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The correlation between the concentration of hepcidin in serum and the occurrence of insulin resistance and hyperandrogenemia in women with polycystic ovary syndrome

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Abstract. – OBJECTIVE: Scarce clinical and experimental studies suggest that hepcidin can be a protein participating in the development of metabolic disorders, while its synthesis and concentration in the circulation outside of the iron metabolism parameters can be influenced by hormones. The aim of the present study was to determine the correlation between the concentration of hepcidin in serum and the occurrence of insulin resistance and hyperandrogenemia in women with PCOS.

PATIENTS AND METHODS: Five groups of women with PCOS were divided based on: correct body mass (17 without hyperandrogenemia and insulin resistance – G1; 17 with hyperandrogenemia and without insulin resistance – G2; 11 without hyperandrogenemia and with insulin resistance – G3; 10 with hyperandrogenemia and insulin resistance – G4), metabolic and hormonal parameters and selected markers of iron metabolism.

RESULTS: Serum glucose levels were significantly higher in the group G3 than G1 and in the group G4 than G1 and G2. Serum insulin levels and HOMA–IR values were significantly higher in the groups G3 and G4 than G1 and G2. Serum androstenedione levels were significantly higher in the group G2 than G1 and G3 than G2. Serum transferrin levels were significantly lower in the group G1 than in the reaming study groups.

CONCLUSIONS: It has been demonstrated that insulin resistance and hyperandrogenemia appear to be the factors decreasing the concentration of transferrin circulation, but not the remaining parameters of the iron metabolism in the studied women. No relationship between the concentration of hepcidin circulation and other studied parameters of the iron metabolism and the parameters of the carbohydrate metabolism was discovered. Androstenedione can stimulate hepcidin synthesis in women with PCOS with correct body mass.

Key Words:
Polycystic ovary syndrome, Hepcidin, Iron metabolism, Insulin resistance.

Introduction

The polycystic ovary syndrome (PCOS) is a metabolic-hormonal disorder inhomogeneous in terms of symptoms. Apart from the rudimentary criteria, which include clinical and/or biochemical hyperandrogenism, menstruation disorders in the form of rare menstruation (also rare ovulation or lack thereof), distinctive image of polycystic ovaries in the ultrasound examination, additional difficulties stem from the following metabolic symptoms: glucose tolerance disorders and type 2 diabetes, lipid disorders and hypertension, excessive body weight, insulin resistance and hyperinsulinemia[1-6]. Moreover, the occurrence of iron metabolism disorders in women with PCOS cannot be ignored[7]. Iron excess often observed in those women can lead to serious consequences.

The laboratory markers for iron metabolism primarily include ferritin, iron, and transferrin. Its homeostasis control is also significantly influenced by one more recently discovered protein – hepcidin. This peptide is mostly synthesized in liver cells. The sources of hepcidin, albeit to a lesser degree, are also macrophages, adipocytes, renal tubular cells, spleen, heart, brain, lungs, thyroid and digestive tract cells[8,9]. Expression of hepcidin is variable and depends mostly on systemic iron supplies. Hepcidin was hailed as the main signal peptide that regulates the iron metabolism[10].

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The relationship between PCOS and metabolic disorders such as glucose intolerance or dyslipidemia is very clear. However, the role of iron metabolism disorders in the pathogenesis of metabolic disorders typical for PCOS requires further studies. The correlation between iron surplus and insulin resistance, abnormal glucose tolerance, and type 2 diabetes has been known for years[12]. On the other hand, the correlation between the occurrence of PCOS and iron metabolism disorders was first noted by Escobar – Morreale in 2012, who also discovered a slight surplus of iron in the organism with this syndrome[11].

Researches suggest that there is a two-way dependence between the iron metabolism and the carbohydrate metabolism disorders in the general populace. On one hand, iron excess influences insulin secretion and the cells sensitivity to it. On the other hand, the insulin itself causes iron to increase its concentration[12]. Iron decreases the insulin's capacity to inhibit gluconeogenesis in the liver. Synthesis and metabolism of insulin are also decreased, which when coupled with increased supplies of iron in the liver, results in hyperinsulinemia[11].

