Expression of Hepcidin and Growth Differentiation Factor 15 (GDF-15) Levels in Thalassemia Patients with Iron Overload and Positive Anti Hepatitis C Virus

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ABSTRACT

Background: *Thalassemia patients who undergo life-long recurrent blood transfusion will experience iron overload in various organs including the liver and possibly suffer from chronic hepatitis C infection which may lead to liver impairment. The liver produces hepcidin, a hormone which plays role in the regulation of iron level in the blood. Various factors may influence hepcidin level in the blood. Chronic hepatitis C causes iron overload and liver impairment. Liver impairment and haemolytic anaemia due to haemoglobinopathy will suppress hepcidin production. Anaemia stimulates growth differentiation factor 15 (GDF-15) to increase erythropoiesis and suppress hepcidin production. Iron overload causes increase in hepcidin level. Presence of factors which decrease or increase hepcidin production will express various levels of hepcidin. This study aimed to identify the expression of hepcidin and GDF-15 levels in thalassemia patients with iron overload and positive anti-HCV. Information on hepcidin and GDF-15 levels are beneficial in the management of iron overload in thalassemia with positive anti-HCV.*

Method: *This study was a descriptive analytic study in thalassemia patients who had received recurrent blood transfusion ≥ 12 times, suffered from iron overload (transferrin saturation > 55% and ferritin > 1,000 ng/mL), which consisted of 31 individuals with positive anti-HCV and 27 individuals with negative anti-HCV. This study was performed in Thalassemia Centre Department of Child Health and Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, in October 2011–January 2012. Serum hepcidin and GDF-15 examinations were performed using enzyme-linked immunosorbent assay (ELISA) method. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) examinations were performed using colorimetry method. Data on ferritin and transferrin saturation were obtained from medical records in the last 3 months. Data was analysed using SPSS Windows version 17 software.*

Results: *Characteristics of subjects in this study included ferritin level, transferrin saturation, AST, and ALT were 5,289 (SD 2,492) ng/mL, 96.7 (SD 9.2)%, 41.8 (SD 26.7) U/L, and 50.6 (24.9) U/L, respectively. It was obtained that the hepcidin levels were within the normal limits with median of 51.5 (19-166) pg/mL, while GDF-15 levels were higher than the normal range with median of 1,936 (643-2,475) pg/mL. There was no significant difference of hepcidin and GDF-15 levels between positive and negative anti-HCV groups, with p value of 0.842 and 0.115, respectively.*

Conclusion: *We obtained that the hepcidin levels were within normal limits and GDF-15 levels were higher than the normal range. There was no significant difference of hepcidin and GDF-15 levels between positive and negative anti-HCV group.*

Keywords: *thalassemia, iron overload, hepatitis C, hepcidin, growth differentiation factor 15 (GDF-15)*

ABSTRAK

Latar belakang: *Pasien thalasemia dengan transfusi darah berulang seumur hidup akan mengalami kelebihan besi di berbagai organ termasuk hati dan kemungkinan terinfeksi hepatitis C kronik yang mengakibatkan kerusakan di hati. Hati memproduksi hepsidin, suatu hormon yang berperan dalam pengaturan kadar besi dalam darah. Berbagai faktor dapat mempengaruhi kadar hepsidin dalam darah. Hepatitis C kronik menimbulkan kelebihan besi dan kerusakan hati. Kerusakan hati dan anemia akan mengurangi produksi hepsidin. Anemia merangsang growth differentiation factor 15 (GDF-15) untuk meningkatkan eritropoiesis dan menekan produksi hepsidin. Kelebihan besi mengakibatkan peningkatan kadar hepsidin. Adanya faktor yang mengurangi dan meningkatkan produksi hepsidin akan memberikan gambaran kadar hepsidin yang bervariasi. Penelitian ini ditujukan untuk mengetahui gambaran kadar hepsidin dan GDF-15 pada pasien thalasemia dengan kelebihan besi dan hepatitis C kronik. Informasi tentang kadar hepsidin dan GDF-15 bermanfaat dalam tatalaksana kelebihan besi pada thalasemia dengan hepatitis C kronik.*

