Letter to the Editor

Effectiveness of Using Mean Corpuscular Volume and Mean Corpuscular Hemoglobin for Beta-thalassemia Carrier Screening in the Guangdong Population of China*

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Beta (β)-thalassemia is one of the most common hemoglobinopathies worldwide, creating major public health problems and social burdens in many regions. Screening for β-thalassemia carriers is crucial for controlling this condition. To investigate the effectiveness of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for screening β-thalassemia, retrospective data were analyzed for 6,779 β-thalassemia carriers subjected to genetic testing following thalassemia screening in Guangdong province between January 2018 and December 2019. Prevalent mutations observed included CD41/42 (-TTCT) (38.43%), IVS-II-654 (C > T) (25.71%), -28 (A > G) (15.78%), CD17 (AAG > TAG) (10.03%), and β^E (GAG > AAG) (3.13%). In the β^0, β^+, and HbE groups, MCV values were 63.8 ± 4.2 fL, 67.0 ± 5.5 fL, and 75.8 ± 5.6 fL, while MCH values were 20.1 ± 1.4 pg, 21.2 ± 1.9 pg, and 24.8 ± 2.0 pg, respectively. Among β-thalassemia carriers, 85 (1.25%) and 28 (0.41%) individuals had MCV ≥ 80 fL and MCH ≥ 27 pg, respectively. Using a combination of MCV and MCH reduced the number of false negative screenings to 15 (0.22%). Therefore, evaluating both MCV and MCH is strongly recommended for screening β-thalassemia carriers.

Key words: Beta-thalassemia; Mean corpuscular volume; Mean corpuscular hemoglobin; Screening; Southern China

Beta (β)-thalassemia is one of the most common hemoglobinopathies worldwide. It is caused by mutations or deletions in the β-globin (*HBB*) gene (OMIM:141900)[1], which result in the reduction or absence of the β-globin chain, thus affecting oxygen transportation in the blood. Cases of β-thalassemia can be classified into two general types based on β-globin chain production: β^0-thalassemia, defined by a total absence of normal β-globin chains, and β^+-thalassemia, with a partial reduction in β-chain synthesis. In general, carriers of β^+-thalassemia mutations have milder hematological abnormalities than those with β^0-thalassemia mutations. The hemoglobin variants (HbVar) represent another form of β-globin gene defects that manifest primarily as mild anemia, while HbE and HbS are the most common β-hemoglobinopathies across the world.

Approximately 1.5% of the world population is estimated to carry the β-thalassemia *HBB* gene. The highest prevalence of β-thalassemia occurs in the Mediterranean region, the so-called "thalassemia belt"[2]. Although thalassemias occur at particularly high frequencies in tropical regions, they have spread worldwide due to migration, creating a major public health problem and social burden in many regions[3]. Thalassemias are reportedly highly prevalent in southern China, particularly in the provinces of Yunnan, Guangdong, Guangxi, Fujian, and Sichuan. A meta-analysis of 16 articles published between 1981 and 2015 indicated that the overall prevalence of α-, β-, and α + β-thalassemia in China was 7.88%, 2.21%, and 0.48%, respectively[4].

The spectrum and prevalence of thalassemias vary in populations from different regions; over 900 different β-globin gene mutations have been discovered worldwide. Genes for β-thalassemia/hemoglobinopathies are inherited in an autosomal recessive manner. Heterozygous carriers of β-thalassemia are asymptomatic or may experience mild anemia, whereas those with homozygous thalassemia and compound heterozygous β-thalassemia (β-thalassemia major or transfusion-dependent β-thalassemia) experience severe anemia requiring lifelong blood transfusions[5], imposing

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considerable financial burdens on their families and society. Therefore, screening and identification of β-thalassemia carriers are important for thalassemia prevention and control.

Carrier screening and prenatal diagnosis have been widely performed in China to prevent the birth of children with homozygous α^0- and β-thalassemias. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are the most commonly used indicators for β-thalassemia screening, with low values exhibited by β-thalassemia carriers. Those with MCV ≥ 80 fL and MCH ≥ 27 pg are considered to be at low risk and do not require further DNA analysis. However, a significant proportion of false negatives may occur in a large-scale population screening of β-thalassemia because several types of β-thalassemia carriers with normal MCV and MCH values have been identified[6].

