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RESEARCH ARTICLE

Hematologic and Clinical Features of Thalassemia Patients with Early or Late Onset Transfusion in East Java, Indonesia

Retno Dwi Wulandari[1,2*], Loeki Enggar Fitri[3], Fatchiyah Fatah[4], Diana Lyrawati[5]

1. *Department of Biomedical, Faculty of Medicine, Wijaya Kusuma Surabaya University, Indonesia.*
2. *Doctoral Program in Medical Science, Faculty of Medicine, Brawijaya University, Indonesia.*
3. *Department of Parasitology, Faculty of Medicine, Brawijaya University, Indonesia.*
4. *Department of Biology, Faculty of Mathematics and Natural Science, Brawijaya University, Indonesia.*
5. *Department of Pharmacy, Faculty of Medicine, Brawijaya University, Indonesia.*

***Corresponding Author: Retno Dwi Wulandari**

Abstract

Thalassemia is a genetic disorder of hemoglobin production which causes chronic anemia. Complete blood count showed lower HbA, MCV, MCH, increase RDW-CV and increase HbA2 (>3,5%) and HbF (>1,5%). Thalassemia major patients need regular and lifelong blood transfusion which started early in life. Thalassemia intermedia, usually with milder symptoms, require blood transfusion at later age, with lower transfusion rate. This study aims to compare hematologic, HbA2 and HbF profiles as well as height, weight, Body Mass Index and spleen size in thalassemia patients grouped based on transfusion start whether in early or later age (before or after 10 yo). Results showed that the hematologic, HbA_2 and HbF profiles were not significantly different in both groups. The clinical profiles i.e. Height and weight were below 3rd percentile in both groups; low BMI and more patients underwent splenectomy in group of those with early onset blood transfusion.

Keywords: *Thalassemia, Transfusion, Hematologic, Height, Weight, BMI.*

Introduction

Thalassemia is a disorder in haemoglobin (Hb) production which can be found all around the world. Haemoglobin A (α2β2) is the main haemoglobin, comprises of 96-98% haemoglobin found in adult, whereas HbA2 (α2δ2) is~3.5% and foetal haemoglobin (α2γ2) less than 1%. Haemoglobin foetal (Hb F) is the predominant haemoglobin (up to 90%) in foetal life, however after two years, it decreases and replaced by HbA [1]. Mutation in the alpha or beta genes which make up the haemoglobin causes imbalance in globin chain production, resulting in defected red blood cells which then easily removed by the body.

Furthermore, it is clinically ascertained as mild, moderate or severe anemia (decreased Hb level). Thalassemia patients also show a decrease in MCV and MCH level. By High performance liquid chromatography, there is an increasing in HbA2 and HbF level in beta thalassemia patients [2], meanwhile both levels are normal in alpha thalassemia [3, 4]. Clinically, beta thalassemia can be divided into minor (TT), intermedia (TI) and major (TM). Thalassemia minor showed no symptoms, or mild anemia; thalassemia major characterized by severe anemia leading to lifelong blood transfusion, while the severity of thalassemia intermedia lies between minor and major. To maintain haemoglobin level, patients get blood transfusion which in beta thalassemia major it started before the age of two years.

However, some patients start transfusion in their late ages or even do not need regular transfusion (thalassemia intermedia) [2]. Some condition is linked with late onset transfusion or transfusion independency, one of which is owing to the high level of HbF in

adults [5].As the consequence of chronic anemia, iron overload due to years of transfusion and inadequately iron chelation, patients with thalassemia major are known to have growth failure [6]. Even, in those with thalassemia intermedia, they may suffer from growth impairment if the dietary intakes are inadequate [7]. The aim of this study is to evaluate haematologic and clinical profiles of patients with early (<10 yo) or late onset blood transfusion (≥10 yo).

Methodology

This research was approved by Ethical Comittee of Health Research of Medical Faculty Wijaya Kusuma Surabaya University (No.10198/SLE/FK/UWKS/2018). The inclusion criteria were thalassemia patients with blood transfusion, age > 10 yo, have completed medical records.

The subject anonymity and confidentiality were kept indefinitely and revealed only to the main researcher. Subject of this study are member of POPTI (Indonesian thalassemia's parents association). After signing informed consent, subjects were measured their height, weight and spleen size. Five milliliter blood was collected in vacutainer for hematologic analysis including HPLC assays. Laboratory investigation for hematologic profile used (Advia 2120 Siemens haematology analyser) and High Performance Liquid Chromatography analysis (Variant II Biorad); whereas the spleen size measured physically and ultrasonographycally by an internist. Samples were then grouped based on the onset of transfusion (before and after 10 years old). Both groups were then compared for height (centimeters), weight (kilograms), BMI (calculated as kg/m^2), hematologic criteria (Hb, MCV, MCH and RDW-CV levels), Hb chromatogram (HbA_2 and Hb F levels) and spleen size. This study used PSPP to compare the average and standard deviation of age, first transfusion and its frequency, haematologic profile, HbA_2 and Hb F levels, and BMI index. P value < 0.05 was considered statistically significant.