Metode: *Penelitian ini merupakan penelitian deskriptif analitik pada pasien thalasemia yang mendapat transfusi berulang ≥ 12 kali, mengalami kelebihan besi (saturasi transferin > 55% dan ferritin > 1.000 ng/mL), yang terdiri dari 31 orang dengan hepatitis C kronik positif dan 27 orang dengan hepatitis C negatif. Penelitian dilakukan di Pusat Thalasemia Departemen Ilmu Kesehatan Anak dan Departemen Patologi Klinik Fakultas Kedokteran Universitas Indonesia (FKUI) Rumah Sakit Cipto Mangunkusumo (RSCM), kurun waktu tahun Oktober 2011– Januari 2012. Pemeriksaan hepsidin serum dan GDF-15 menggunakan metode enzyme-linked immunosorbent assay (ELISA). Pemeriksaan aspartate aminotransferase (AST), alanine aminotransferase (ALT) dengan metode kolorimetri. Data feritin dan saturasi transferin diambil dari data rekam medik 3 bulan terakhir. Data diolah menggunakan perangkat lunak SPSS Windows versi17.*

Hasil: *Karakteristik subjek penelitian antara lain kadar feritin, saturasi transferin, AST, dan ALT berturut-turut 5.289 (SD 2.492) ng/mL, 96,7 (SD 9,2)%, 41,8 (SD 26,7) U/L, dan 50,6 (24,9) U/L. Didapatkan kadar hepsidin dalam rentang nilai rujukan dengan median 51,5 (19-166) pg/mL dan kadar GDF-15 didapatkan lebih tinggi dari nilai rujukan, dengan median 1.936 (643-2.475) pg/mL. Tidak didapatkan perbedaan bermakna kadar hepsidin dan GDF-15 antara kelompok hepatitis C positif dan negatif, dengan nilai p berturut-turut 0,842 dan 0,115.*

Simpulan: *Didapatkan kadar hepsidin dalam batas normal dan kadar GDF-15 lebih tinggi dari nilai rujukan. Tidak didapatkan perbedaan bermakna kadar hepsidin dan GDF-15 antara kelompok hepatitis C kronik positif dengan negatif.*

Kata kunci: *thalasemia, kelebihan besi, hepatitis C, hepsidin, growth differentiation factor 15 (GDF-15)*

INTRODUCTION

Thalassemia is a hereditary genetic abnormality and is marked by globin chain synthesis impairment due to lack or loss of one or more globin chain formation.[1] Data in Paediatric Outpatient Unit Cipto Mangunkusumo Hospital until the end of 2008 revealed that total major thalassemia patients reached the number of 1,453 patients with addition of new patients approximately 70-80 patients per year.[2] Thalassemia patients will suffer from anaemia due to ineffective erythropoiesis and intravascular haemolysis, thus they need life-long recurrent blood transfusions.[1,3] Life-long blood transfusion in thalassemia patients leads to iron overload in various organs in the body and these patients are potentially infected by blood borne diseases, such as hepatitis C.[1,3] In Indonesia, the rate of hepatitis C infection in thalassemia patients is quite high. Roesli in the year 2001, obtained that the rate of positive anti-HCV in thalassemia patients was 84.4% (54/64).[4] Data from Thalassemia Centre Department of Child Health, Cipto Mangunkusumo Hospital at the end of 2011 showed that the incidence of positive anti-HCV was 16% (116/716).[5] While in Pakistan, Younus et al in year 2004, found anti-HCV seropositive rate of 42%.[6]

