Clinical Research

Frequency of thalassemia carrier and Hb variant and the quality of stored donor blood

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Abstrak

Latar belakang: *Penelitian ini bertujuan untuk mengetahui frekuensi pembawa sifat talasemia dan Hb varian pada donor darah. Di samping itu, juga dilihat kualitas darah simpan yang dihasilkan sampai dengan hari ketujuh, dengan persentase hemolisis secara in vitro.*

Metode: *Penelitian ini menggunakan disain potong lintang dengan sampel berasal dari 138 donor darah. Dilakukan skrining talasemia dan Hb varian pada keseluruhan sampel, yang meliputi pemeriksaan hitung sel darah lengkap, analisis Hb metode HPLC dan analisis DNA untuk deteksi pembawa sifat talasemia α. Uji kualitas darah simpan dilakukan melalui penghitungan persen hemolisis sel darah merah pada komponen* whole blood *(WB) dan dibandingkan antara hari pertama dan hari ketujuh penyimpanan.*

Hasil: *Dari 138 sampel, ditemukan pembawa sifat talasemia α pada 5 subjek (3,6%) yang salah satu diantaranya disertai ovalositosis herediter tipe Asia Tenggara (*Southeast Asian Ovalositosis/SAO*), pembawa sifat talasemia β 3 subjek (2,2%), dan HbE 3 subjek (2,2%). Donor pembawa sifat talasemia dan Hb varian serta SAO tersebut mempunyai persen hemolisis darah simpan pada hari pertama dan tujuh hari penyimpanan kurang dari satu persen.*

Kesimpulan: *Frekuensi total pembawa sifat talasemia dan Hb varian pada populasi donor darah di UTD PMI DKI Jakarta adalah sebesar 8%. Keseluruhan sampel pembawa sifat talasemia dan Hb varian mempunyai kualitas darah simpan cukup baik pada satu minggu penyimpanan.*

Abstract

Background: This study was aimed to determine the frequency of thalassemia and Hb variant in blood donor. In addition, we also wanted to know the quality of blood from the donor up to seven days of storage, by calculating percentage of hemolysis *in vitro*.

Methods: This cross-sectional study was conducted on 138 blood donor specimens at Red Cross Blood Centre Unit in Jakarta. All specimens were tested for thalassemia and Hb variant by complete blood count (CBC) and Hb analysis with HPLC method and DNA analysis for the detection of α thalassemia carrier. To analyze the quality of stored blood, the calculation of hemolytic rate of red blood cells (RBCs) on whole blood (WB) was compared between the first and seventh days of storage.

Results: Out of the 138 specimens, 5 samples (3.6%) were diagnosed for α thalassemia carrier in which, one of them is co-inherited with ovalositosis hereditary (Southeast Asian Ovalositosis/SAO), 3 samples (2.2%) for β thalassemia carrier, and 3 samples (2.2%) for Hb E. Meanwhile, the hemolytic rates of RBCs on WB in first day and seven day of storage were below one percent.

Conclusion: The frequency of thalassemia carrier and Hb variants in blood donors at Red Cross Blood Centre Unit in Jakarta was 8%. The quality of stored blood until seven day of storage was quite good.

Keywords: donors, Hb variant, hemolytic rate, thalassemia carier

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Thalassemia and hemoglobin (Hb) variant are the most common genetic disorders in Southeast Asia.[1] The Southeast Asian region accounts for about 50% for the world's carrier of thalassemia.[2] Based on the mean corpuscular hemoglobin (MCH), the frequency of α° thalassemia carrier among Javanese, Sumatra and Sulawesi population was 2.6 - 3.2% and the frequency of -α^{+} thalassemia carrier in the Javanese, South Sumatra and South Sulawesi population were 2.7%, 10% and 11% respectively.[3] Hb E is the most common Hb variant found in Indonesia[1] and its frequency is 1.5 - 36%.[4]

The prevalence of thalassemia and Hb variant (Hb E) in Indonesia is quite high. Currently, blood donors are not routinely screened for these disorders. The thalassemia carriers do not usually show clinical symptoms, carry normal Hb concentration, therefore, these subjects may pass the donor selection that only rely on the Hb concentration.[5,6]

The thalassemia carrier and Hb variant, like Hb E individu, has an unstable Hb. The abnormal hemoglobin may affect the structure and function of red blood cells (RBC) membrane, making the RBCs more susceptible to damage and lysis.[7] Blood from donor who are carrier for these disorders might affect RBCs' survival. Therefore, if such red blood cells are to be donated and transfused to a recipient may encountering a stressful event.[8]

