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Relationship between the amount of blood transfusion and the amount of iron chelation with blood and salivary ureum levels in children with beta thalassemia major

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ABSTRACT

Introduction: Patients with thalassemia experience a synthesis of one of the beta chains of the globin gene, resulting in reduced hemoglobin formation. Patients with beta-thalassemia major require routine transfusions and iron chelation. Both of these cause impaired kidney function. This study aims to determine the relationship between the amount of blood transfusion and the amount of iron chelation with blood urea and saliva levels in children with beta-thalassemia major.

Methods: An observational cross-sectional study was conducted at Moewardi Hospital, Solo using patients with beta-thalassemia major as subjects, taken by consecutive sampling from September to November 2018. The data on blood transfusion, iron chelation, and blood urea levels were obtained from medical records. Unstimulated saliva was taken in the morning. The urease method was used to assess the salivary urea levels and all data was analyzed using Pearson correlation analysis.

Result: The results showed the mean amount of transfusion was 71.9 ± 34.3, the amount of iron chelation 61.0 ± 32.1, blood urea levels 4.41 ± 1.1 mg / dL, and salivary urea levels 35.6 ± 12.5 mg / dL. Correlation analysis showed a positive correlation between the amount of blood transfusion and the amount of iron chelation with blood and saliva urea levels. There was also a positive correlation between blood urea and saliva levels.

Conclusion: It can be concluded that the amount of blood transfusion and iron chelation affects urea levels in blood and saliva in children with beta-thalassemia major. The close correlation between blood and saliva urea indicates that salivary urea can be used as an alternative assessment of blood urea.

Keywords: Beta Thalassemia Major, Blood Urea Level, Saliva Urea Level
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INTRODUCTION

Thalassemia is a hemoglobin synthesis disorder inherited autosomal recessively due to reduced production of one or more globin chains and caused by chronic hemolytic anemia. The genetic anomaly causes an imbalance in the production of globin chains because of the disturbance in the synthesis of the polypeptide chain that composes the globin molecule in hemoglobin (Hb). Interference with this synthesis can result in inadequate production of red blood cell counts, decreasing the number of red blood cells. Hemoglobin has the same tetrametric form, consisting of 2 pairs of globin chains bound to heme.[1]

Based on the disturbed globin chain, there are several types of thalassemia, namely α and β thalassemia. In Indonesia, which is often found is β thalassemia. Data from WHO states that there are more than 332,000 pregnancies and births every year with hemoglobin abnormalities. About 56,000 of them are thalassemia major.[2]

According to Riskesdas 2007, 8 provinces with a higher prevalence of national prevalence, including Aceh Province (13.4%), DKI Jakarta (12.3%), South Sumatra (5.4%), Gorontalo (3.1%), Riau Islands (3.0%), West Nusa Tenggara (2.6%), Maluku (1.9%), and West Papua (2.2%). Based on YTI and POPTI data in 2014, from the results of screening in the general public from 2008 to 2017, traits were obtained by 699 people (5.8%) of 12,038 people examined, while the results of screening in the thalassemia (ring 1) family in 2009-2017 were 1,184 (28.61%) of 4,137 people. Whereas based on RSCM data, up to October 2016, there were 9,131 thalassemia patients registered throughout Indonesia.[3]

Based on the severity of clinical features, thalassemia b is classified into (1) thalassemia major, which is highly dependent on transfusion, (2) usually asymptomatic thalassemia minor, and (3) intermedia thalassemia.[4] Thalassemia major requires regular blood transfusions to maintain quality of life. Patients must get lifelong blood transfusions to overcome anemia and maintain Hb levels of 9-10 grams/dl.[5,6] In thalassemia patients, repeated transfusions and ineffective erythropoiesis would result in excessive iron accumulation in various

organs.[5] Iron accumulation will spur the emergence of superoxide radicals and increase the amount of iron in the heart, liver, kidneys, and endocrine glands resulting in damage and disruption of organ function.[1] These superoxide radicals oxidize lipid cell membranes, proteins, and organelle membranes, causing cell damage and death.[7,8]

