

Original Article

Thalassemia gene detection and the prenatal diagnosis outcomes of pregnant women in Guiyang City

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Abstract: Objective: To study thalassemia gene detection and to analyze the prenatal diagnoses of pregnant women at the Guiyang Maternity and Child Health Care Hospital and Guiyang Children's Hospital. Methods: Prenatal diagnostic genetic testing was performed on pregnant women with anemia (n = 2,306) at Guiyang Maternity and Child Health Care Hospital and Guiyang Children's Hospital. For each positive patient whose spouse also agreed to undergo thalassemia genetic testing, genetic testing in the amniotic fluid for prenatal diagnosis was carried out as their offspring were more likely to have intermediate or severe thalassemia. Results: Among the 2,306 samples, there were 213 positive cases, including 114 cases of α -thalassemia, 85 cases of β -thalassemia and 14 cases of α/β -thalassemia. $-^{SEA}/\alpha$ and $-\alpha^{3.7}/\alpha$ were the most common mutation types of the α -thalassemia gene in pregnant women, while β^{41-42}/β^N and $\beta^{IVS-II-654}/\beta^N$ were the most common mutation types of the β -thalassemia gene. Genetic testing in the amniotic fluid was performed for 82 couples with high-risk gene combinations. Sixty-four cases were positive, and 18 cases were completely normal. Conclusion: Genetic testing for thalassemia and early prenatal diagnosis can effectively avoid the birth of infants with intermediate and severe thalassemia by choosing to terminate the pregnancy, which is of great significance for the improvement of eugenics and postnatal care.

Keywords: Pregnant women, thalassemia, gene detection, prenatal diagnosis

Introduction

Anemia in pregnant women has a higher incidence in developing countries than in developed countries, and it occurs in over 60% of pregnant women in China [1]. Thalassemia anemia, a common genetic disease in clinical practice, is very common in Guangxi, Guangdong, and Hainan in China [2]. Thalassemia is classified into three types, namely, α -thalassemia, β -thalassemia and α/β -thalassemia. In addition, based on the clinical manifestations and outcomes of genetic testing, it can be divided into the minor, intermediate, or major clinical types [3, 4]. Fetuses may be born with thalassemia due to this genetic disease. Although children with thalassemia minor and intermediate need no treatment since they have no obvious anemia symptoms, those with thalassemia major require long-term blood transfusions and iron chelation therapy, treatment that places a burden on society and on the family [5, 6]. At present, premarital screening and prenatal

diagnosis are the main early diagnostic and preventive measures to determine the potential conditions of children [7, 8]. Therefore, in this study, thalassemia screening and prenatal diagnosis were carried out for pregnant women at the Guiyang Maternity and Child Health Care Hospital and Guiyang Children's Hospital in order to provide further eugenic guidance.

Materials and methods

Patients

This study was approved by the Ethics Committee of Guiyang Maternity and Child Health Care Hospital and Guiyang Children's Hospital. Informed consents were signed by the patients included in this study. From May 2014 to May 2020, pregnant women with anemia (n = 2,306) receiving prenatal diagnosis in Guiyang Maternity and Child Health Care Hospital and Guiyang Children's Hospital were recruited as the study cohort. These pregnant women of

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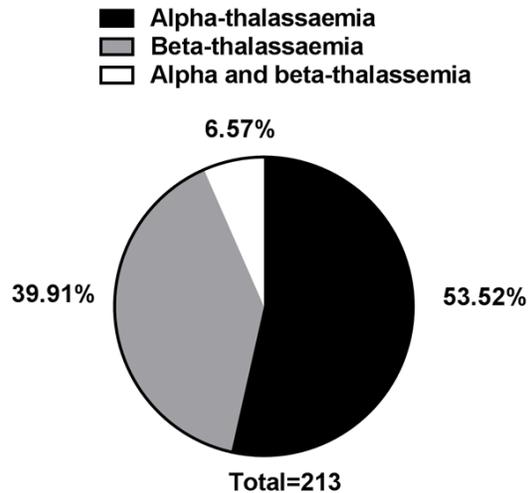


Figure 1. Proportional distribution of the different types of thalassemia.

Han ethnicity ranged from 20-43 years old, with an average age of 36.8 ± 7.3 years. They had been pregnant 1-3 times and were within the gestational range of 17-22 weeks (19.3 ± 3.6 weeks on average). Pregnant women with iron deficiency anemia were excluded from the study.

Methods

According to the "Guidelines for Thalassemia Prevention and Control Programme", 5 mL of venous blood was collected from each patient at 8:00 am on the testing day, and the collected blood samples were stored in a sterile anticoagulant tube (ml017974, Shanghai Enzyme Technology Co., Ltd., China) and treated with ethylenediaminetetraacetic acid. Venous blood (2-3 mL) was collected into the EDTA-2K anticoagulation tube to test the blood routine. Patients whose mean corpuscular volume (MCV) was less than 80 fl and/or whose mean corpuscular hemoglobin (MCH) was less than 27 pg were considered positive and in need of further genetic testing for thalassemia. For each positive genetic testing patient her spouse was also asked to undergo thalassemia genetic testing. Based on the outcome of each couple's genetic testing, an amniotic membrane puncture was performed if their offspring were likely to have intermediate or major thalassemia.

There were two methods of genetic screening. For the first method, venous blood was collected from the couple for polymerase chain reac-

tion (PCR) testing combined with membrane hybridization to detect the α -thalassaemia and β -thalassaemia genes. The kits were purchased from Promega Corporation. For the second method, transabdominal amniocentesis was performed under ultrasound guidance. Amniotic fluid (20 mL) was extracted and centrifuged to collect the amniotic fluid cells. After the cell resuspension, DNA was released and purified by adsorption, rinsing, and elution in a spin column. DNA kits (AR303-01, Beijing Tiangen Biochemical Technology Co., Ltd., China) were used for the genetic testing.