The iron surplus in the organism can become a risk factor in the development of glucose tolerance disorders in PCOS as well. The state of mild excess of iron in the organism can be manifested through increased concentration of iron, ferritin, and transferrin in the circulation, as well as the decreased concentration of hepcidin[11,13]. The very rise in ferritin concentration can increase the risk of glucose tolerance disorders by a factor of 2[14,15.] The sensitivity of the target tissue to insulin is inversely proportional to the concentration of ferritin[15].

The state of mild excess of iron in women with PCOS can also stem from other metabolic processes[7]. The excess of androgens, relatively common in PCOS, stimulates erythropoiesis, which when connected with insulin resistance causes the decrease in hepcidin concentration and is a stimulus for intensified intestinal absorption of iron[15]. The description of the negative correlation between the concentration of hepcidin and the concentration of free testosterone in the circulation confirms the correlation between hyperandrogenemia and the iron metabolism disorders[22]. The main aim of this paper is to evaluate the hepcidin concentration and the selected iron metabolism markers in the serum of women with PCOS, insulin resistance and/or hyperandrogenemia, as well as to evaluate the correlation between the hepcidin concentration and the selected iron metabolism markers and the concentration of androgenic hormones in the serum of the aforementioned women.

Patients and Methods

Patients qualified for the study include women hospitalized in the Clinic of Gynecological Endocrinology of the Medical University of Silesia. The study group consisted of 55 women with regular BMI and with PCOS diagnosed at the age of 18-35, including: 17 women without hyperandrogenemia and insulin resistance – G1; 17 women with hyperandrogenemia and without insulin resistance – G2; 11 women without hyperandrogenemia and with insulin resistance – G3; 10 women with hyperandrogenemia and with insulin resistance – G4.

PCOS was diagnosed based on the occurrence 2 out of 3 of the Rotterdam criteria. The ultrasound examination was performed with the Voluson 730 Expert. The studied women were 12 hours from their last meal, on an empty stomach, in the follicular phase between the 2nd and 5th day of the menstrual cycle. Anthropometric examination (weight, height, and waist circumference) was performed, and blood was drawn for the laboratory markings. Using the colorimetric method the markings in the lipid profile serum, glucose, iron, and transferrin (analyzer AU 680 with reagents from Beckman Coulter (Brea, CA, USA)) were made. Using the method of chemiluminescence (with microparticles and chemiluminescence marker (CMIA) and reagents by Abbott (Architect i2000SR; Chicago, IL, USA)), the following serum concentrations were marked: estradiol, FSH, LH, total and free testosterone, 17-OH-progesterone, androstenedione, cortisol, DHEAS, SHBG, and insulin. The ferritin and hepcidin concentrations were marked using the ELISA method (hepcidin – reagent by Peninsula Laboratories International, Inc. (San Carlos, CA, USA); ferritin – reagent by Biovendor (Karasek, Czech Republic).

The evaluation of insulin resistance was conducted using the indirect method with the HOMA-IR index. Insulin resistance was diagnosed at the value HOMA-IR > 1.7.

Free androgen index (FAI) was calculated from the formula: FAI = (total testosterone/SHBG) × 100%.

Adopted nominal values FAI < 5%.

Table I. Characteristics of studied groups.

	G1	G2	G3	G4
Age (years)	26 ± 4	24 ± 4	25 ± 4	24 ± 5
Body mass (kg)	55.2 ± 7.4	54.2 ± 5.8	65.6 ± 6.6	61.2 ± 5.6
BMI (kg/m^2)	20.8 ± 2.2	19.9 ± 2.0	23.2 ± 1.9	22.5 ± 1.8
Waist circumference (cm)	71 ± 6	68 ± 5	87 ± 12	76 ± 8

Nutritional status based on the BMI index was calculated according to WHO criteria.