Humans do not have a mechanism for excreting excess iron, so iron chelation therapy is needed. Iron flatfoot therapy has proven to reduce iron content in thalassemia patients who get transfusions. Iron flatness is useful, but it is dangerous because it contains chemicals. Iron chelation drugs are absorbed and circulated for several hours. Long periods will increase kidney burden in excretion, which can cause kidney damage.[7,9]

The existence of complications from the pathological process in patients with beta-thalassemia major can interfere with the function of various organs, one of which is the kidney. The involvement of the kidneys greatly affects the urea levels in the body. Impaired kidney function is characterized by protein in the urine (proteinuria or albuminuria), blood in the urine (hematuria), and increased urea levels in the blood. Beta thalassemia major has impaired renal function characterized by the emergence of toxic urea and can be detected to determine impaired renal function.[10-13]

Interest in rapid and noninvasive diagnostic tests has developed in the past few decades. One of them is using saliva as a biological liquid for clinical diagnosis. Saliva has advantages compared to blood and urine, which are easier to collect and have lower protein components than blood serum.[14,15] Saliva can be used as a substitute for blood samples for diagnostic tests of urea levels in patients with kidney disorders because it is noninvasive, simple, and inexpensive. This method is also more cost-effective for screening large populations.[13]

METHODS

Study Design

This study used an observational cross-sectional design with a consecutive sampling method. The study subjects were 27 beta thalassemia major patients treated at Moewardi Hospital in Solo who received blood transfusions and iron chelation. The research time range from September - November 2018.

Saliva Sampling

The salivary collection uses the spitting method. The salivary collection is carried out for 10 minutes. Every 1-minute interval, the subjects were asked to remove the saliva collected in the mouth into the measuring tube through a measuring funnel.[15]

The saliva collected was unstimulated saliva. The patients were instructed to fast for 45 minutes before collecting saliva to prevent acidity due to the effects of salivary stimulation during food chewing and no increase in protein concentration.[16] The collected saliva was put into the Sample Separator Tube (SST) and then taken directly to the Clinical Pathology Laboratory in Moewardi Hospital Solo.

Examination of Saliva Urea Levels

Saliva urea levels were measured using the UV urease / GLDH (fixed time) method. The tool used is Automatic Analyzer with advia 1800 Siemens.

Blood Urea Data Collection

Blood urea level calculations take data from patients' medical records in Moewardi Hospital Solo.

RESULTS

This study is about the relationship between the amount of blood transfusion and iron flatness with blood urea levels and salivary urea levels in beta-thalassemia major patients. Consecutive sampling techniques took the research subjects of patients who fulfilled the inclusion and exclusion criteria as many as 27 patients. The description of the research subject is presented in Table 1.

Based on Table 1, the subject of the study was 27 subjects, divided into age groups, namely toddlers (0-5 years), children (6-11 years), and adolescents (12-17 years). The most age group is children (6-11 years) of 12 (44.4%). The research subjects based on sex groups were male groups of (55.6%) and women (44.4)%.

The mean and standard deviation of urea levels based on blood transfusion, the amount of iron chelation, blood urea levels and salivary urea levels are presented in Table 2.

The amount of transfusion was 71.9 with a standard deviation of 34.3, while the amount of iron chelation was 61.0 with a standard deviation of 32.1. On the other hand, the mean of blood urea levels was

Table 1. The description of the research subject

Characteristics	Total	Percentage
Age		
Toddlers 0-5 Years Old	5	18.5
Children 6-11 Years Old	12	44.4
Adolescents 12-17 Years Old	10	37.0
Sex		
Male	15	55.6
Female	12	44.4

Table 2. The average and standard deviation of the amount of blood transfusion, the amount of iron seams, blood urea levels, and salivary urea levels

Subject	N	The Amount of Blood Transfusion	The Amount of Iron Chelation	Blood Ureum Levels (mg/dl)	Saliva Ureum Levels (mg/dl)
			$\bar{x}$ ± SD		
Children with Beta-Thalassemia Major	27	71,9±34,3	61,0±32,1	4,41±1,1	35,6±12,5

4.41 mg / dL with a standard deviation of 1.1 mg / dL, while the mean levels of salivary urea were 35.6 mg / dL with a standard deviation of 12.5 mg / dL.