Ineffective erythropoiesis and recurrent blood transfusion in thalassemia patients causes iron overload which will be deposited in various organs. Liver is the largest reticuloendothelial organ and the main site of iron deposit.[1,7] Iron overload in thalassemia patients causes free iron in the blood (non-transferrin bound iron/NTBI). Free iron reacts with peroxide and forms hydroxyl free radical which will cause oxidative stress and inflammation in various tissues in the body.[1,7] Thalassemia patients with iron overload and positive hepatitis C will cause more severe liver impairment

compared to those without hepatitis C infection.[1,7] Patients with chronic hepatitis C frequently suffer from iron overload through mechanism which remains unknown.[8,9] Liver impairment influences hepcidin synthesis in the liver.[10-13] Hepcidin is a regulatory protein which plays role in the regulation of iron which is released by macrophages and erythrocytes into the blood circulation by interacting directly with ferroportin (FPN1) in basolateral surface of erythrocytes, macrophages, and hepatocytes. Hepcidin synthesis is influenced by iron level, erythropoiesis activity, and inflammation.[10-14] Other factors which may influence hepcidin synthesis include hypoxia, erythropoiesis, iron level in the blood, and infection or inflammation, as shown in Figure 1.[10-14] Hypoxia will suppress hepcidin synthesis. The increase of erythropoiesis due to anaemia will supress hepcidin synthesis, which thus causes the increase of iron in the blood as the main resource of erythropoiesis. Low level of hepcidin will increase iron release from cell and iron absorption in the gut. High iron level in the blood will stimulate hepcidin synthesis to increase, causing reduced iron in the blood by decreasing iron release from reticuloendothelial cells and decreasing iron absorption in the gut. Inflammation will elevate hepcidin synthesis, thus iron release from reticuloendothelial cell is inhibited. Currently, many researchers start to see hepcidin as a target of therapy in iron overload cases. High hepcidin level is expected to decrease the amount of iron circulating in the blood, which therefore decrease iron deposit in various organs.

Hepcidin expression is influenced by growth differentiation factor 15 (GDF-15).[15,16] Anaemic condition in thalassemia patients will stimulate erythropoiesis in the bone marrow through growth differentiation factor 15 (GDF-15) protein stimuli. GDF-15 is a protein which plays role in the regulation of erythropoiesis by increasing erythropoiesis activity in the bone marrow and suppress hepcidin synthesis in the liver, so that there is enough iron for erythropoieisis.[15,16] Thalassemia patients with iron overload and hepatitis C have many factors which influence hepcidin synthesis, including erythropoiesis activity, high iron level, and hepatitis C infection.[13] High erythropoiesis activity due to anaemia will suppress hepcidin synthesis. Iron overload will increase hepcidin level to decrease iron level in the blood. Hepatitis C infection causes liver impairment which leads to decreased hepcidin synthesis. The presence of interactions with various factors, such as erythropoiesis activity, iron level, and infection will influence hepcidin synthesis, thus will express various levels of hepcidin. This study aimed to identify levels of hepcidin and GDF-15 in thalassemia patients suffering from iron overload and positive and negative chronic hepatitis C infection. Information on hepcidin and GDF-15 levels are beneficial in the management of iron overload in thalassemia with positive anti-HCV.

METHOD

The design of this study was cross sectional study and reported in the form of descriptive analytic study. Study was performed in Thalassemia Centre Department of Child Health and Department of Clinical Pathology Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital in October 2011-January 2012. Study subjects were paediatric patients who had been diagnosed with major thalassemia by paediatrician according to the criteria in Cipto Mangunkusumo Hospital, had undergone blood transfusion at least 12 times, and suffered from iron overload (transferrin saturation > 55% and ferritin > 1,000 ng/mL). Subjects were divided into 2 groups, which were positive and negative chronic hepatitis C. Data on transfusion history, ferritin level, transferrin saturation, and hepatitis C status were obtained from medical record in the previous 3 months. Collected data from medical records included data in the last month from 3 months earlier.