Despite the quality of blood from carrier of thalassemia and Hb variant due to their unstable Hb is still questionable, it could also be related to blood collecting and storing, where RBCs can change and subsequently undergo hemolysis that will decrease the effectiveness of transfusion.[8] A recent study shows that hemolysis rate at the end of storage period correlates not only with the hemolytic rate at the start of the storage period but also with individual donors who, having poor storage characteristics. According to US applied standard, the hemolysis rate of RBCs at the point of transfusion, must be lower than 1%, to avoid transfusing hemolytic products to patients.[9]

The aim of this study was to determine the frequency of thalassemia and Hb variant among blood donors from the Indonesian Red Cross (IRC) Blood Centre Unit in Jakarta. The study was also reviewing the quality of stored blood by hemolytic rate of red blood cells (RBC's) in whole blood (WB) on first day and seven day of storage. This protocol is in accordance with the policy at the IRC Blood Centre Unit in Jakarta that the maximum storage time for WB before they are processed into Packed Red Cell (PRC) is seven days.

METHODS

Thalassemia and Hb variant screening was performed on 138 specimens from blood donors at the IRC Blood Centre Unit in Jakarta. Ethical clearance was obtained from Ethical Committee of Faculty of Medicine, Unversitas Indonesia No. 452/ PT02.FK/ETIK/2012. Blood samples were collected in ethylene diaminetetraacetic acid (EDTA) tubes. All samples were first tested by automated analyzer for complete blood count (CBC), continued by preparation of blood smears. The presence of abnormal Hb and the relative quantity of normal and abnormal Hb were examined by high performance liquid chromatography (HPLC).[10] Moreover, the DNA analysis was used for the detection of α thalassemia carrier using multiplex PCR method (for deletions in one or two α globin gene) and PCR RFLP (for thalassemia α non-deletional mutations, Hb Adana and Hb CS).[3,11]

Examination on quality of blood was performed by hemolytic rate, calculated on first and seven day of storage in CPD-A whole blood (WB). The hemolytic rate was calculated by comparing the supernatant Hb concentration with the total Hb concentration.[9]

Hb and hematocrit (Ht) levels were measured using an automated hematology analyzer. Hemolytic rate calculation is as follows:[9]

$$\text{Hemolytic rate} = \frac{\text{(supernatant Hb (g/L) x (100-Ht)}}{\text{Total Hb (g/L)}}$$

RESULTS

Based on CBC and Hb analysis we detected three donors (2.2%) with β thalassemia carrier, three donors (2.2%) for Hb E carrier, and 39 donors suspected of α thalassemia from 138 donors. One of donors suspected of α thalassemia also had hereditary ovalositosis (Southeast Asian Ovalositosis/ SAO). Donors with suspected α thalassemia trait and α thalassemia silent carrier in this study had concentration of MCH < 25 pg and MCH 25 - 29 pg, respectively. As determination of alpha thalassemia carrier should be confirmed by the DNA analysis, we caried out multiplex PCR for one gene deletion on 39 suspected of thalassemia. We found three donors

carrier for one gene deletion type 3.7 kb ($-\alpha^{3,7kb}$) and two donors carrier for non deletion mutation that produces Hb variant Constant Spring (Hb CS). Subsequently, the hemolytic rate calculation showed that all thalassemia carrier and Hb variant donors have the hemolytic rate on first day and seven day of storage below one percent. Whereas among normal donors, there were six samples (4.35%) with a hemolytic rate in seven day over 1% (Figure 1).

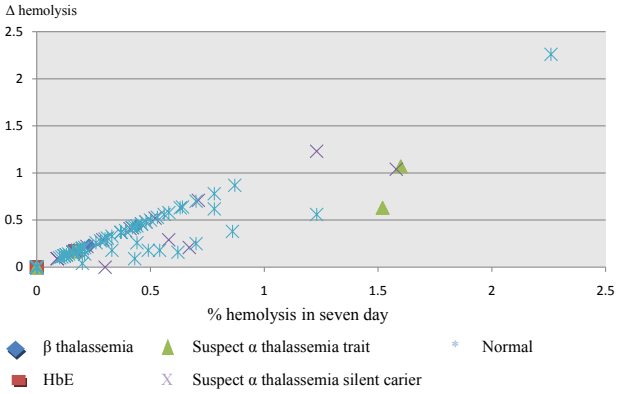

Figure 1. Hemolytic rate in seven days and the difference hemolytic rate between first day and seventh day of storage (Δ hemolysis)

DISCUSSION

Thalassemia screening on general population and pre-marriage thalassemia counseling are the thalassemia prevention program that currently used.[12] However, this program has not been fully implemented in Indonesia. The thalassemia screening in this study was conducted on the blood donor population. The donor selection process with Hb testing is currently implemented in all IRC blood centres to ensure that potential donors are in good health. Therefore, donors with minor red cell abnormalities such as thalassemia carrier and Hb variant are accepted to donate their blood as long as they are in good health and passed the pre-donation Hb test.[5] In this study, there were 11 donors (8%) with thalassemia carrier and Hb variant who passed the pre-donation Hb test and donated their blood, and even some of them have been donating their blood regularly.