Statistical Analysis

The data was presented as an average ± SD with median and lower and upper quartiles, minimum and maximum values. Qualitative data was presented as percentages. Normalcy of the result distribution was calculated based on the Anderson – Darling test and the Q-Q quantile chart. To compare the dichotomic variables, the χ^2-test was used. If the anticipated number was less than 5 – either the χ^2-test with Yates correction or the χ^2-test for trend were used. The analysis of the quantitative variables was conducted with: univariate parametrical analysis of variables for data with normal distribution or after logarithmic function normalization, Tukey test post-hoc, univariate non-parametrical analysis of variables Kruskal-Wallis for data with heavily-skewed distribution and the Student *t*-test for independent variables.

Correlations between the variables are presented in the form of multidimensional model of linear regression using the reverse step method and the Pearson linear correlation coefficient. The outlier data was identified based on the Cook distance. While preparing the regression models, the occurrence of variables collinearity based on variance inflation factor (VIF) was simultaneously evaluated. The VIF should not exceed 5. Statistically significant parameters were variables with level of significance p less than 0.05. The calculations were made with the following software: Statistica 10.0 PL, Excel MS Office.

Results

Significant statistical differences between the particular groups in the average values of body mass, BMI index and waist circumference were found (Table I).

By evaluating the metabolic parameters, the serum in G3 was found to have slightly increased concentration of glucose than in G1, while G4 had significantly higher concentration than G1 and G2. The concentration of insulin and the HOMA-IR value was significantly higher in G3 and G4 than in G1 and G2 (Table II).

The concentration in the PRL serum was significantly higher in G3 than in G1, G2, and G4. The concentration of 17-OH-progesterone in the serum was significantly higher in G1 than in G2, in G4 than in G1 and in G2 and in G3 than in G2. The concentration of DHEAS was significantly higher in G2 than in G1, in G3 than in G2 and in G4 than in G1 and in G2. The concentration

Table II. Metabolic parameters.

	G1	G2	G3	G4
Total cholesterol (mg/dl); [mmol/l]	187.0 ± 25.1 [4.84 ± 0.65]	159.2 ± 18.4 [4.12 ± 0.48]	185.0 ± 23.0 [4.78 ± 0.59]	172.4 ± 20.3 [4.46 ± 0.52]
LDL cholesterol (mg/dl); [mmol/l]	103.6 ± 19.3 [2.68 ± 0.5]	81.1 ± 30.5 [2.1 ± 0.79]	113.7 ± 22.6 [2.94 ± 0.58]	99.4 ± 38.5 [2.57 ± 1]
HDL cholesterol (mg/dl); [mmol/l]	67.8 ± 9.3 53.2 ± 8.4	[1.75 ± 0.24] [1.38 ± 0.22]	65.5 ± 12.4 67.4 ± 13.8	[1.69 ± 0.32] [1.74 ± 0.36]
Triglycerides (mg/dl); [mmol/l]	77.6 ± 19.2 90.8 ± 20.9	[0.88 ± 0.22] [1.03 ± 0.24]	67.6 ± 24.6 89.3 ± 33.5	[0.76 ± 0.28] [1.01 ± 0.38]
Glucose (mg/dl); [mmol/l]	85.6 ± 7.7 [4.7 ± 0.4]	87.2 ± 4.7 [4.8 ± 0.3]	91.3 ± 3.9 [5.1 ± 0.2]	95.3 ± 3.9 [5.3 ± 0.2]
Insulin(IU/ml)	4.5 ± 1.1	4.2 ± 1.6	11.3 ± 2.0	11.5 ± 1.6
HOMA–IR	1.0 ± 0.2	0.9 ± 0.4	2.8 ± 0.9	2.7 ± 0.4

Table III. Hormonal parameters.