To determine the effectiveness of MCV and MCH indicators for β-thalassemia screening, we analyzed retrospective data from 1,237,465 subjects who participated in the thalassemia prevention and control program in Guangdong province, China, between January 2018 and December 2019. The participants ranged from 20 to 54 years old. Collected information included nationality, ID number, sex, and full name to provide a comprehensive description of the study subjects. Informed consent forms were signed or thumb-printed by the participants. Only apparently healthy women (i.e., with an absence of clinical signs and symptoms of disease) were included in the study. Individuals with certain conditions, such as chronic diseases or inflammation, and currently taking iron tablets over the course of the past 3 months, were excluded. All studies were approved by the Ethics Committee of Family Planning Research Institute of Guangdong Province.

Hematological testing was performed at the district (county) family planning service stations of Guangdong province. Peripheral blood samples (2 mL) anti-coagulated with 4.0 mg of K2-EDTA were obtained from each subject. Hematological parameters, including MCV and MCH, were determined *via* standard laboratory procedures using automated blood cell analyzers (Mindray Blood Analyzer; BC5180, BC1900, and BC3000Plus, Shenzhen Mindray, Inc., Shenzhen, China). Subjects with MCV ≥ 80 fL and/or MCH ≥ 27 pg were regarded as noncarriers of β-thalassemia. When one member of a couple had a positive result of MCV < 80 fL and/or MCH < 27 pg, both individuals underwent thalassemia genetic testing.

Thalassemia genetic testing of 24,794 subjects was performed by Guangzhou KingMed Diagnostics Group Co., Ltd. Genomic DNA of suspected carriers detected by thalassemia screening was isolated from peripheral blood leukocytes using TIANamp Genomic DNA kits (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. A total of 17 β-globin mutations frequently observed in the Chinese population were detected by PCR-reverse dot blot (RDB) assays using the β-thalassemia gene detection kit (Yaneng Bioscience Co., Ltd., Shenzhen, China), including $\beta^{CD41/42\ (-TTCT)}$, $\beta^{CD43\ (GAG > TAG)}$, $\beta^{IVS\text{-}II\text{-}654\ (C > T)}$, $\beta^{-28\ (A > G)}$, $\beta^{-29\ (A > G)}$, $\beta^{-32\ (C > A)}$, $\beta^{CD71/72\ (+A)}$, $\beta^{E\ (GAG > AAG)}$, $\beta^{CD17\ (AAG > TAG)}$, $\beta^{CD31\ (-C)}$, $\beta^{CD14/15\ (+G)}$, $\beta^{CD27/28\ (+C)}$, $\beta^{IVS\text{-}I\text{-}1\ (G > T)}$, $\beta^{IVS\text{-}I\text{-}5\ (G > C)}$, $\beta^{CAP\ (-AAAC)}$, $\beta^{Initiation\ CD\ (ATG > AGG)}$, and $\beta^{-30\ (T > C)}$.

The IBM SPSS Statistics v.26 software (IBM Corp., Armonk, NY, USA) was used to perform statistical analyses. Differences in mean values were assessed using *t*-tests, and $P < 0.05$ was considered statistically significant.

Thalassemia genetic screening was carried out in 24,794 subjects, of which 6,779 (27.34%) were diagnosed with β-thalassemia gene abnormalities, including 16 different mutations identified by RDB methods (Table 1). Among the mutations, CD41/42 (-TTCT) (38.43%) was the most frequent, followed by IVS-II-654 (C > T) (25.71%), −28 (A > G) (15.78%), CD17 (AAG > TAG) (10.03%), and β^E (GAG > AAG) (3.13%). The total number of subjects with any of these five mutations accounted for more than 93% of β-thalassemia carriers in the Guangdong province.

The mutation spectrum of β-thalassemia varies in different geographic areas and races, and regional differences may play a significant role. For example, IVSII-654 (C > T) is a common mutation in China, but is not very common in Southeast Asian countries, except in the Chinese Malaysian population[4]. In the present study, CD41-42 (-TTCT) was the most frequent β-thalassemia mutation. This observation is in agreement with results from Southern China, although the exact ratios differed[7]. This finding contrasted with results from a study conducted in the Fujian province, where IVS-II-654 (C > T) was the most prevalent β-thalassemia mutation[8]. The mutation spectrum distribution across different geographical locations also reflected the heterogeneity of β-thalassemia.

