INTRODUCTION

Thalassemia is a disorder in hemoglobin production which is inherited in a family and first found in the United States as well as Italy at the same time between 1925 and 1927. Thalassemia is a genetic disease passed through generations, from parents to their children, in an autosomal recessive pattern. This disease is caused by hemoglobin synthesis disorder; it decreases the production of one or more globin chain, which causes an imbalance in globin chain production as one of the main component consisting of the hemoglobin.1

Thalassemia has various clinical spectrum, starting from mild (heterozygote), called as minor thalassemia or trait until the most severe symptom (homozygote) called as major thalassemia. Minor thalassemia (heterozygote) is inherited by one of the parents suffering from thalassemia, while both parents suffering from the thalassemia inherits the homozygote form. Clinically, thalassemia is divided into three groups, which are: minor, intermediate and major thalassemia.1,2

Thalassemia is found across the whole world, prominently in a Mediterranean race, Middle-East, India until South East Asia. The World Health Organization reported that around 7% of citizen’s population in the world is a thalassemia carrier and approximately 300,000 until 500,000 of infants were born each year with this disorder. The thalassemia carrier genes are spreading across Mediterranean countries such as Italy, Greece, Malta, Sardinia, and Cyprus with the prevalence of 10-16%, while the prevalence of thalassemia carrier in Asia such as in China, Malaysia, and Indonesia are around 3-10%. The prevalence of thalassemia carrier in North Sumatera, particularly in Medan and its surrounding can reach 7.69%; it consists of alpha thalassemia of 3.35% and beta thalassemia of 4.07%. West Java Province, with the most considerable amount of citizens in Indonesia, has the thalassemia carrier population of 5.5-6%. One of the reasons for a large number of thalassemia cases...
in Indonesia is migration and marriage among race in Indonesia.\textsuperscript{3,4} Beta thalassemia trait (BTT) and iron deficiency anemia (FDA) patients will suffer from a gradual disorder in the hemoglobin synthesis, causing abnormality in the erythrocyte structure then make them hypochromic and microcyclic, and slowly causing the concentration of hemoglobin in the blood will decrease and anemia, both clinically and laboratory. The individual with beta thalassemia trait also has a risk in inheriting the disease to their descendants.\textsuperscript{5,7}

The identification of thalasemia carrier holds a vital role in preventing the inheriting this disease.\textsuperscript{6} It is important to know the diagnostic and epidemiology approach regarding the cause of anemia in thalassemia patient’s family since they have a higher risk in inheriting thalassemia to their descendants.\textsuperscript{3,8,9}

Various value of calculated erythrocyte indices has been formulated to differ between iron deficiency anemia (FDA) and beta-thalassemia trait (BTT). Iron deficiency anemia and beta thalassemia trait are the most frequent cause of hypochromic microcyclic anemia. The use of calculated erythrocyte indices value based on the calculation of several routine hematology parameters as Mentzer, England & Fraser, Srivastava, Shine & Lal Indices is useful to and differences between the iron deficiency anemia and the beta-thalassemia trait. Besides, these test is particularly useful in the region with health facility and laboratory limitation for further tests, such as hemoglobin electrophoresis which becomes the modality in diagnosing beta-thalassemia trait.\textsuperscript{6,10,11} This study is aimed to know the compatibility of Mentzer, England & Fraser, Shine & Lal and Srivastava indices with the hemoglobin electrophoresis in the transfusion-dependent thalassemia patient’s family.

\section*{METHODS}

This is a cross-sectional study, the subject of the study is the transfusion-dependent thalassemia patient’s family, who participated in the screening on 5\textsuperscript{th} of March 2017 in Eijkman Building, held by the partnership among Association of Clinical Pathology Specialist Doctor of West Java, Indonesian Thalassemia Foundation, as well as PT Sysmex Indonesia.

The subject of this study was requested for their inform consent, and have 2cc of their blood taken by the EDTA. The blood samples then examined by Hematology Analyzer KX-21 and continued by the hemoglobin electrophoresis test in the Medical Research Unit of the Faculty of Medicine of Padjadjaran University. The inclusion criteria of the thalassemia patient’s family are the ones with MCV <80 fL while the exclusion criteria were when the patient had a blood transfusion within the last 3 months.

The data resulting from the routine hematology test and hemoglobin electrophoresis after such screening are used as secondary data in this study. Microsoft Excel and SPSS 17.0 process the data of this study in order to obtain descriptive data on the routine hematology test of the subjects, it is followed by the categorical analysis with chi-square to seek for significance, the p-value is used to determine such significance, if p < 0.05 then this study has significant means. The data further analyzed using the compatibility test between the test result of calculated erythrocyte indices and the hemoglobin electrophoresis test result of the subjects. One of the many ways to assess the compatibility is by determining the kappa (k) value. Compatibility value is considered as poor if the value of k < 0.20, weak if the value of k is 0.21-0.40, fair if the value of k is 0.41-0.60, good if the value of k 0.61-0.80, and considered as excellent if the value of k > 0.81.

