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Hepcidin and Matriptase-2 as a potential biomarker for responsiveness to oral iron supplementation in adolescents female with iron deficiency anemia

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ABSTRACT

Background: In Indonesian the prevalence of IDA in female adolescents is high. Higher levels of hepcidin are related to non-responsiveness to oral iron supplementation. Matriptase-2 is a major enzyme which regulates hepcidin secretion. The aim of this study was to investigate whether or not female adolescents were responsive to oral iron supplementation.

Methods: This was an analytical observational study with prospective cohort approach. Subjects were 173 female adolescents who studied in year 10 and 11 of secondary schools in Boyolali regency. Hemoglobin (Hb) levels were measured using the cyanmethemoglobin method and ferritin serum used ELISA. Sixty-eight IDA subjects were given ferrous-fumarate 1 tablet/day for 1 month. After treatment, Hb levels were determined using the same method and serum levels of hepcidin and matriptase-2 were detected by ELISA. All data were analyzed using independent t-test and Mann-Whitney test with 95% significance level.

Results: Around 50% (87/173) female adolescents had IDA and 38.2% was responsive to iron supplementation ($\Delta Hb \geq 1$ g/dL) while 61.8% was non-responsive ($\Delta Hb < 1$ g/dL). Hepcidin levels of non-responsive subjects (6.8 ng/mL) were higher than that of responsive subjects (6.4 ng/mL), but it did not reach significant difference (p=0.302). Lower levels of matriptase-2 were observed in non-responsive subjects (666.3 pg/mL), compared with responsive subjects (1133.0 pg/mL) (p=0.074).

Conclusion: More than 50% of female adolescents is not responsive to oral iron supplementation with higher levels of hepcidin and lower levels of matriptase-2. Both proteins are a potential biomarker for detection of responsiveness to oral iron supplementation.

Key words: Hepcidin, IDA, Matriptase-2, Responsiveness, Oral iron supplementation
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INTRODUCTION

Anemia, which is one of the nutritional disorders, is found worldwide either in developed or developing countries.[1] Many factors contribute to the etiology of anemia such as massive blood loss, vitamin B12 and folic acid, blood diseases, infectious diseases and inflammation, but iron deficiency is a main cause of anemia. Approximately 2 billion people around the world suffer iron deficiency anemia (IDA), and almost all age groups are vulnerable to IDA.[2] In Indonesia, the prevalence of anemia varies among age groups, 21.7% is in children, 29% is school age 22.7% is adolescent and 37.1% in pregnant women.[3] Persisted IDA in female adolescents will manifest in reproductive women who get pregnant in future. In recent years, there is no government program to treat it. Iron supplementation for 90 days during pregnancy is the government program to treat IDA in pregnant women. In the last ten years, the anemia prevalence in pregnant women is still high although 89.1% has got iron supplementation.[4] It is probably caused by non-responsiveness to oral iron supplementation. A study which was done in general population indicates that 37.5% adult women are responsive to oral iron supplementation and 62.5% women are non-responsive after oral iron treatment for one month.[5] Furthermore, non-responsive to iron supplementation will increase the risk of insulin resistance, metabolic syndrome and type-2 diabetes mellitus.[6]

Hepcidin hormone is the main regulator of iron homeostasis.[7] The hepcidin is an oligopeptide which is synthesized in the liver and regulates serum iron levels through intestinal absorption, iron recycle in macrophages and iron release in their storages.[8,9] The expression of hepcidin is regulated by bone morphogenetic protein-sons of mothers against decapentaplegic homolog (BMP-SMAD) cell signaling, hemojuvelin and hemochromatosis-associated membrane proteins (HFE).[7] Increased hepcidin levels inhibit iron absorption and iron release from macrophages and storages, leading to decrease of serum iron, and erythropoiesis.[10,11] A recent study has reported that hepcidin levels become an accurate parameter for determination of responsiveness to oral iron supplementation with 41.3% sensitivity, 84.4% specificity and 81.6% Positive Predictive Value (PPV). This parameter is better than serum ferritin levels and transferrin saturation.[5]

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Matriptase-2 is a serine protease that plays a major role in the regulation of hepcidin. In vitro studies have shown that the matriptase-2 cleaves hemojuvelin which downregulates BMP-SMAD signaling and inhibits hepcidin expression. Decreased hepcidin expression results in an increase of iron absorption and erythropoiesis.[12,13] Patients who have polymorphism of matriptase-2 or TMPRSS6 gene show high serum levels of hepcidin, low iron status and non-responsive to oral iron therapy.[11]

Thus, hepcidin and matriptase-2 are potential biomarkers for IDA to distinguish whether or not responsive to oral iron supplementation. The aim of this study was to investigate hepcidin and matriptase-2 levels in female adolescents who got oral iron supplementation for one month.