	G1	G2	G3	G4
LH (IU/l)	5.2 ± 3.0	6.1 ± 4.6	3.7 ± 1.0	5.0 ± 4.5
FSH (IU/l)	5.3 ± 1.9	4.9 ± 1.6	4.3 ± 1.0	3.7 ± 1.3
PRL (mIU/mL)	8.6 ± 4.2	8.5 ± 4.6	13.2 ± 5.4	7.7 ± 1.9
Estradiol (pg/ml)	40.0 ± 17.8	9.9 ± 14.4	34.1 ± 11.7	42.4 ± 19.3
17–OH–progesterone (ng/ml)	1.3 ± 0.3	1.9 ± 0.4	1.2 ± 0.2	2.2 ± 0.6
DHEAS (ng/dl)	295.1 ± 68.9	458.3 ± 157.5	310.7 ± 86.7	597.1 ± 222.4
Androstenediones (ng/ml)	2.2 ± 0.5	3.4 ± 1.2	2.2 ± 0.9	3.2 ± 1.9
Cortisol (nmol/l)	19.0 ± 6.1	18.6 ± 5.4	21.0 ± 5.0	20.7 ± 5.0
Total testosterone (ng/dl)	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.2
Free testosterone (pg/ml)	1.2 ± 0.8	2.4 ± 1.5	1.4 ± 0.9	3.2 ± 1.9
SHBG (nmol/l)	101.4 ± 32.9	75.6 ± 31.9	53.7 ± 16.8	47.6 ± 24.5
FAI	0.5 ± 0.3	0.8 ± 0.4	0.8 ± 0.4	1.8 ± 1.0

of androstenedione was significantly higher in G2 than in G1 and in G3 than in G2. The concentration of free testosterone was significantly higher in G2 than in G1 and in G4 than in G1 and G3. The concentration of total testosterone was significantly higher in G4 than in G1 and G3. The concentration of SHBG was significantly lower in G3 and G4 than in G1. FAI values were significantly higher in G4 than in G1 and in G2 and G3 (Table III).

In the group without hyperandrogenemia and insulin resistance (G1), a lower concentration of transferrin compared to the other groups was observed. However, it was comparable in groups G2, G3, and G4. There were no differences in the iron and ferritin concentration between the groups. The same can be said about the concentration of hepcidin in all of the studied groups (Table IV).

No statistical differences in the distribution of the positive results in the Ferriman – Gallwey scale between the studied groups was noted. However, there were significant statistical differences in the occurrence of acne – increasing from G1 to G4.

The statistically lower concentration of hepcidin in the serum was noted in patients with abundant bleeding than in patients with scarce and mediocre bleeding (6.46 ± 8.19 *vs.* 14.85 ± 10.30; $p < 0.01$).

Moreover, a significant moderate positive correlation between the concentration of hepcidin and androstenediones was shown ($r = 0.306$; $p < 0.05$).

Discussion

The present work has shown no correlation between the concentration of hepcidin and the analyzed parameters of iron metabolism and the concentration of glucose and insulin on an empty stomach and the HOMA-IR values. Those results differ from those previously identified in experimental research conducted on cell cultures, where hepcidin was one of the factors in the impairment of insulin secretion by the beta cells of the pancreas caused by the glucotoxicity induced by the Pdx-1 expression inhibition.

The obtained results are also in contradiction with the observations that the liver hepcidin expression is proportional to the body mass and the parameters of the carbohydrate metabolism.

This paper has shown a significant positive correlation between the hepcidin and androstenediones concentrations in the serum. There was no observable correlation between the concentration of total and free testosterone and the hepcidin concentration. The mechanism of relationships between the hepcidin and androstenediones concentration is difficult to explain.

Table IV. Parameters of iron metabolism and the hepcidin concentration.

	G1	G2	G3	G4
Transferrin (mg/dl)	231.1 ± 23.4	258.3 ± 24.7	261.2 ± 35.2	285.8 ± 28.7
Ferritin (ng/ml)	25.5 ± 12.1	23.3 ± 12.3	18.4 ± 12.8	16.1 ± 11.7
Iron (g/dl)	104.3 ± 50.9	105.1 ± 41.3	104.9 ± 36.9	106.3 ± 54.4
Hepcidin (ng/ml)	15.3 ± 11.8	12.3 ± 8.6	11.2 ± 11.7	9.3 ± 10.8