Sample used in this study was blood serum. Hepcidin examination was performed using ELISA method (kit Hepcidin 25C Terminal Elisa, DRG, EIA 4705), normal range 13.3-54.4 pg/mL.[17] GDF-15 examination was performed using ELISA method (kit Quantikine Human GDF-15 Immunoassay No. catalogue DGD 15), normal range 337-1,060 pg/mL.[17] ALT and AST examinations were conducted using Cobas 501 equipment. ALT examination was conducted using alanine aminotransferase acc./IFCC without pyridoxal phosphate activation (ALTL) instrument from Roche, catalogue number: 20764957322.[19] AST examination was done using aspartate aminotransferase IFCC (ASTL) instrument from Roche, catalogue number 20764949322.[20]

The data was presented in the form of tables and graphs. Numerical data distribution was analysed using Kolmogorov Smirnov test. Significant difference between negative and positive anti-HCV was analysed using Mann-Whitney test.[21] Data analysis was performed using SPSS Windows version 17 software.

This study has passed ethical evaluation from Faculty of Medicine Universitas Indonesia Ethical Clearance Committee under letter number 166/PT02.FK/ETIK/2011.

RESULTS

In this study, we obtained 58 research participants with research subject characteristics as shown in Table 1. Ferritin level and transferrin saturation were found to be high and fulfilled iron overload criteria (transferrin saturation > 55% and ferritin > 1,000 ng/mL). AST and ALT activities were found to be higher than the normal range. There was no significant difference of ferritin level, transferrin saturation, AST, ALT, hepcidin level, and GDF-15 level between negative and positive hepatitis C groups.

In this study, we obtained hepcidin level from all subjects were within normal limit (13.3-54.4 pg/mL) with median value of 51.5 (19-166) pg/mL. GDF-15 level from all subjects were higher than the normal range (337-1,060 pg/mL), with median value of 1,936 (643-2,475) pg/mL. There was no significant difference of hepcidin and GDF-15 levels in positive and negative Anti-HCV groups with p value of 0.842 and 0.115, respectively.

DISCUSSION

In this study, we obtained normal hepcidin levels with median of 51.5 (19-166) pg/mL, different from those found in the study conducted by Tanno et al and Fujita et al.[16,21] Tanno et al, found lower hepcidin level in thalassemia population compared to healthy control.[16] Tanno et al assumed that the lower hepcidin level was found due to chronic anaemia.[16] Low hepcidin synthesis in anaemic condition causes iron release from erythrocytes, macrophages, and hepatocytes.[23] Further, iron in the circulation will increase because it is needed as the main resource for erythropoiesis.[22] Fujita obtained lower hepcidin level in chronic hepatitis C population compared to control. Fujita et al, presumed that the low hepcidin level happened as a result of liver impairment due to chronic hepatitis C.[22]

In this study, we found that the hepcidin level was within normal range due to influences from various factors which could increase or decrease hepcidin level. Hepcidin as an iron homeostasis regulator protein in the body will influence the amount of iron released by enterocytes, placenta, and macrophages.[11,12] In iron overload condition, the body will decrease iron release into the blood by increasing hepcidin production, while anaemia and liver impairment will decrease hepcidin level.[11-13, 23,24]

Tsochatzis et al reported the association between serum hepcidin level with severity of lesion in the liver seen through biopsy histopathologic examination.[25] In this study, parameter of liver impairment being used was AST and ALT activities. Pradat identified the association between elevated ALT activity > 2.25 x normal range and liver impairment.[26] In this study, activities of AST and ALT were slightly increased (< 2 x normal range), thus it could not be confirmed whether the impairment was mild or severe; therefore, it need to be confirmed by further examination.

In this study, there was no significant difference in hepcidin level between positive and negative anti-HCV groups. This result was different with the results achieved by Fujita et al and Lee et al.[22,27] Fujita et al found significantly lower hepcidin level in hepatitis C group compared to hepatitis B patients and healthy control.[22] Lee et al, obtained lower prohepcidin serum level in patients with chronic hepatitis C infection.[24] Different result of this study was because patients in the study performed by Fujita et al and Lee et al were chronic hepatitis C patients who did not suffer from iron overload; hence, the hepcidin level was only affected by the liver impairment factor.