In this study, a normality test was conducted to see the normality of the data. The results of the normality test used were the Shapiro-Wilk test presented in Table 3.

Bivariate analysis in Table 4 shows there was a significant relationship between the amount of blood transfusion with blood urea levels (p = 0.000, correlation coefficient 0.749) and with salivary urea levels (p = 0.000, correlation coefficient 0.773). There was also a significant relationship between the amount of iron chelation with blood urea levels (p = 0.000, correlation coefficient 0.785) and with salivary urea levels (p = 0.000, correlation coefficient 0.710). There is a significant relationship between blood urea levels and salivary urea levels (p = 0.000, correlation coefficient 0.775). Multiple regression tests were then carried out to determine the contribution of the amount of transfusion and the amount of iron chelation, and the results are presented in Table 5 and 6.

Based on Table 5, it was found that there was a significant effect between the amount of transfusions and the amount of iron chelation on blood urea levels (p <0.05). The coefficient value of the amount of transfusion and the amount of iron chelation indicate that every 1 scale increase in transfusion would increase blood urea levels by 0.013 and every I scale increase in iron chelation would increase blood urea levels by 0.017. The influence of the two variables on blood urea levels was 70.2%; other factors outside the study influenced the remaining 29.8%. The line equation obtained is y = 2,435 + 0,013 x (amount of blood transfusion) + 0,017 x (amount of iron chelation).

Based on Table 6, it was found that there was a significant effect on the amount of transfusion and the amount of iron chelation on salivary urea levels (p <0.05). The regression coefficient of the amount of transfusion and the amount of iron chelation indicates that every 1 scale increase in transfusion would increase blood urea levels by 0.196. In contrast, an increase in iron chelation would increase the urea by 0.134. The magnitude of the influence of the two variables on urea was 66.0%; other factors outside the research influenced the remaining 34.0%. The line equation obtained is y = 13,331 + 0,196 x (amount of blood transfusion) + 0,134 x (amount of iron chelation).

Table 3. Shapiro-Wilk Normality Test on The Amount of Transfusion, Amount of Iron Seams, Blood Urea Levels, Salivary Urea Levels

The normality of the data	*Shapiro-Wilk*	N	Sig.
The Amount of Blood Transfusion	0.939	27	0.118
The Amount of Iron Chelation	0.951	27	0.228
Blood Ureum Levels	0.973	27	0.696
Saliva Ureum Levels	0.908	27	0.060

Table 4. Table summary of Pearson correlation test

Source	r	p
The Amount of Blood Transfusion vs Blood Ureum Levels	0.749	0.000
The Amount of Blood vs Saliva Ureum Levels	0.773	0.000
The Amount of Iron Chelation vs Blood Ureum levels	0.785	0.000
The Amount of Iron Chelation vs Saliva Ureum Levels	0.710	0.000
Blood Ureum Levels vs Saliva Ureum levels	0.775	0.000

Table 5. The result of multiple linear regression tests assessing the effect of the amount of transfusion and the amount of iron chelation on blood urea levels.

Variable	Regression Coefficient (B)	p	CI 95%	R^2
(Constant)	2.435	0.000	1.83-3.03	
The Amount of Blood Transfusion	0.013	0.014	0.003-0.023	0.702
The Amount of Iron Chelation	0.017	0.003	0.007-0.028	

Table 6. The result of multiple linear regression tests assessing the effect of the amount of transfusion and the amount of iron chelation on saliva urea levels

Variable	Regression Coefficient (B)	p	CI 95%	R^2
(Constant)	13.331	0.001	5.95-20.71	
The Amount of Blood Transfusion	0.196	0.003	0.074-0.319	0.660
The Amount of Iron Chelation	0.134	0.045	0.003-0.264	

DISCUSSION

This study was conducted on patients with beta-thalassemia major who were treated at Moewardi Hospital Solo. The study was conducted at Moewardi Hospital Solo because there was a POPTI community (Association of Parents with Thalassemia in Indonesia) under the guidance of Moewardi Hospital Solo.