RESEARCH DESIGN AND METHODS

This was an analytical observational study with prospective cohort approach. Adolescents female students who studied at year 10 and 11 in senior high schools in Boyolali were screened using criteria such as pregnancy, menstruation, chronic illness, severe anemia, blood diseases supplementation, and drug consumption. This study has been approved by Ethical Review Committee School of Medicine Sebelas Maret University Surakarta Number 660/VIII/HREC/2016.

Determination of oral iron responsiveness

Determination of IDA used Hemoglobin (Hb) and serum ferritin levels. The Hb was measured using cyanmethemoglobin method and serum ferritin levels used ELISA kit (Elabscience®). Female adolescents were considered suffering IDA if Hb was less than 12 mg/dL and ferritin was less than 15 ng/mL. All subjects with IDA were given Ferro-fumarate one tablet/day for 30 days. During supplementation, iron consumption was regularly monitored. Hb levels were measured using the same method at the end of iron supplementation. If the Hb levels increased less than 1 g/dL, subjects would be classified as non-responsiveness or vice versa.[5]

Biochemical assays

Serum of IDA subjects was collected using serum separator tube from 2 mL of whole blood after 1500 rpm centrifugation. Serum hepcidin and matriptase-2 levels pre and post supplementation were measured using the methods described in manufacturer instruction. Serum hepcidin levels were determined using Human hepcidin ELISA kit (Elabscience®) and matriptase-2 levels used Matriptase-2 ELISA kit (Elabscience®). The absorbance values of serum samples were measured with OD_{450}.

Statistical analysis

All data in this study were analyzed using independent sample t-test and Mann-Whitney test with SPSS version 23 (IBM Corp.). A significant level was set up at $p<0.05$.

Table 1 Characteristics of research subjects with or without anemia

Characteristics subjects	Anemia (n=87)	Non Anemia (n=86)	P value
Hb pre supplementation (g/dL)	10.7±1.0	13.3±0.9	0.000*
Body mass index (BMI) (kg/m²)	24.1±4.9	23.0±4.6	0.108†

Note: *independent sample t-test, †Mann-Whitney test

Table 2 Determination of oral iron iron responsiveness in IDA subjects

Characteristics	Mean ± SD		P value
	Non Responsive (n=42)	Responsive (n=26)	
Body Mass Index (BMI) (kg/m²)	25.5±5.0	21.7±4.5	0.002*
Hb Pre supplementation (g/dL)	11.1±0.6	10.3±1.2	0.05*
Hb Post Supplementation (g/dL)	11.3±0.7	12.1±0.9	0.01*
Serum Ferritin Levels (ng/mL)	13.4±8.3 (n=18)	13.8±6.4 (n=14)	0.878†

Note :*independent sampel t-test, † Mann-Whitney test.

RESULTS

A total of 173 female adolescents met the criteria from which 87 (50.3%) subjects suffered anemia (Hb <12 g/dL) and 86 not anemic (Hb>12 g/dL). Table 1 showed that Hb levels and BMI of anemia and healthy subjects were different. Anemia subjects had lower Hb (10.7±1.0 g/dL) than healthy subjects (13.3 ± 0.9 g/dL). The Hb difference of both subjects is statistically significant ($p<0.001$). In contrast to Hb levels, a higher BMI (24.1±4.9) was observed in anemic subjects, compared with healthy subjects (23.0±4.6) but not significantly different ($p=0.108$).

After 30 days supplementation, Hb levels were measured to evaluate whether or not IDA subjects had a better response to oral iron. There were only 68 IDA subjects who completed this study. Table 2 indicated that 61.8% IDA subjects were nonresponsive and the remaining subjects were responsive. BMI and Hb pre supplementation in nonresponsive subjects were significantly higher (25.5±5.0 and 11.1±0.6 g/dL) than that of responsive subjects (21.7±4.5 and 10.3±1.2) with $p=0.002$ and 0.05 respectively. However, the nonresponsive subjects

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Table 3 Serum hepcidin and matriptase-2 levels in IDA subjects during oral iron supplementation

Parameter		Mean ± SD		P value
		Non-responsive (n=25)	Responsive (n=16)	
Hepcidin (ng/mL)	Pre	8.7±1.2	8.2±0.7	0.131*
	Post	6.8±1.5	6.4±1.0	0.302*
Matriptase-2 (pg/mL)	Pre	331.4±425.8	1,030.7±1276.1	0.204†
	Post	666.3±437.3	1,133.0±810.8	0.074†

Note : *independent sampel t-test, † Mann-Whitney test

Table 4 Difference average of hepcidin and matriptase-2 levels during oral iron supplementation

Parameter	Non Responsive (n=25)	Responsive (n=16)	P value
Δ Hepcidin (ng/mL)	-1.6±1.3	-2.6±1.5	0.034†
Δ Matriptase-2 (pg/mL)	376±244	440±398	0.856†

Note : † Mann-Whitney test

had Hb post supplementation at 11.3±0.7 g/dl, lower than responsive subjects (12.1±0.9 g/dl). The average difference of Hb levels in nonresponsive subjects is 1/10 fold of responsive subjects. It differed statistically (p=0.01). For iron storage, both subjects had similar ferritin levels (13.4±8.3 vs. 13.8±6.4).