In this study, we observed an increased level of GDF-15. This result was similar to the results attained by Tanno et al.[16] This increase of GDF-15 showed active erythropoiesis as a compensation towards chronic anaemia. Increased GDF-15 will suppress hepcidin production, thus iron release by macrophages

Table 1. Characteristic of study subjects (n = 58)

	Total subjects (n = 58)	Negative Anti HCV (n = 27)	Positive Anti HCV (n = 31)	p
Age (years old)	22.1 (SD 7.9)*	20.5 (SD 11.6)*	24.2 (SD 6.2)*	
Sex				
Male	34 (59%)	11(41%)	23 (74%)	
Female	24 (41%)	16 (59%)	8 (26%)	
Ferritin (ng/mL)	5,289 (SD 2,492)*	5,214 (SD 2,564)*	5,355 (SD 2,467)*	0.765
Transferrin saturation (TS) (%)	96.7 (SD 9.2)*	96 (SD 10.8)*	97 (SD 7.7)*	0.522
AST (U/L)	41.8 (SD 26.7)*	43.6 (SD 20.0)*	56.6 (SD 27.4)*	0.059
ALT (U/L)	50.6 (SD 24.9)*	36.2 (SD 25.4)*	46.6 (SD 27.3)*	0.427
Hepcidin (pg/mL)	51.5 (19-166)**	53.0 (SD 23.5)*	55.8 (SD 28.7)*	0.842
GDF-15(pg/mL)	1,936 (643-2,475)**	1,788 (SD 413)*	1,826 (SD 332)*	0.115

*mean, SD; **median, min-max; HCV: Hepatitis C Virus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GDF-15: growth differentiation factor 15

and erythrocytes would increase. Elevated iron in the blood was meant to fulfil the requirement of erythropoiesis.

Growth differentiation factor 15 (GDF-15) is known as macrophage inhibitory cytokine-1 (MIC-1) and placental bone morphogenetic protein which belongs to the family of TGF-β superfamily. Growth differentiation factor 15 is synthesized in the form of inactive 40kDa protein precursor and later will undergo breakdown in terminal C fragment area and become an active homodimer with the size of 28 kDa which is connected by disulphide bond. Growth differentiation factor 15 (GDF-15) plays role in the regulation of erythropoiesis by increasing erythropoiesis activity in the bone marrow and suppress hepcidin synthesis in the liver, so that there is an adequate amount of iron for erythropoiesis.[13]

In this study, we found an increased GDF-15 level with median of 1,936 (643-2,475) pg/mL. GDF-15 results obtained in this study were similar to those obtained by Tanno et al.[16] Tanno et al reported higher GDF-15 in thalassemia patients compared to healthy individuals (66,000 ± 9,600 pg/mL, 4,800-248,000 pg/mL, $p < 0.05$ to 450 ± 50 pg/mL).[16] Tanno et al assumed that increase of GDF-15 level would suppress hepcidin synthesis, thus iron was released from macrophages and erythrocytes into blood circulation.[16] Iron which was released into the blood was needed in erythropoiesis process. This increased iron level would even augment iron overload in thalassemia patients.

In this study, we found no significant difference of GDF-15 level between positive and negative Anti-HCV. GDF-15 production was not influenced by liver condition, thus liver impairment due to hepatitis C did not affect GDF-15 production.

CONCLUSION

In this study, we found that hepcidin levels were within normal limits and GDF-15 levels were increased in all research subjects (positive and negative anti-HCV). There was no significant difference of hepcidin and GDF-15 level between positive and negative anti-HCV groups.

