108: 3204-9
15. Babitt J.L., Huang F.W., Wrighting D.M., Xia Y., Sidis Y., Samad T.A., Campagna J.A., Chung R.T., Schneyer A.L., Woolf C.J., Andrews N.C., Lin H.Y.: Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat Genet* 2006; 38: 531-9
16. Fleming R.E., Sly W.S.: Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. *Proc Natl Acad Sci USA* 2001; 98: 8160-2

17. Bridle K.R., Frazer D.M., Wilkins S.J., Dixon J.L., Purdie D.M., Crawford D.H., Subramaniam V.N., Powell L.W., Anderson G.J., Ramm G.A.: Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *Lancet* 2003; 361: 669-73
18. Dallalio G., Fleury T., Means R.T.: Serum hepcidin in clinical specimens. *Br J Haematol* 2003; 122: 996-1000
19. Ulukol B., Orhon F.S., Hanoluk A., Akar N.: Serum Pro- Hepcidin Levels and Relationship with Ferritin in Healthy Non- Anaemic Infants. *Acta Haematol* 2007; 118: 70-2
20. Benedict Ch., Ghio A.J., Gehring H., Schultes B., Peters A., Oltmanns K.M.: Transient hypoxia and downregulation of circulating prohepcidin concentrations in healthy young men. *Haematologica* 2007; 92: 125-6
21. Laskowska-Klita T., Chelchowska M., Ambroszkiewicz J., Swiatek E., Leibschang J.: Serum pro-hepcidin and iron markers during uncomplicated pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2007; 130: 273-4
22. Kemna E.H., Tjalsma H., Podust V.N., Swinkels D.W.: Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. *Clin Chem* 2007; 53: 620-8
23. Weinstein D.A., Roy C.N., Fleming M.D., Loda M.F., Wolfsdorf J.I., Andrews N.C.: Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood* 2002; 100: 3776-81
24. Malyszko J., Malyszko J.S., Hryszko T., Pawlak K., Mysliwiec M.: Is hepcidin a link between anemia, inflammation and liver function in hemodialyzed patients? *Am J Nephrol* 2005; 25: 586-90
25. Jasiniewska J., Zekanowska E.: Hepcidin- New diagnostic parameter In iron metabolism disturbances ?" *Diagn Lab* 2006; 42: 485-93
26. Kulaksiz H., Gehrke S.G., Janetzko A., Rost D., Bruckner T., Kallinowki B., Stremmel W.: Pro- hepcidin: expression and cell specific localisation in the liver and its regulation in hereditary haemochromatosis, chronic renal insufficiency, and renal anaemia. *Gut* 2004; 53: 735-43
27. Tiker F., Celik B., Tarcan A., Kilicdag H., Ozbek N., Gurakan B.: Serum pro- hepcidin levels and relationships with iron parameters in healthy preterm and term newborns. *Pediatr Hematol Oncol* 2006; 23: 293-7
28. Luukkonen S., Punnonen K.: Serum pro-hepcidin concentrations and their responses to oral iron supplementation in healthy subjects manifest considerable inter-individual variation. *Clin Chem Lab Med* 2006; 44: 1361-2
29. Lehmann P.: Anaemia: A major medical problem in the world. *Europ Clin Lab* 2001; 20: 26-30
30. Chelchowska M., Laskowska-Klita T., Leibschang J.: Stężenie ferrytyny, transferyny i żelaza w ocenie niedoborów tego pierwiastka u zdrowych kobiet w wieku reprodukcyjnym. *Pol Merk Lek* 2007; 127: 25-7
31. Demirag M.D., Haznedaroglu S., Sancak B., Konca C., Gulbahar O., Ozturk M.A., Goker B.: Circulating hepcidin in the crossroads of anemia and inflammation associated with rheumatoid arthritis. *Intern Med* 2009; 48: 421-6
32. Christiansen H., Saile B., Hermann R.M., Rave-Fränk M., Hille A., Schmidberger H., Hess C.F., Ramadori G.: Increase of hepcidin plasma and urine levels is associated with acute proctitis and changes in hemoglobin levels in primary radiotherapy for prostate cancer. *J Cancer Res Clin Oncol.* 2007; 133: 297-304
33. Howard C.T., McKakpo U.S., Quakyi I.A., Bosompem K.M., Addison E.A., Sun K., Sullivan D., Semba R.D.: Relationship of hepcidin with parasitemia and anemia among patients with uncomplicated *Plasmodium falciparum* malaria in Ghana. *Am J Trop Med Hyg.* 2007; 77: 623-6
34. Nagashima M, Kudo M, Chung H, Ishikawa E, Hagiwara S, Nakatani T, Dote K: Regulatory failure of serum prohepcidin levels in patients with hepatitis C. *Hepatol Res.* 2006; 36: 288-93