However, the lack of connections between the hepcidin and testosterone concentration stands in contradiction with the results of the previously published studies, which have shown the influence of this particular hormone on the hepcidin concentration. One of the studies has shown the inhibitory effect on the hepcidin concentration during the use of testosterone in men with hypogonadotropic hypogonadism and type 2 diabetes[18]. Angeli et al[19] on the cell culture and mice showed that spironolactone, a non-specific antagonist of aldosterone that exhibits anti-androgen activity, inhibited the expression of hepcidin[19]. Moreover, the study has shown a lower concentration of hepcidin in women with diagnosed PCOS and abnormal menstrual bleeding that in women with scarce and moderate bleeding. Such impact of the menstrual bleeding on the hepcidin concentration was confirmed by the results of the HEPMEN study, indicating a variability of the iron metabolism parameters during the menstruation cycle, which needs to be taken into account during hepcidin measurement for diagnostic purposes[20].

It should be emphasized that in other works, women with PCOS have shown a higher concentration of both iron and hepcidin that in the control group. Moreover, the concentration of hepcidin in the multivariate analysis was dependent on the parameters of the iron metabolism, free androgen index and the concentration of CRP, glucose, and insulin on an empty stomach[21]. Such correlations were not observed in this particular report.

In reference to the observed higher concentrations of hepcidin in women with PCOS, it can be assumed that this protein can be a marker of metabolic risk in women with this syndrome. One of the studies has shown that inadequate lowering of the hepcidin concentration in relation to the systemic supplies of iron is an independent predictive factor for the development of type 2 diabetes[22]. However, the confirmation of this hypothesis requires further prospective research.

Insulin resistance and hyperandrogenemia in women with PCOS are not factors that significantly impact the parameters of iron metabolism and the concentration of ferritin and hepcidin. The severity of menstrual bleeding and thus the loss of iron are, however, significant. The potential participation of hepcidin in the pathogenesis of hormonal metabolic disorders in PCOS requires further studies.

Conclusions

Insulin resistance and/or hyperandrogenemia in women with PCOS and regular body mass seem to be the factors that decrease the concentration of transferrin, but not the concentration of other evaluated parameters of iron metabolism, such as iron, ferritin, and hepcidin.

Women with PCOS and regular body mass have shown no correlation between the concentrations of hepcidin and other parameters of iron metabolism and the parameters of the carbohydrate metabolism.

Androstenedione can be an androgen stimulating the synthesis of hepcidin in women with PCOS and regular body mass.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Franik G, Skałba P. Clinical observations and hormone screenings of patients with non-standard hypertrophy of the adrenal cortex. Endokrynol Pol 2011; 62: 230-237.
2) Łebkowska A, Kowalska I. Anti-Müllerian hormone and polycystic ovary syndrome. Endokrynol Pol 2016; 68: 74-78.
3) Orlik B, Madej P, Owczarek A, Skałba P, Chudek J, Olszanecka-Glinianowicz M. Plasma omentin and adiponectin levels as markers of adipose tissue dysfunction in normal weight and obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 2014; 81: 529-535.
4) Kozakowski J, Zgliczyński W. Body composition, glucose metabolism markers and serum androgens - association in women with polycystic ovary syndrome. Endokrynol Pol 2013; 64: 94-100.
5) Olszanecka-Glinianowicz M, Madej P, Zdun D. Are plasma levels of visfatin and retinol-binding protein 4 (RBP4) associated with body mass, metabolic and hormonal disturbances in women with polycystic ovary syndrome? Eur J Obstet Gynecol Reprod Biol 2012; 162: 55-61.
6) Skałba P, Cygal A, Madej P. Is the plasma anti-Müllerian hormone (AMH) level associated with body weight and metabolic, and hormonal disturbances in women with and without polycystic ovary syndrome? Eur J Obstet Gynecol Reprod Biol 2011; 158: 254-259.
7) Pluta D, Madej P, Maksym M. Rola gospodarki żelazowej u kobiet z zespołem policystycznych jajników. Ginekol Położ 2014; 4: 32-41.
8) Krawiec P, Pac-Kożuchowska E. Rola hepcydyny w metabolizmie żelaza w przebiegu nieswoistych

zapaleń jelit. Postepy Hig Med Dosw 2014; 68: 936-943.