MCV and MCH values were compared among carriers in the β^0, β^+, and HbE groups. The MCV values were 63.8 ± 4.2 fL, 67.0 ± 5.5 fL, and 75.8 ± 5.6 fL, whereas MCH values were 20.1 ± 1.4 pg, 21.2 ± 1.9 pg, and 24.8 ± 2.0 pg, respectively (Table 2).

Comparing β^0 and β^+groups, β^0 and HbE groups, and β^+ and HbE groups revealed significant differences ($P < 0.05$).

Effective carrier screening and accurate diagnosis are important steps in the prevention and control of thalassemias. Values of MCV < 80 fL and MCH < 27 pg are typically used as criteria for thalassemia screening. Although most β-thalassemia carriers exhibited MCV < 80 fL and MCH < 27 pg, several forms of β-thalassemia may have normal MCV and MCH values. For example, carriers with β^E-thalassemia (GAG > AAG) are clinically asymptomatic and exhibit almost normal erythrocyte indices in heterozygotes. In a study by Sirichotiyakul et al.[9], MCV testing was recommended as a suitable tool for screening β-thalassemia in pregnant women, but β^E-thalassemia was excluded because MCV has a low sensitivity for it (37.7%). There were 15 β-thalassemia carriers with MCV ≥ 80 fL and MCH ≥ 27 pg in the current study, of which 10 subjects presented with HbE (Table 3). These people may not have been considered high risk in the screening program and were not obliged to undergo further genetic analysis. Unfortunately, if the spouse is also a β-thalassemia carrier, the risk of having children with HbE/β-thalassemia is 25% in every pregnancy. These results are consistent with those reported in a study by Mirzakhani et al.[10], in which it was noted

Table 1. Frequency of different types of β-thalassemia mutations identified in this study

β-thal mutation	HGVS name	*N*	Proportion (%)	Type	Total
CD41/42 (-TTCT)	HBB:c.124_127delTTCT	2,605	38.43		
CD17 (AAG > TAG)	HBB:c.52A > T	680	10.03		
CD71-72 (+A)	HBB:c.216_217insA	172	2.54		
CD27/28 (+C)	HBB:c.84_85insC	74	1.09		
CD43 (G > T)	HBB:c.130G > T	62	0.91	β^0	3,684
IVS-I-1 (G > T)	HBB:c.92+1G > T	45	0.66		
CD14/15 (+G)	HBB:c.45_46insGa	35	0.52		
Initiation Condon (ATG > AGG)	HBB:c.2T > G	10	0.15		
CD31(-C)	HBB:c.94delC	1	0.01		
IVS-II-654 (C > T)	HBB:c.316-197C > T	1,743	25.71		
−28 (A > G)	HBB:c.-78A > G	1,070	15.78		
−29 (A > G)	HBB:c.-79A > G	60	0.89		
Cap (-AAAC)	HBB:c.-11_-8delAAAC	6	0.09	β^+	2,883
IVS-I-5 (G > C)	HBB:c.92+5G > C	2	0.03		
−32 (C > A)	HBB:c.-82C > A	2	0.03		
−30 (T > C)	HBB:c.-80T > C	0	0		
β^E (GAG > AAG)	HBB:c.79G > A	212	3.13	HbE	212
Total			100.00		6,779

Note. Abbreviations: β^0, production of normal β-globin chains is completely absent; β^+, production of normal β-globin chains is partially reduced; HGVS, Human Genome Variation Society; HBB, hemoglobin β-globin.

Table 2. Comparison of MCV and MCH values among β^0, β^+, and HbE groups

Variables	β^0 (*n* = 3,684) Mean ± SD	β^+ (*n* = 2,883) Mean ± SD	HbE (*n* = 212) Mean ± SD	β^0 *vs*. β^+ *P*-value	β^0 *vs*. HbVar *P*-value	β^+ *vs*. HbE *P*-value
MCV (fL)	63.8 ± 4.2	67.0 ± 5.5	75.8 ± 5.6	< 0.05	< 0.05	< 0.05
MCH (pg)	20.1 ± 1.4	21.2 ± 1.9	24.8 ± 2.0	< 0.05	< 0.05	< 0.05

Note. Significance level: $P < 0.05$.

that some carriers are not considered in every screening program. Our results indicated that such false negatives during β-thalassemia screening occurred when using MCV < 80 fL and MCH < 27 pg as the cutoff values.