Thalassemia and Hb variant screening result with CBC and Hb analysis can directly identify donors with β thalassemia and Hb E. In this study, three donors were detected as carriers of β thalassemia and have the concentration of MCV ≤ 76 fL and MCH ≤ 27 pg. The identification of donors with β thalassemia and Hb E was indicated by the presence of increased levels of hemoglobin A_2 (HbA_2).[13] Previous population studies noted that the concentration of HbA_2 in β thalassemia was 3.5 - 6%.[7] The three suspected carrier of β thalassemia identified in this study had HbA_2 concentration of 4.9 to 5.7%.

HPLC method gives Hb E a similar retention time as HbA_2, thereby the concentration of HbA_2 was found to be greatly increased up to 29.4 ± 2.3%.[13] In this study, three donors identified with HbE have HbA_2 concentration of 27.2 - 28.9%. Meanwhile, the MCV and MCH values were 71.6 - 73.9 fL and 23.2 - 24.1 pg, respectively.

The DNA analysis resulted in three α thalassemia donors with one gene deletion type 3.7 kb ($-\alpha^{3.7kb}$). This deletion is produced by the reciprocal recombination of homologous subsegments of the α globin gene duplication unit.[3] DNA analysis also detected non deletion mutations that produced Hb variant Constant Spring (Hb CS) in two donors suspected of α thalassemia. This mutation changes the stop codon (UAA) to another amino acids (CAA) and allow mRNA translation to continue to the next in-phase termination codon (UAA) located within the polyadenylation signal (AAUAAA) and causing the α chain extended by 31 amino acids from the natural C-terminal arginine (codon 141).[7]

The screening were also resulted in one donor with ovalositosis hereditary (Southeast Asian Ovalocytosis/SAO). SAO is a blood disorders with the morphology of an ovalocyte red blood cell with distinctive dark center line. SAO is caused by abnormalities of the membrane protein band 3 in red blood cells which is the result of a 27 bp deletion in the gene that encodes a protein band 3 (AE1).[14]

An imbalance of globin chain synthesis is the major cause of red cell abnormalities in thalassemia and affects cell survival and deformability. In α and β thalassemia, the relative excess of unpaired β and α globin chains, respectively, result in accumulation of excessive amounts of unpaired globin chains that precipitate, and by different mechanisms, damage the cell membrane and cause red cell premature destruction. Detailed analysis of cellular and membrane deformability characteristics showed that thalassemic red blood cells have excess surface area in relation to cell volume and increased membrane dynamic rigidity. The ability of the RBCs to deform is a very important requirement for these cells to adapt in narrow capillaries *in vivo*.[7] A decrease

in deformability or membrane defect may play a significant role in the spontaneous or storage-induced hemolysis.[15]

Among six samples with hemolysis rate over 1% in seven day of storage, there were two donors with suspected α thalassemia silent carrier, two donors suspected of α thalassemia trait and two normal donors. All of these samples have been confirmed negative for α and β thalassemia, Hb E and SAO. The hemolysis could be caused by several factors including other blood disorders such as G6PD deficiency or inappropriate temperature during blood processing. The hemolytic rate over 1% could also occur on blood from any kind of donors, usually will not affect the color of plasma. However, inappropriate temperature during shipping, storing or processing of blood, can affect the quality of blood, especially to unstable and deformed RBCs membranes from thalassemic donors.[15,16] This condition could occur on four donors with suspected α thalassemia even though they had no evidence of having a common α thalassemia. The possibility of iron deficiency in these four donors could not be proven because we did not measure the iron level of donors in this study.

Blood from donor carrier of thalassemia and Hb variant was theoretically unstable and prone to hemolysis. In this study, the hemolysis rate of blood from thalassemia and Hb variant donors after seven days of storage was below 1%. However, the changes of hemolysis rate *in vivo* if this blood is transfused are not yet known. Therefore, a potential risk of hemolytic complications could still occur, especially if the blood is used for susceptible patients such as neonates that need exchanged transfusion or blood transfusion dependent recipients such as thalassemia, Hb variant or patients with the other blood disorder.

In conclusion, the frequency of thalassemia carrier and Hb variants in blood donors at Red Cross Blood Centre Unit in Jakarta was 8%. The quality of stored blood until seven day of storage was quite good.

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Conflict of interest

The authors affirm no conflict of interest in this study.

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