9) Šimetić L, Zibar L. Laboratory use of hepcidin in renal transplant recipients. Biochem Med (Zagreb) 2016; 26: 34-52.

10) Ganc T, Nemeth T. Hepcidin and iron homeostasis. Biochim Biophys Acta 2012; 1823: 1434-1443.

11) Escobar-Morreale HF. Iron metabolism and the polycystic ovary syndrome. Trends Endocrinol Metab 2012; 23: 509-515.

12) Eskind IB, Franklin W, Lowell FC. Insulin-resistant diabetes mellitus associated with hemochromatosis. Ann. Intern. Med 1953; 38, 1295-1306.

13) Sam AH, Busbridge M, Amin A, Webber L, White D, Franks S, Martin NM, Sleeth M, Ismail NA, Mat Daud N, Papamargaritis D, Le Roux CW, Chapman RS, Frost G, Bloom SR, Murphy KG. Hepcidin levels in diabetes mellitus and polycystic ovary syndrome. Diabet Med 2013; 30: 1495-1499.

14) Martinez-Garcia MA, Luque-Ramirez M, San-Millan JL, Escobar-Morreale HF. Body Iron stores and glucose intolerance in premenopausal women: role of hyperandrogenism, insulin resistance and genomic variants related to inflammation, oxidative stress and iron metabolism. Diabetes Care 2009; 32: 1525-1530.

15) Escobar-Morreale HF, Luque-Ramirez M. Role of androgen-mediated enhancement of erythropoiesis in the increased body iron stores of patients with polycystic ovary syndrome. Fertil Steril 2011; 95: 1730-1735.

16) Mao X, Chen H, Tang J, Wang L, Shu T. Hepcidin links gluco-toxicity to pancreatic beta cell dysfunction by inhibiting Pdx-1 expression. Endocr Connect 2017; 6: 121-128.

17) Dhindsa S, Ghanim H, Batra M, Kuhadiya ND, Abuaysheh S, Green K, Makdissi A, Chaudhuri A, Dandona P. Effect of testosterone on hepcidin, ferroportin, ferritin and iron binding capacity in patients with hypogonadotropic hypogonadism and type 2 diabetes. Clin Endocrinol (Oxf) 2016; 85: 772-780.

18) Mleczko-Sanecka K, da Silva AR, Call D, Neves J, Schmeer N, Damm G, Seehofer D, Muckenthaler MU. Imatinib and spironolactone suppress hepcidin expression. Haematologica 2017; 102: 1173-1184; Doi:10.3324/haematol.2016.162917.

19) Angeli A, Lainé F, Lavenu A, Ropert M, Lacut K, Gissot V, Sacher-Huvelin S, Jezequel C, Moignet A, Laviolle B, Comets E. Joint Model of Iron and Hepcidin During the Menstrual Cycle in Healthy Women. AAPS J 2016; 18: 490-504.

20) Kim JW, Kang KM, Yoon TK, Shim SH, Lee WS. Study of circulating hepcidin in association with iron excess, metabolic syndrome, and BMP-6 expression in granulosa cells in women with polycystic ovary syndrome. Fertil Steril 2014; 102: 548-554.

21) Pechlaner R, Weiss G, Bansal S, Mayr M, Santer P, Pallhuber B, Notdurfter M, Bonora E, Willeit J, Kiechl S. Inadequate hepcidin serum concentrations predict incident type 2 diabetes mellitus. Diabetes Metab Res Rev 2016; 32: 187-192.

22) Luque-Ramírez M, Álvarez-Blasco F, Alpañés M, Escobar-Morreale HF. Role of decreased circulating hepcidin concentrations in the iron excess of women with the polycystic ovary syndrome. J Clin Endocrinol Metab 2011; 96: 846-852.