Results

A total of 52 subjects comprising of 31 males and 21 females, between the ages of 10 and 51 years old. Samples with the onset of transfusion ≥10 years old was designated to group A, while those with the onset <10 years old were in B. In group A, 50% (8/16) were male between the age of 22 to 53 yo (mean 33.06). Group B consists of 33 samples: 66.7% (22/33) was male between the age of 10 to 51 yo (mean 24.83). Results showed no difference in transfusion frequency, haematologic index, and HbA2 and HbF levels between groups (Table 1).

Table 1: Haematologic and clinical features of thalassemia patients with early or later onset of blood transfusion

	Group A **Onset>10 yo** mean±SD	Group B **Onset <10 yo** mean±SD	***p* value**
Sex: **Male** **Female**	8 8	 23 13	
Age (y.o)	33.06±9,86	24.82±8.65	0.004
1st transfusion (y.o)	24.06±13.05	4.64±2.59	0.000
Freq. transfusion (Times/year)	8.25±3.97	8.75±3.84	0.674
Hb (g/dL)	7.14±0.88	7.12±1.01	0.964
MCV (fl)	70.29±5.45	70.98±8.20	0.760
MCH (pg)	21.54±2.88	21.99±3.51	0.658
RDW-CV (%)	25.39±3.69	25.35±6. 15	0.963
HbA2 (%)	27.49±14.58	27.92±15.66	0.927
HbF (%)	7.67±4.12	6,24±4.85	0.316
BMI index	19.95±2.32	18.28±1.97	0.012

The data of height and weight (Table 2) were categorized using CDC scale [8]. The height and weight of 3 patients in group A and 3 patients in group B belong to 50th percentile, while the majority of patients belong to below 3rd percentile.

Table 2: Height and weight of thalassemia patients with early or later onset of blood transfusion

Measurement	Sex (N)	percentile				
		<3rd	3-10th	11-25th	26-50th	>50th
Group A (later onset of blood ttansfusion)						
Height (cm)	Male (N=8)	5	2	1		
	Female (N=8)	3	2		2	1
	Total 16	8 (50%)	4 (25%)	1(6.25%)	2(12.5%)	1(6.25%)
Weight (kg)	Male (N=8)	6	2			
	Female (N=8)	4	1	1		2
	Total 16	10 (62.5%)	3 (18.75%)	1(6.25%)	0 (0%)	2(12.5%)
Group B (early onset of blood transfusion)						
Height (cm)	Male (N=22)	14	5	2		1
	Female (N=11)	6	4			1
	Total 33	20(60.6%)	9(27.2%)	2(6.1%)	0 (0%)	2(6.1%)
Weight (kg)	Male (N=22)	5	8	4	4	1
	Female (N=11)	8	2	1		
	Total 33	13(39.4%)	10(30.3%)	5(15.2%)	4(12.1%)	1(3%)

Majority of the patients showed spleen enlargement in all groups, however only three in group B requiring splenectomy (Table 3).

Table 3: Spleen size measurement

Group	Spleen size			Total
	3-10 cm	>10 cm	Splenectomy	
A	2 (12.5%)	14 (87.5%)	0	16
B	6 (18.2%)	24(72.7%)	3(9.1%)	33

Discussion

Beta thalassemia inter media refers to patients with independency to regular transfusion with the condition shows wide range of clinical manifestation and occurs later, frequently in the 3rd or 4th decade of life [9]. Ho, et al (1998), described that TI patient's phenotype between TT (asymptomatic) and TM, and at the severe state, it presents between the age of 2 and 6 years old [10]. Group A in the present study was considered as TI patients, as they took first transfus.ion later in life, mostly during early three decades of life) whereas group B were TM patients.

