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THE ASSOCIATION BETWEEN SERUM FERRITIN LEVELS AND ASPARTATE AMINOTRANSFERASE TO PLATELET RATIO INDEX SCORE IN THALASSEMIA PATIENTS

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Abstract

Background: In thalassemia patients, routine transfusion is one of the given therapies, but this can lead to accumulation of iron which possibly leads to liver fibrosis. Serum ferritin levels are used to evaluate iron over load and correlate with liver iron concentration. Assessment of liver fibrosis, biopsy remains a gold standard, but the method is invasive. However, today many noninvasive methods are developed simple, one of which is the AST to platelet ratio index (APRI).

Objectives: To analyze the association of serum ferritin levels and AST to platelet ratio index scores in thalassemia patients who received the chronic transfusion.

Methods: Thalassemia patients who have had routine transfusions for more than 5 months or more than 10 bags regularly and no splenectomy has ever been performed. Serum ferritin levels were measured by the Enzyme-Linked Fluorescence Assay method, APRI scores were calculated using the formula (AST/threshold on normal AST value) x 100]/platelet count (10^9/L). Statistical analysis of the relationship between serum ferritin and APRI score by using Spearman correlation test ($p < 0.05$).

Results: Most of the subjects were male (66.67%) and gained thalassemia beta major (61.11%) by age 26.14 ± 7.39 years. The results of measurement of serum ferritin 5011.49 ± 2595.33 ng/ml and APRI score 1.16 ± 1.10. Statistical analysis showed that there was a weak and significant positive correlation between serum ferritin level and APRI score in thalassemia patients with chronic transfusion ($r = 0.355$; $p = 0.033$).

Conclussion: In this study, APRI scores have not been used to assess the presence of liver fibrosis that caused by elevated serum ferritin level.

Keywords: *serum ferritin, APRI score, thalassemia, fibrosis*

Introduction

In thalassemia patients, routine or chronic transfusion is one of the therapies given, but this can lead to iron over accumulation. Humans can not actively remove excess iron. This cumulative effect of iron can damage various organs, one of which is the liver [*Mishra AK, Tiwari A, 2013*]. The liver is the main organ of the body's iron storage, the accumulation of iron along with the reactive oxygen species (ROS) can damage the hepatocytes, resulting in fibrosis [*Kountouras D et al., 2013, Soliman A et al., 2014*]. Iron overload, hepatitis C and a large number of transfusions are factors that can lead to the progression of liver fibrosis [*Wang W et al., 2017*]. Serum ferritin is used to evaluate iron overload in thalassemia patients and correlates with liver iron concentration [*Mishra AK, Tiwari A, 2013*]. To assess liver fibrosis and measure LIC, biopsy remains a gold standard, but the method is invasive, expensive and susceptible to sampling error. Because of

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this, many non-invasive, simple, reliable, and inexpensive non-invasive methods for identifying liver fibrosis, including APRI (Aspartate Aminotransferase to Platelet Ratio Index) [*Lin Z et al., 2011; Yilmaz Y et al., 2011*]. Up until now, APRI scores in thalassemia patients have not been widely studied, especially in Indonesia.

Morbidity and mortality in thalassemia patients are caused by iron overload effects resulting from chronic transfusions. Liver disease is still the leading cause of death in thalassemia patients [*Shelton E et al., 2015*]. Mortality in thalassemia-related patients with cirrhosis and even hepatocellular carcinoma resulting from severe iron overload. The reported prevalence of fibrosis in thalassemia major 40-80% and liver cirrhosis by 10-40% [*Li CK et al., 2002*]. Children who were diagnosed with thalassemia major after the age of 15-16 years on average showed a picture of cirrhosis of the liver [*Jean G et al., 1984*]. Liver fibrogenesis is a dynamic process and can improve; unlike cirrhosis, are irreversible, therefore fibrosis detection is important for thalassemia patients [*Wang W et al., 2010; El-Shabrawi M et al., 2012; Shelton E et al., 2015*]. Ten years free survival of thalassemia patients with serum ≥800 *ng/ml* serum levels were 0% while serum ferritin serum ≤300 *ng/ml* was 100% (log-rank test p = 0.001) [*Musallam KM et al., 2014*]. Then, 76% of thalassemia patients have serum ferritin levels above 2500 *ng/ml*. This can increase the associated complications of iron overload, one of which is hepatic hemosiderosis resulting in fibrosis and cirrhosis [*Ikram N et al., 2004*]. The risk of cirrhosis of the liver increased at serum ferritin levels> 1000 *ng/ml* [*Shivaraja A et al., 2017*]. Therefore, iron overload complications, especially in the liver, are important and can not be underestimated.