Based on Table 1, it can be seen that research subjects are as many as 27 subjects; the age group is divided into 3 groups, namely toddlers (0-5 years), children (6-11 years), and adolescents (12-17 years). The most age group is children (6-11 years) at 44.4%. The research subjects were based on sex groups; the male group was bigger, as much as 55.6%, and the women 44.4%. According to the Indonesian Ministry of Health (2009), the age of children 6-11 years are included in the category of childhood age.[16] Patients with beta-thalassemia major will appear normal at birth, but symptoms of anemia will begin at the age of 3-18 months. This is in accordance with the theory that the clinical symptoms of thalassemia have been seen at 2 years. Still, thalassemia sufferers are treated at 4-6 years because they are increasingly paler, causing sufferers to require regular transfusions.[17]

In Table 4, the study results show a strong positive correlation between the

amount of blood transfusion and blood urea levels and salivary urea levels. Blood transfusion aims to suppress erythropoiesis but can increase iron accumulation. Survival rates in beta-thalassemia major sufferers depend on blood transfusions every 2-5 weeks for life to overcome anemia and maintain Hb levels of 9-10 gr/dl. Iron deposits due to hyperhemolysis of erythrocytes resulting from erythropoiesis and chronic blood transfusions are the main causes of kidney failure in patients with beta-thalassemia major. In blood transfusions, every 1 ml of red blood cells will increase the iron load in the body by 1 mg. Impaired kidney function is characterized by protein in the urine (proteinuria or albuminuria), blood in the urine (hematuria), and increased urea levels in the blood. Beta thalassemia major has impaired kidney function characterized by the onset of urea and can be used to determine impaired kidney function. The kidney's function is to filter the remaining digestive products of protein in the blood, especially urea which will be released through urine and saliva.[4,10,11,18-20]

The results also showed a strong positive correlation between the amount of iron chelation with blood urea levels and salivary urea levels. Iron chelation therapy aims to reduce the toxicity of iron deposits in the tissues, prevent excess iron organs, and remove iron from the red blood cell membrane. Iron flatness will overcome iron accumulation which can cause heart, liver and endocrine system dysfunction. Treatment using iron chelation therapy can have toxic effects on the kidneys.[21] Excessive iron chelation therapy results in vacuolization of proximal renal cells and accumulation of nephrotoxic components, which cause direct toxicity to the kidneys. An increase in urea in the blood is associated with kidney abnormalities, especially the glomerular and tubular parts. Urea analysis of saliva with kidney disease reflects the level of urea in the blood associated with the presence of abnormalities in the kidneys, especially the glomerular and tubular parts. Urea analysis of saliva with kidney disease reflects blood urea levels.[22]

The mechanism of blood urea migrated to saliva through 3 pathways. The first pathway is passive diffusion through 5 layers, namely capillary wall, interstitial space, basal cell membrane from acinus cells or ductal cells, acytoplasmic cells or ductal cells, and luminal cell membranes. The second pathway is blood urea which enters the saliva by active transport through secretory gland cells. The third pathway is transporting from the bloodstream to saliva through the space between the acinus and ductal cells. Ureum has no electric charge and is fat soluble so it can diffuse easily through biological membranes and distribute through body fluids. Diffusion between intracellular and extracellular compartments only takes a few minutes. Balance is achieved faster in membranes with ureum transporters such as red blood cells, hepatocytes and epithelial cells.[23,24] The results showed a positive and strong correlation between blood urea levels and salivary urea levels, meaning that analysis of salivary urea levels could be used as a diagnostic biomarker for renal disorders in major beta-thalassemia. Saliva can be used as a substitute for blood samples for diagnostic tests of urea levels in patients with kidney disorders in major beta-thalassemia. Using blood as a diagnostic tool is an invasive process and is usually associated with stress and tension.

CONCLUSION

Based on the results of this study, it can be concluded that the amount of blood transfusion and iron chelation affects the level of urea in blood and saliva. It also revealed that salivary urea level might reflect the level of urea in the blood. However, further study is needed to confirm and verify this finding and validate the association between blood and salivary urea level by controlling the age of the subjects. Also, direct assessment of blood urea is recommended as secondary data was prone to operator bias.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this article

ETHICS APPROVAL

This study had been ethically approved with an ethical clearance number 001555/KKEP/FKG-UGM/EC/2018

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AUTHOR CONTRIBUTION

All authors contributed equally in the study, writing and revising this article

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