Serum hepcidin and matriptase-2 levels before and after supplementation were determined in nonresponsive and responsive IDA to investigate their roles in iron metabolism. Hepcidin levels decreased in both subjects after iron supplementation, but matriptase-2 levels also increased in both subjects after oral iron supplementation (Table 3). Before iron based treatment was commenced, hepcidin levels were similar in IDA subjects which were nonresponsive or responsive to oral iron. Matriptase-2 levels in nonresponsive IDA (331.4±425.8) were lower than responsive IDA (1,030.7±1276.1) but not statistically different (p=0.204). Furthermore, a similar decrease in serum hepcidin levels was found in both subjects after 30 days supplementation. After iron supplementation, matriptase-2 levels (666.3±437.3) doubled in nonresponsive subjects compared with matriptase levels before iron supplementation (331.4±425.8), but responsive subjects just had 10% more matriptase-2 levels after iron supplementation.

The differences of hepcidin and matriptase-2 levels during iron supplementation were determined to evaluate the involvement of both proteins in iron regulation in IDA subjects. Table 4 exhibited that nonresponsive subjects had lower average differences of hepcidin and matriptase-2 compared with responsive subjects. The average difference of hepcidin in nonresponsive subjects (-1.6±1.3) decreased slightly, but in responsive subjects, the mean difference of hepcidin was higher (-2.6±1.5). The differences of hepcidin in both subjects reached statistical significance (p=0.034). In contrast with hepcidin levels, the average increase of matriptase-2 levels in nonresponsive subjects (376±244) was lower than the average increase of matriptase-2 levels in responsive subjects (440±398). However, it did not differ significantly (p=0.856).

DISCUSSION

We have demonstrated in the first time that the average difference in Hb levels during supplementation can be used to distinguish between responsive and nonresponsive subjects. In this study, we found that the average difference in Hb levels in responsive subjects increased ten times compared with nonresponsive subjects. Average BMI in responsive subjects was normal while nonresponsive subjects were overweight. Increased Hb levels in responsive subjects are followed by a decrease of 1.6 times hepcidin levels and an increase of 1.2 matriptase-2 levels compared with nonresponsive subjects.

Our data is in line with data from the previous study[5] regarding Hb and hepcidin levels. It was reported that 240 IDA subjects were given with 325 mg sulfate-ferrous three times for 28 days and only 37.5% subjects had increased Hb levels more than 1 g/dL which was considered as responsive subjects.[5] In contrast to this study, Hb levels in responsive subjects in our study increased (1.9 g/dL). In addition, we administered single oral dose 250 mg ferrous fumarate for 30 days in IDA subjects. Therefore, IDA subjects which are nonresponsive to oral supplementation may result in genetic variability.

Our finding also shows that average BMI in nonresponsive subjects is 25.5±5.0 kg/m^2. Higher BMI is related to higher fat accumulation through all the body. Fat accumulation subsequently induces chronic inflammation through secretion of pro-inflammatory cytokines like IL-6 and TNF-alfa.[14] As a result, increased IL-6 levels in blood circulation stimulates the release of hepcidin from liver and adipocytes.[15] Our data indicate that hepcidin levels in nonresponsive subjects remain high after 30 days iron supplementation. Hepcidin-induced inflammation more likely inhibits iron absorption by an increase of Ferroportin (FPN) degradation.[16] Therefore, inflammation may contribute to the failure of responsiveness to oral iron supplementation in nonresponsive subjects.

A number of studies have shown that activity of matriptase-2 enzyme predominantly regulates hepcidin expression.[12,13,17] Our finding supports these previous studies that iron responsive subjects with IDA have lower hepcidin levels and higher matriptase-2 levels than iron nonresponsive subjects with IDA. So far, regulation of hepcidin expression involves BMP (*Bone Morphogenetic Protein*)/SMAD (son-mothers against decapentaplegic) protein signaling.[17] Higher matriptase levels will then cleave hemojuvelin (Hjv), leading to suppression of hepcidin expression. However, our study does not investigate this signaling pathway.[17,18] Other limitations of our study are we do not collect data on iron intake from daily consumption, measure waist circumference, serum IL-6 and TNF-alfa levels, and determine genetic variability in TMPRSS6 gene. Moreover, we just measure soluble matriptase-2 in blood circulation, but it does not reflect the total of matriptase-2 because the majority of matriptase-2 is expressed in the liver cell membrane.