APRI scores only require AST and platelet data, where the data is done routinely with formulas and easy calculations and can be done both in outpatients and inpatients [*Loaeza-del-Castillo A et al., 2008; Kruger FC et al., 2011*]. Significant correlations between serum ferritin levels and liver iron concentration (LIC) (r 0.718; p <0.001) in thalassemia patients with transfusion-dependent [*Majd Z et al., 2015*]. Based on the above description, we are interested to examine the relationship between serum ferritin level and APRI score in thalassemia patients receiving the chronic transfusion.

Material and Methods

The subjects of this study were thalassemia patients who underwent outpatient in Oncology Poly One Medical Roof Outpatient Installation at Dr. Soetomo General Hospital Surabaya, Indonesia that meets the criteria of inclusion and exclusion. Inclusion criteria include thalassemia patients (in 2014) aged 15-60 years who have received routine transfusions of more than 10 bags and no splenectomy has ever been performed. Exclusion criteria include obese, inflammatory, inflammatory, smokers, kidney function, Immune Thrombocytopenic Purpura (ITP), aplastic anemia, leukemia, hepatitis B, patients with a history of alcohol consumption, diabetes mellitus, autoimmune hepatitis, non-alcoholic fatty liver disease NAFLD), musculoskeletal trauma, malignancy, and patients taking influential drugs, ROS ferritin, and liver function. Subjects who are willing to follow the research first fill out the informed consent sheet.

First of all, the researcher conducts the ethical test in Dr.Soetomo General Hospital Surabaya, Indonesia. The number of thalassemia patients treated in 2015 was 438 patients and 150 patients each performed blood transfusions. After creating using consecutive sampling method obtained by 36 subjects.

Subjects were subjected to anamnesis related to the general characteristic data including sex, age, type of thalassemia, early age diagnosed, length of transfusion, iron chelation therapy, hemoglobin. The subjects were subjected to blood venous taking stored in EDTA vial and analyzed blood composition. Furthermore, blood was measured using a mini VIDAS (Biomerieux Inc., Singapore) with Enzym linked fluorescence assay (ELFA) method and denoted by units *ng/ml*. APRI score calculation [*Loaeza-del-Castillo A et al., 2008*] calculation based on aspartate aminotransferase (AST) and platelet counts using APRI formula below:

$$\text{APRI} = \frac{\frac{\text{AST level}}{\text{upper Limit of AST normal value}}}{\text{platelet}} \times 100$$

The measurement data is divided into two, the frequency data and the average data. The researchers analyzed data using SPSS version 17.0 (SPSS, Inc., Chicago, IL). The data of the measurement result before the first statistical test was performed by Shapiro Wilk test (p> 0.05). Further data were analyzed using Pearson test if the data was normally distributed and use spearmen test if otherwise.

RESULTS

Subject characteristic

Most of the subjects were male (66.67%) and gained thalassemia beta major (61.11%). The majority of patients treated with deferasirox (52.78%) iron chelation therapy and the majority of subjects had hepatitis C anti-HCV (-) of 83.33% (Table 1). The average subject aged 26.14 ± 7.39 years with the youngest age was 19 years old and the oldest age was 55 years. The first median age of diagnosis was 4 years with the youngest age was 0.5 years or 6 months and the oldest age was 52 years. The thalassemia patients routinely receive monthly transfusions with an average of 2 packed red cell or WE (washed erythrocytes) bags each month with an average chronic transfusion for 16.29 ± 8.48 years, the fastest being for 1 year, the longest getting a transfusion 29 year (Table 2).

The mean of Hb subjects was 6.56 ± 1.37 g/dL with range 2.20-9.48 *g/dL* and IMT value of 18.73 ± 2.31 kg/m^2 with range 13.85-25.20 kg/m^2. The mean aspartate transaminase (AST) of the study subjects was 61.22 ± 29.39 *U/L*, while median alanine transaminase (ALT) 41 *U/L* with the lowest value of 16 *U/L* and the highest value of 135 *U/L*. Median platelet values were found to be 140.1 x $10^3/\mu L$ with the lowest values of 29 x $10^3/\mu L$ and the highest score of 373 x $10^3/\mu L$ (Table 2).

Serum Ferritin Levels

The results of measurement of serum ferritin 5011.49 ± 2595.33 *ng/ml*, with the lowest serum ferritin level were 1287.92 *ng/ml* and the highest serum ferritin level was 9699.23 *ng/ml*. No research subjects had serum ferritin <1000 *ng/ml*, serum ferritin> 2500 *ng/ml* of 83.3%. There was a tendency to increase serum ferritin levels with the duration of transfusion, the longer the transfusion the higher the serum ferritin level. The mean serum ferritin levels in the anti-HCV (+) group were higher when compared with mean serum ferritin levels in the anti-HCV group (-) with 6308.7 ± 2520.9 *ng/ml* and 4752.0 ± 2572.13 *ng/ml*.