This research also showed that thalassemia patients were short and underweight, which is consistent with other studies. Low haemoglobin level, iron overload, iron chelator drugs and nutrition intake are possible contributors to this parameter. The short stature in thalassemia major patients persists despite the introduction of major advances in the treatment [11, 13]. When comparing for average BMI level, both groups were significantly different. According to WHO (2004)[14], group A belongs to normal BMI level, while group B belongs to mild thinness. Malnutrition, toxic effects of long term iron chelation, and the disease severity were thought to be the possible aetiologies. Kaur et al (2018) reported that BMI decreases along with the advancing age and progression of the disease [15]. Red cell indices are important in thalassemia diagnosis. The main component in CBC include: Hb, MCV, MCH and RDW-CV. Thalassemia patients generally classified as hypochromic and microcytic anemia, with MCV as high as 72 fL are presumed as thalassemia syndrome [16].

Although the onset of transfusion in both groups was significantly different, it appeared that the mean values of Hb, MCV and MCH result was similar (p=0.964, 0.760, and 0.658, respectively). The mean haemoglobin level in this research was similar to the study conducted by Wahidayat *et al* (2018) which showed Hb level for the intermediate thalassemia were 7.5g/dL [17].

Khera et al (2015), who investigated 87 cases of thalassemia disorder stated that MCV and MCH were almost similar in thalassemia major, inter media and minor [18]. As thalassemia beta major and HbE/beta thalassemia patients need blood transfusion, they showed very high degree of anisocytosis which indicated by high value of RDW-CV [19]. Anisocytosis describe the presence of two red cell's populations, which one of the possibilities is mixed between patient and donor cells population. The two cell-populations may have varying size, leading to an increase in RDW level [20].

The level of—RDW-CV in this study was higher than normal (11.5-14.5%). Khera *et al* (2015) investigating haemoglobin profile in thalassemia syndrome and haemoglobinopathies, reported increasing RDW-CV (26.5 ± 6.7) (range 20.2-36.2) similar with this study for both groups (25.39 ±3.69 and 25.47 ±5.95). According to Khera et al (2015), anisocytosis and poikilocytosis in thalassemia major/intermedia, was moderate to severe, and mild in thalassemia trait [18]. An interesting study by Sharma et al (2012) showed that the mean RDW value was higher in HbE/ ßthal (35.76) rather than HbE/HbE (22.3) and HbE trait (18, 6). Therefore, HbE/ßthal mutation should be considered in patients with microcytic hypochromic anemia with increasing RDW level aside from Iron Deficiency Anemia [21]. In globin gene disorder, Haemoglobin Foetal level varies greatly[22]. The level of HbF is around 80% at birth and steadily decrease after birth until reach less than 1% in normal adult [23].

While in ß thalassemia heterozygotes its level is slightly increased, in $ß^{+}$ thalassemia homozygotes or $ß^{0}/ß^{+}$ compound heterozygotes, the level lies between 70-90%, whereas in $ß^{+}$/HbE it ranges from 2% to 76%. The high level of HbF in adult with thalassemia due to delayed switch from γ-globin to ß-globin gene expression [23].

In this study, the mean HbF level in both group was 7.67±4.12 and 6.24±4.85 respectively, with the two highest HbF level was belong to group B (21.1% and 19.1%). Others cause of increasing HbF level are alpha thalassemia co-inheritance, gamma chain synthesis and C>T polymorphism in gamma gene promoter [22,24].

The increasing HbF level in this study still needs investigation to determine the underlying molecular basis. Thalassemia patients are characterized by ineffective erythropoiesis and peripheral haemolysis, which cause anemia and lead to chronic anemia [25]. Although some patients with Thalassemia intermedia show no symptom until adult life, they may suffer from moderate anemia with the need for transfusion might be eliminated or delayed, whereas the remaining are symptomatic even from young age.

Though the onset of transfusion may be delayed, it becomes frequent with advancing age, during infection, pregnancy and when there is spleen enlargement [26]. To overcome chronic anemia, the body responses by increasing EPO production, but still erythroid marrow is unable to respond properly to EPO signal. This cause the erythroid cells fail to differentiate and die prematurely, which then lead to bone marrow expansion and enlargement of spleen size. The spleen becomes larger as the consequence of its major role in removing the red cells damage in blood stream [27]. Both group in this study showed that the majority had spleen size of above 10 cm (splenomegaly). Although, no one in group A had splenectomy, compared to 3 (9.1%) samples in group B. Splenectomy can increase HbF level as shown in study conducted by Mussallam et al [28]. However in this study, the mean HbF level in the splenectomized sample was 5.9, below the mean HbF level in all group B's sample (6.24 range 0.2 – 21.1).

Conclusion

Both groups showed similar weight and height but significantly differ in BMI index; no difference in haematologic profiles, despite the different onset of blood transfusion. The low BMI index of thalassemia patients, regardless of transfusion onset, may associated with the risk for morbidity and mortality.

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