\section*{RESULTS}

As many as 99 subjects of the study fulfill the inclusion criteria with the characteristic described in Table 1 as follow. From the study, it was found that 58\% of respondents were 15-55 years old group and predominantly by women (55\%).

The data resulting from the routine hematology test in the study subjects further analyzed descriptively and served in Table 2 as follow. From the Table it showed that the average value of hemoglobin was 11.2 ± 1.1 g/dL, following by hematocrit (33.3 ± 4.52\%), and red blood cell (RBC) (5.65 ± 0.76 x 10\(^{12}\)/mm\(^3\)).

Analysis of several calculated erythrocytes indices then calculated to know the amount and percentage of beta-thalassemia trait on the study subjects, as served in Table 3. Based on the Mentzer Indices, the amount of β- Thalassemia Trait was 46.5%, then followed by Shine & Lal Indices.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|}
\hline
\textbf{Variables} & \textbf{Percentage (\%)} \\
\hline
\textbf{Age} & \\
0-14 Years Old & 39 (39\%) \\
15-55 Years Old & 57 (58\%) \\
>56 Years Old & 3 (3\%) \\
\hline
\textbf{Gender} & \\
Men & 45 (45\%) \\
Women & 54 (55\%) \\
\hline
\end{tabular}
\caption{Demographic data of respondents}
\end{table}
The hemoglobin electrophoresis on the study subjects can be seen in Table 4 below. From the Table, it can be seen that most results were normal (28.3%), followed by β-Thalassemia Trait (47.5%), Hb E-Trait (18.2%), and Hb-E (6.1%).

Data of compatibility test result between calculated erythrocyte indices and Hb-electrophoresis on the study subjects were statistically significant (P<0.05).

**DISCUSSION**

Based on Table 3 that the study subjects are mostly women with the age range of 15-55 years old (58%). This productive age is also called a childbearing age, which has the risk to inherit genetic disorder to their descendants; for that reason, they are chosen to participate in the thalassemia screening activity. The result of this study is in line with a similar study conducted by Majeed (2013) in Pakistan, which shows that the largest age group comes from people who are 15-55 years old, as much as 48%.1

The result of this study also shows that the average of hemoglobin is 11.2 ± 1.1 gr/dL and hematocrit is 33.3 ± 4.52% with the average of MCV as much as 68.5 ± 6.9 fl; this shows that the study subjects have minor anemia according to WHO laboratory criteria. The result of this study is not far from the similar study by Ullah (2016) in Pakistan, which obtained that the average of hemoglobin level is 10.7± 1.2 gr/dL and the average of MCV is 67.2 ± 6.2 fl. The most often clinical manifestation of BTT and FDA is microcytic anemia. Several similarities of the two disease are that the decrease of hemoglobin, hematocrit, MCV , MCH level as well as the existence of microcytic and hypochromic picture on the peripheral blood smear.7

Various mathematical formula based on erythrocyte indices have been used to determine the BTT. However there are no single indices that is 100% specific and sensitive enough to filter such condition. The frequency of BTT based on the Mentzer Indices was found in 46.5% of study subjects, England & Fraser Indices was found in 55.6% study subjects, Srivastava Indices was found in 55.6%, Shine & Lal Indices was found in 54.5% study subjects. The result of hemoglobin electrophoresis on the study subjects shows that there were 28.3% of subjects obtained a normal result, 18.2% obtained Hb E-trait, 6.1% obtained HbE, and 47.5% of subjects obtained beta-thalassemia trait. The study from Majeed (2013) in Pakistan shows the BTT case as much as 52% based on hemoglobin electrophoresis.1 This is not far from the result of this study which shows that there are around 44.4%-55.6% cases of BTT among the study subjects based on the indices calculation, and 47.5% based on the hemoglobin electrophoresis.13 Various studies disclosed that the percentage of BTT in thalassemia patient’s family is quite high, around 50-60%. Such a high frequency of BTT can be caused by the inheritance of genetic disorder that constructs the globin.13-14 The iron status test was not conducted in this study. Hence we cannot exclude the possibility of comorbidities among the BTT and ADB study subjects.