APRI Score

APRI scores were obtained abnormal distribution, the average value of 1.16 ± 1.10 with the lowest score was 0.22 and the highest score 4.97. APRI score consisted of AST and platelet variable with mean AST value was 61.22 ± 29.39 *U/L* and platelet average 139.12 ± 62.22 $10^3/\mu L$, from the data got the average score of APRI score was 1.16 ± 1.1. APRI score was not correlated with the duration of transfusion, it was found that the increasing of APRI score against transfusion duration, the longer the transfusion the higher the APRI score. Apri score> 1.5 only found as much as 22.22%, and at most on APRI score between 0.5-1.5 that was equal to 47.22%. The higher serum ferritin level, the higher the APRI score and the highest serum ferritin level was found in APRI> 1.5 scores of 6385.27 *ng/ml*.

Correlation of Serum Ferritin Levels and APRI Scores

Association of serum ferritin level and APRI score got significant correlation (p = 0.033; r = 0.355). The positive correlation between the two variables shows a weak correlation between the two variables. The association of serum ferritin level with APRI score was positive or unidirectional, which means that with increasing serum ferritin, the APRI score will increase. The association between

TABLE 1.

Frequency distribution of subject characteristics

Characteristics	Category	%
Sex	Male	66.67
	Female	33.33
Thalassemia	Beta mayor	61.11
	Beta minor	11.11
	HbE disease	22.22
	Beta Intermedia	5.56
Iron chelation therapy	Deferasirox	52.78
	Deferiprone	47.22
Hepatitis C	Anti HCV (+)	16.67
	Anti HCV (-)	83.33

TABLE 2.

The mean of subject characteristics

Variables	mean±SD
Age (year)	26.14±7.39
Early age was diagnosed (year)	9.85±12.70
Transfusion duration (year)	16.29±8.48
IMT	18.73±2.31
Hb (g/dL)	6.56±1.37
ALT (*U/L*)	52.03±30.21
AST (U/L)	61.22±29.39
Platelet ($10^3/\mu L$)	139.12±62.22
APRI	1.16±1.10

serum ferritin and APRI scores was seen in the scatter diagram of Figure 1. After removing the presence of hepatitis C, a statistical test showed an association between serum ferritin levels with significant APRI scores (p = 0.040; r = 0.377; figure 2). Several factors affecting serum ferritin levels and APRI scores can be seen in Table 3.

DISCUSSION

Serum ferritin was used to evaluate the iron overload in thalassemia patients, the higher serum ferritin levels will result in liver cell damage due to iron buildup and lipid peroxidase formation. The high serum ferritin levels will be followed by the tearing of fibrosis. The progressive damage of hepatocytes causes AST to be released into the circulation and decreases the clearance of AST by liver cells so that the levels increase during the liver damage process become chronic, in chronic liver disease also decreases the production of thrombopoietin causing the process of megakaryopoiesis and platelet maturation disrupted [*Asopari AA et al., 2014*]. In Indonesia, there has been no published study of the association between serum ferritin levels and APRI scores to assess liver fibrosis in thalassemia patients receiving a chronic transfusion.

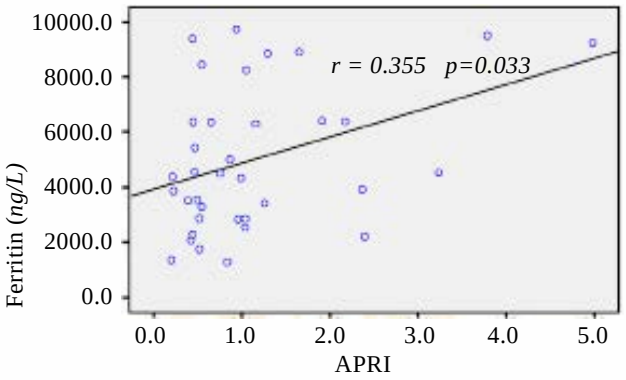

FIGURE 1. *Ferritin Serum Diagram of APRI*

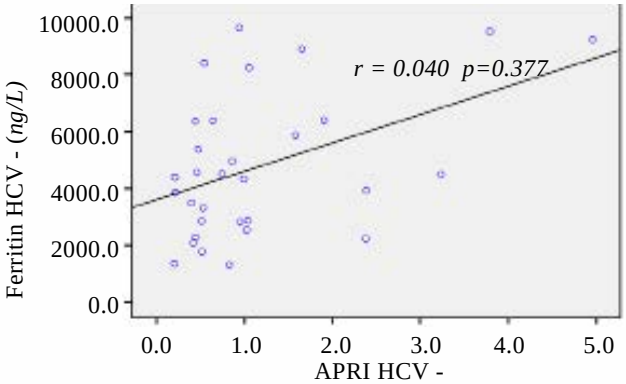

FIGURE 2. *Serum Ferritin Diagram of APRI in HCV-*

TABEL 3.