REFERENCES

1. Olivieri NF. The B thalassemias. N Engl J Med 1999;341:99-109.
2. Ananta Y. Perbandingan efektivitas deferipron pada anak thalassemia dengan atau tanpa infeksi virus hepatitis B/C: uji klinis tanpa randomisasi [Tesis]. Jakarta: Universitas Indonesia 2009.
3. Timan IS, Aulia D, Atmakusuma D, Sudoyo A, Windiastuti E, Kosasih A. Hematological disorders in the developing countries: some hematological problems in Indonesia. Int J Hematol 2002;76:286-90.
4. Marie, Bousse, Kerdilès, Martyré C, Samson M. Cellular and molecular mechanisms underlying bone marrow and liver fibrosis: a review. European Cytokine Network 2008;19:69-80.
5. Roesli T. Gangguan fungsi hati pada penderita thalassemia beta mayor : telaah khusus hubungan dengan saturasi transferrin dan infeksi virus hepatitis B dan C [Tesis]. Jakarta: Universitas Indonesia 2001.
6. Data Pusat Thalasemia. Departemen Ilmu Kesehatan Anak Rumah Sakit Cipto Mangunkusumo, 2012.
7. Younus M, Hassan K, Ikram N. Hepatitis C virus seropositivity in repeatedly transfused thalassemia major patients. Int J Pathol 2004;2:20-3.
8. Di-Bisceglie AM, Axiotis CA, Hoofnagle JH,Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology 1992;102:2108-13.
9. Boucher E, Bourienne A, Adams P, Turlin B, Brissot B, Deugnier Y. Liver iron concentration and distribution in chronic hepatitis C before and after interferon treatment. Gut 1997;41:115-20.
10. Domenico ID, Ward DM, Kaplan J. hepcidin regulation: ironing out the details. J Clin Invest 2007;117:1755-8.
11. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anaemia of inflammation. Blood 2003;102:783-8.
12. 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.
13. Fleming RE, Ponka P. Mechanisme of disease : iron overload in human disease. N Engl J Med 2012;366:348-59.
14. Collins JF, Wessling-Resnick M, Knutson MD. Hepcidin regulation of iron transport. J Nutr 2008;138:2284–8.
15. Finkenstedt A, Bianchi P, Theord I. Regulation of iron metabolism through GDF 15 and hepcidin in pyruvate kinase deficiency. Br J Haematol 2009;144:789-93.
16. Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, et al. High levels of GDF 15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nature medicine 2007;13:1096-101.
17. Manual user Hepcidin ELISA EIA-4705 dari GDR.
18. Manual user Kit dari Quantikine Human GDF-15 Immunoassay.
19. ALT-L. In Roche, eds. Manheim. Indianapolis: Roche Diagnostic; 2011.p.1-3.
20. AST-L. In Roche, eds. Manheim. Indianapolis: Roche Diagnostic; 2011.p.1-3.
21. Dahlan MS. Statistik untuk kedokteran dan kesehatan. 4th ed. Jakarta: Penerbit Salemba Medika.2009.p.29-58, 59-71, 155-63.
22. Fujita N, Sugimoto R, Takeo M, Urawa N, Mifuji R, Tanaka H, et al. Hepcidin expression in the liver: relatively low level in patients with chronic hepatitis C. Mol Med 2007;13:97-104.
23. Means RT. Hepcidin and anaemia. Blood Reviews 2004;18:219–25.
24. Kwapisz J, Slomka A, Zekanowska E. Hepcidin and its role in iron homeostasis. The Journal of the International federation of Clinal Chemistry and Laboratory Medicine 2009;20:1-5.
25. Tsochatzis E, Papatheodoridis V, Koliaraki V, Hadziyannis E, Kafiri G. Serum hepcidin levels are related to the severity of liver histological lesions in chronic hepatitis C. Journal of viral hepatitis 2010;17:800-6.

26. Pradat P, Alberti A, Poynard T, Esteban JI, Weilan O, Marcellin P, et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. Hepatology 2002;36:973-7.
27. Lee SH, Jeong SH, Park YS, Hwang JH, Kim JW, Kim N, et al. Serum prohepcidin levels in chronic hepatitis C, alcoholic liver disease, and non-alcoholic fatty liver disease. Korean J Hepatol 2010;16:288-94.