Among the 6,779 β-thalassemia carriers, over 98% exhibited MCV < 80 fL and MCH < 27 pg, 85 (1.25%) subjects had MCV ≥ 80 fL, whereas only 28 (0.41%) had MCH ≥ 27 pg (Table 3), indicating that MCH values generated fewer false negatives than MCV values (P < 0.05). Therefore, this finding confirmed that MCH was a better screening marker for β-thalassemia than MCV. MCH is also more stable than MCV during blood specimen storage[11]. This characteristic is particularly important in regions where blood samples must be obtained in remote areas. When the two indicators, MCV and MCH, were used together as a combined screening tool, the number of false negatives was reduced from 85 (MCV used alone) and 28 (MCH used alone) to 15. In addition, 99.8% of β -thalassemia carriers were detected. The present results confirmed that a combined MCV and MCH test for β-thalassemia screening is highly recommended to minimize the number of false negatives.

Of the 85 β-thalassemia carriers with MCV ≥ 80 fL, 36 were identified as β^+-thalassemia carriers using DNA analysis, accounting for 1.25% of all β^+-thalassemia carriers, while 42 were genetically identified as HbE carriers (19.81% of all HbE carriers). The remaining seven subjects (0.19%) were identified as β^0-thalassemia carriers *via* DNA analysis (Table 3), including CD41/42 (-TTCT) (n = 5) and CD17 (A > T) (n = 2).

Among the 28 β-thalassemia carriers with MCH ≥ 27 pg, 18 subjects were identified as HbE carriers *via* DNA analysis, accounting for 8.49% of all HbE carriers. Seven individuals were genetically identified as β^+-thalassemia carriers (0.24% of all β^+-thalassemia carriers). The remaining three subjects (0.08%) were identified as β^0-thal carriers *via* DNA analysis (Table 3), including CD41/42 (-TTCT) (n = 1), CD17 (A > T) (n = 1), and CD43 (G > T) (n = 1).

When MCV and MCH were combined as a screening tool, the number of false negative results was reduced to 15 (0.22%), which included 10 HbE carriers (4.72% of all HbE carriers) and five β^+-thalassemia carriers (0.17% of all β^+-thalassemia carriers).

A combined MCV and MCH test can identify most of the thalassemia carriers. However, iron deficiency, which has similar blood characteristics, could not be excluded. Thus, hemoglobin analysis, dichlorophenolindophenol, and red cell indices, such as red blood cell count, red cell distribution width, reticulocyte hemoglobin, and formulas derived from these indices, which are more specific and efficient in the initial discrimination of β-thalassemia traits from iron deficiency, should be used in future studies[12].

In conclusion, our study demonstrated the heterogeneity and spectrum of β-thalassemia mutations in the Guangdong population. The reported findings confirmed that MCH generated fewer false negatives and is therefore a better screening marker for β-thalassemia than MCV. Using a combination of MCV and MCH for β-thalassemia screening is highly recommended to reduce the chance of obtaining inaccurate results. Although the combined use of MCV and MCH has many advantages, such as low cost and ease of measurement, the poor ability to identify HbE hemoglobinopathies is one of the drawbacks of using this index as the only criterion for β-thalassemia screening.

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Table 3. Comparison of false negative subjects during β-thalassemia screening

Variables	Total	MCV ≥ 80 (fl)		MCH ≥ 27 (pg)		MCV ≥ 80 (fl) and MCH ≥ 27 (pg)	
		N	Percentage (%)	*N*	Percentage (%)	*N*	Percentage (%)
β^0-thalassemia	3,684	7	0.19	3	0.08	0	0
β^+-thalassemia	2,883	36	1.25	7	0.24	5	0.17
HbE	212	42	19.81	18	8.49	10	4.72
Total	6,779	85	1.25	28	0.41	15	0.22

Note. Abbreviations: *N*, number; %, proportion of the total number.

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