Factors affecting ferritin and APRI

Characteristics	Ferritin	APRI
Transfusion duration	0.021*	0.005*
Iron chelation theraphy	0.235	0.107
Hepatitis C	0.201	0.418
AST	<0.000*	<0.000*
ALT	<0.000*	0.170
Platelet	0.410	<0.000*

NOTE: *AST=aspartate transaminase, ALT=alanine transaminase *p<0.05*

A total of 63 major thalassemia and 22 patients with intermediate thalassemia has a mean age of 22.79 ± 7.1 years that identified the association between serum ferritin level and liver iron concentration was obtained a significant relationship between the two. Known serum ferritin and liver iron concentration are both used to know iron overload on the liver [*Majd Z et al., 2015*]. In Italy, 119 thalassemia patients with and without hepatitis C patients aged 32.8 ± 9.3 years had a significant correlation between serum ferritin levels with transient elastography [*Ferraioli G et al., 2016*]. The presence of iron overload is associated with liver damage resulting in fibrosis and cirrhosis. While the relationship between serum ferritin levels with APRI Score has not been studied previously. The APRI scores for assessing liver fibrosis in thalassemia patients should be used with caution because the APRI formula uses platelets, and one of the therapies to reduce transfusion requirements is splenectomy so that it can influence the interpretation of APRI scores [*Poustchi H et al., 2013*], while in this study excluded patients who have done splenectomy. APRI scores can be used to predict moderate-grade fibrosis and its primary role is to exclude significant fibrosis and cirrhosis [*Shaheen A, Myers R, 2007*].

After eliminating the presence of hepatitis C analysis, the association between serum ferritin and APRI score remained significant. Hepatitis C and hepatic iron overload are risk factors that can lead to the progression of fibrosis in patients with thalassemia [*Angelucci E et al., 2002*], but in this study, there was no difference in the results due to the small number of samples on anti HCV + that was only 16.67%. This weak correlation was allegedly due to many factors that can affect the increase of serum ferritin levels. Serum ferritin levels are commonly used for cases of haemochroma-

tosis because they are cheap, easy, non invasive and reliable but have low specificity because they can be affected by inflammation, especially inflammation of the liver, liver steatosis, neoplastic, diabetes mellitus, alcohol consumption, viral hepatitis infection, and another cell necrosis, one of the causes of elevated serum ferritin levels was also due to hepatitis C but no difference was found between serum ferritin levels in the anti-HCV-anti-HCV + group, due to the small number of group samples with anti-HCV + (16.67%), other factors have been excluded but the limitations of this study are some exclusion criteria based only on the history and physical examination. Serum ferritin levels are commonly used to determine the body's iron status on iron overload, but they are not fully accurate, as many factors can affect serum ferritin levels, so an evaluation is required to look at the accumulation of iron in the liver by measuring the good use of liver iron concentration MRI or by using a biopsy, a combination of serum ferritin and MRI may provide more precise information, but not all health services are available for MRI and at a higher cost than serum ferritin [*Angulo IL et al., 2008*]. A combined evaluation of serum ferritin levels with MRI may provide more precise information, high serum ferritin levels accompanied by low MRI T2 liver, a higher sailing dose is required and then reevaluated after 6 months [*Fahmy HS et al., 2015*].

Other factors were suspected because APRI scores were not able to identify patients in early fibrosis, but on significant fibrosis of F2-F4, APRI scores in thalassemia patients were best used to diagnose F4 fibrosis with 87.5% sensitivity and 37.7% [*Poustchi H et al., 2013*]. Liver damage that occurs may not reach the stage of severe fibrosis/cirrhosis so that the amount of Thrombopoietin produced by the liver has not been too low, and the low number of platelets is affected by splenomegaly due to Ineffective hematopoiesis from the bone marrow causing extramedullary hematopoiesis. Thrombopoietin is a hormone produced mainly by hepatocytes, which can promote platelet production by bone marrow. The Thrombopoietin level correlates with liver fibrosis levels and hepatocyte function failure [*Garjani A et al., 2015*]. Fibrosis in thalassemia patients was associated with alanine transaminase, aspartate transaminase, gamma-glutamil transferase, bilirubin and International Normal Ratio but not related to platelet count, probably due to the high proportion of patients who have undergone splenectomy [*Shelton E et al., 2015*]. Low platelet counts can be a marker of portal hypertension. However, it was not recommended to predict cirrhosis by using only platelets and ALT in hemochromatosis.

Conclusion

Most of the male thalassemia patients and the type major are much experienced. There was a correlation between serum ferritin level and APRI score in thalassemia patients with weak correlation strength. The increase of serum ferritin level to increase APRI score is relatively small, so APRI score cannot be used to know the existence of liver fibrosis and still need further research.

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