CONCLUSION

In conclusion, more than a half of female adolescents is not responsive to oral iron supplementation with higher levels of hepcidin and lower levels of matriptase-2, compared with responsive female adolescents. Both proteins are a potential biomarker for detection of responsiveness to oral iron supplementation. Further investigation is required for unraveling nonresponsiveness to oral iron supplementation in female adolescents.

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REFERENCES

1. de Benoist B, McLean E, Egli I, Cogswell M. Worldwide prevalence of anemia 1993-2005 WHO global database on anemia. Geneva, Switzerland: WHO Press; 2008.
2. Petry N, Olofin I, Hurrell RF, Boy E, Wirth JP, Moursi M, et al. The proportion of anemia associated with iron deficiency in low, medium, and high human development index countries: a systematic analysis of national surveys. Nutrients. 2016;8:693 http://dx.doi.org/10.3390/nu8110693
3. Badan Litbangkes RI. Riset Kesehatan Dasar. Jakarta: Badan Litbangkes, Kementerian Kesehatan RI; 2013
4. Kementerian Kesehatan RI. Profil Kesehatan Indonesia 2010. Jakarta: Kementerian Kesehatan RI; 2011.
5. Bregman DB, Morris D, Koch TA, He A, Goodnough LT. Hepcidin levels predict nonresponsiveness to oral iron therapy in patients with iron deficiency anemia. Am J Hematol. 2013;88: 97-101 http://dx.doi.org/10.1002/ajh.23354
6. Vari I, Balkau B, Kettaneh A, Andre P, Tichet J, Fumeron F, et al. Ferritin and Transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population. Diabetes Care. 2007: 30(7) : 1795-1801. http://dx.doi.org/10.2337/dc06-2312.
7. Ganz T. Systemic iron homeostasis. Physiol Rev. 2013;93:1721-1741 http://dx.doi.org/10.1152/physrev.00008.2013
8. Ganz T and Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol. 2006;290: G199-G203 http://dx.doi.org/10.1152/ajpgi.00412.2005
9. Ganz T. Molecular control of iron transport. J Am Soc Nephrol. 2007;18: 394-400 http://dx.doi.org/10.1681/ASN.2006070802
10. Fleming MD. The Regulation of hepcidin and its effects on systemic and cellular iron metabolism. Hematology Am Soc Hematol Educ Program. 2008;1:151-158 http://dx.doi.org/10.1182/asheducation-2008.1.151
11. Nai A, Pagani A, Silvestri L, Campostrini N, Corbella M, Girelli D, et al. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. Blood. 2011;118(16):4459-4462 http://dx.doi.org/10.1182/blood-2011-06-364034
12. Bergamaschi G and Villani L. Serum hepcidin: a novel diagnostic tool in disorders of iron metabolism. Hematol 2009;94(12):1631-1633 http://dx.doi.org/10.3324/haematol.2009.013615
13. Lemos AD, Ismael LAS, Boato CCM, Borges MTF, Rondó PHD. Hepcidin as a biochemical parameter for the assessment of iron deficiency anemia. Rev Assoc Med Bras. 2010;56(5):596-599 http://dx.doi.org/10.1590/S0104-4230201000500024
14. Sindhu S, Thomas R, Shihab P, Sriraman D, Behbehani K, Ahmad R. Obesity is a positive modulator of IL-6R and IL-6 expression in the subcutaneous adipose tissue: significance for metabolic inflammation. PLoS ONE. 2015;10(7):e0133494 http://dx.doi.org/10.1371/journal.pone.0133494*
15. Collins JF, Wessling-Resnick M, Knutson MD (2008). Hepcidin regulation of iron transport. JNutr 2008;138(11):2284–2288 http://dx.doi.org/10.3945/jn.108.096347
16. Tussing-Humphreys L, Pustacioglu C, Nemeth E, Braunschweig C (2012). Rethinking Iron Regulation and Assessment in Iron Deficiency, Anemia of Chronic Disease, and Obesity: Introducing Hepcidin. Acad Nutr Diet. 2012;112(3):391–400. http://dx.doi.org/10.1016/j.jada.2011.08.038
17. Finberg KE, Whittlesey RL, Fleming MD, Andrews NC. 2010. Down-regulation of Bmp/Smad signaling by Tmprss6 is required for maintenance of systemic iron homeostasis. Blood 2010;115:3817-3826 http://dx.doi.org/10.1182/blood-2009-05-224808
18. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab 2008;8:502-511 http://dx.doi.org/10.1016/j.cmet.2008.09.012