### Table 2 Description of Routine Hematology Test Result in the Study Subjects

| Parameter   | Range     | Average ± SD | Unit  |
|-------------|-----------|--------------|-------|
| Hemoglobin  | 10.1-12.9 | 11.2 ± 1.1   | g/dL  |
| Hematocrit  | 27.0-36.1 | 33.3 ± 4.52  | %     |
| RBC         | 3.17-7.67 | 5.65 ± 0.76  | x 10^6/mm^3 |
| MCV         | 52.6-79.8 | 68.5 ± 6.9   | fl    |
| MCH         | 15.6-34.8 | 22.2 ± 3.4   | Pg    |
| MCHC        | 28.0-38.6 | 32.73 ± 3.56 | g/dL  |

### Table 3 Percentage of Beta Thalassemia Trait on Study Subjects

| Calculated Erythrocyte Index | Range             | Amount (% of β-Thalassemia Trait) |
|------------------------------|-------------------|-----------------------------------|
| Mentzer Indices             | 7.18-17.94        | 46 (46.5%)                        |
| England & Fraser Indices    | -18.57-21.13      | 55 (55.6%)                        |
| Srivastava Indices          | 2.44-7.3          | 55 (55.6%)                        |
| Shine & Lal Indices         | 431.61-1985.0     | 54 (54.5%)                        |

### Table 4 The Hemoglobin Electrophoresis Result on the Study Subjects

| Hb Electrophoresis Result | Amount (%) |
|---------------------------|------------|
| Normal                    | 28 (28.3%) |
| Hb E-Trait                | 18 (18.2%) |
| Hb-E                      | 6 (6.1%)   |
| β-Thalassemia Trait       | 47 (47.5%) |

### Table 5 Compatibility test between calculated erythrocyte indices and Hb-electrophoresis on the study subjects

| Erythrocyte Indices | Kappa value | p-value |
|---------------------|-------------|---------|
| Mentzer Indices with Hb Electrophoresis | 0.663 | 0.0001 |
| England & Fraser Indices with Hb Electrophoresis | 0.636 | 0.002 |
| Srivastava Indices with Hb Electrophoresis | 0.558 | 0.018 |
| Shine & Lal Indices with Hb Electrophoresis | 0.527 | 0.015 |
The compatibility test between Mentzer indices and the hemoglobin electrophoresis shows the kappa value of as much 0.663 with the p-value of 0.0002, this means there is a good and significant compatibility, while the compatibility of England & Fraser indices with the Electrophoresis was 0.636 with p-value of 0.001, which also shows good and significant compatibility, compatibility between Srivastava indices with Hb electrophoresis were 0.558 with p-value of 0.018, which means it has fair and significant compatibility, and the compatibility between Shine & Lal indices and the hemoglobin electrophoresis were 0.527 with p-value of 0.015, which means it has fair and significant compatibility. Based on the prior study form Batebi (2012) in Iran, there was no erythrocyte indices which has 100% sensitivity, the Mentzer indices has 94.5% sensitivity and 93.7% specificity, the Shine & Lal indices has 86.4% sensitivity and 98.9% specificity, the England & Fraser indices has 96% sensitivity and 88.2% specificity, and the Srivastava indices has 92.4% sensitivity and 90.2% specificity. The result of England & Fraser and Mentzer indices in this study have the highest sensitivity, according to this study whereas the two indices have a higher value of compatibility.

The calculated erythrocyte indices test was only conducted based on several parameter calculations on the routine hematology test; it is different with hemoglobin electrophoresis that can analyze the fractions in hemoglobin, which means it can support the diagnose of beta-thalassemia trait, hence if it is compared, there might be incompatibility of a result. The possibility of iron deficiency can exist at the same time with BTT, and it is not superseded at the iron status test in such patient. Based on this study, the use of such indices can be utilized as a tool to screen beta-thalassemia trait, since it has good and fair compatibility with the hemoglobin electrophoresis. The possibility of other incompatibilities can also occur due to the influence of the clinical condition of the study subjects at the routine hematology test, such as infection or other inflammation that can cause anemia. The result of this study has several limitations, which are: other clinical data of the study subjects cannot be traced, as well as there was no Fe status test conducted in order to supersede the possibility of Fe deficiency, particularly the ones happening at the same time with the beta-thalassemia trait.

CONCLUSION
There is good and significant compatibility between the Mentzer indices and England & Fraser with the hemoglobin electrophoresis, as well as fair and significant compatibility between the Shine & Lal indices and Srivastava with the hemoglobin electrophoresis. Such calculated erythrocyte indices can screen thalassemia, particularly in the risk group (thalassemia patient’s family with anemia or MCV decrease).

ETHICAL CLEARANCE
This research had been approved by the Ethics Committee prior to a study conducted.

CONFLICT OF INTEREST STATEMENT
The authors declare that there was no conflict of interest in this research.

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AUTHOR CONTRIBUTION
All authors have contributed to all process in this research, preparation, drafting, review, and approval of this manuscript.

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