Statistical analysis

SPSS 22.0 statistical software was used, and the count data were expressed as a rate (%).

Results

Distribution of the different types of thalassemia in pregnant women

The preliminary thalassemia screening tests found 752 positive patients, accounting for 32.61% of the total (752/2,306). Through further genetic testing, 213 of these positive patients were diagnosed with thalassemia (28.32%, 213/752), including 114 cases of α -thalassaemia, 85 cases of β -thalassaemia and 14 cases of α/β -thalassaemia. See **Figure 1**.

Analysis of the mutation types of the α -thalassaemia gene in the pregnant women

We found that $^{-SEA}/\alpha\alpha$ and $^{-\alpha^{3.7}}/\alpha\alpha$ were the most common α -thalassaemia gene mutation types in the pregnant women. See **Table 1**.

Analysis of the mutation types of the β -thalassaemia gene in the pregnant women

We found that β^{41-42}/β^N and $\beta^{IVS-II-654}/\beta^N$ were the most common β -thalassaemia gene mutation types in the pregnant women. See **Table 2**.

Analysis of the mutation types of the α/β -thalassaemia gene in the pregnant women

We found that the most common mutation type in the α/β -thalassaemia gene was $^{-SEA}/\alpha\alpha$ composite $\beta^{CD41-42}/\beta^N$. See **Table 3**.

Genetic testing in the amniotic fluid

For the 213 positive genetic testing patients, their spouses also agreed to undergo thalassemia genetic testing. Among them, the fetus-

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Table 1. Analysis of the mutation types of the α -thalassemia gene in pregnant women

Type of α -thalassemia gene mutation	Cases (n)	Percentage (%)
$_{-SEA}/\alpha \alpha$	73	64.04
$-\alpha^{3.7}/\alpha \alpha$	24	21.05
$-\alpha^{4.2}/\alpha \alpha$	8	7.02
$-\alpha^{CS}/\alpha \alpha$	3	2.63
$-\alpha^{3.7}/-\alpha^{4.2}$	3	2.63
$-\alpha^{3.7}/_{-SEA}$	2	1.75
$-\alpha^{3.7}/-\alpha^{CS}$	1	0.88
Total	114	100

Table 2. Mutation types of the β -thalassemia gene in pregnant women

Type of β -thalassemia gene mutation	Cases (n)	Percentage (%)
β^{41-42}/β^N	33	39.28
β^{-17}/β^N	12	14.28
$\beta^{IVS-II-654}/\beta^N$	25	29.76
β^{-28}/β^N	4	4.76
β^E/β^N	3	3.57
β^{-90}/β^N	1	2.38
Chinese G $\gamma + (A \gamma \delta \beta) O/\beta^N$	2	3.57
β^{27-28}/β^N	1	1.19
β^{71-72}/β^N	1	1.19
B^{-29}/β^N	1	1.19
β^{14-15}/β^N	1	1.19
Total	85	100

Table 3. Mutation types of the α/β -thalassemia gene

Type of α -thalassemia gene mutation	Type of β -thalassemia gene mutation	Cases (n)	Percentage (%)
$_{-SEA}/\alpha \alpha$	β^{41-42}/β^N	3	21.42
	$\beta^{IVS-II-654}/\beta^N$	2	14.28
	β^{-28}/β^N	2	14.28
	β^{-17}/β^N	1	7.14
	β^E/β^N	1	7.14
$-\alpha^{3.7}/\alpha \alpha$	$\beta^{IVS-II-654}/\beta^N$	1	7.14
	β^{41-42}/β^N	1	7.14
	β^{71-72}/β^N	1	7.14
$-\alpha^{4.2}/\alpha \alpha$	β^{-17}/β^N	1	7.14
	β^{41-42}/β^N	1	7.14
Total		14	100

es of 82 couples were estimated to have intermediate or severe thalassemia after we analyzed the gene combinations. There were 64 positive cases and 18 negative cases according to the genetic testing in the amniotic fluid in

those pregnant women. 39 cases of the α -thalassemia gene mutation, 42 cases of the β -thalassemia gene mutation, and 1 case of the $\alpha\beta$ -thalassemia gene mutation were observed in the mothers, and 38 cases of the α -thalassemia gene mutation, 43 cases of the β -thalassemia gene mutation, and 1 case of the $\alpha\beta$ -thalassemia gene mutation were found in the fathers. Genetic testing in the amniotic fluid showed that there were 39 cases of α -thalassemia gene mutations, 42 cases of β -thalassemia gene mutations, and 1 case of $\alpha\beta$ -thalassemia gene mutation. See **Tables 4 and 5.**

Pregnancy outcomes

Informed of the amniotic fluid outcome, 29 pregnant women with intermediate, severe, and composite thalassemia voluntarily chose to terminate their pregnancies after full consultation and consideration. Eighteen normal cases and 35 pregnant women with minor thalassemia chose to continue their pregnancies. Follow-up and neonatal thalassemia genotypes were then carried out after delivery. The testing results were consistent with the prenatal diagnoses.

Discussion

Thalassemia, one of the most common genetic diseases in the world, has been included in genetic screening. Significant regional differences in the distribution of thalassemia have been observed in China, although it's not the region of highest incidence. A meta-analysis revealed that the rates of α -thalassemia, β -thalassemia and α/β -thalassemia in China were 7.88%, 2.21%, and 0.48%, respectively [2]. And surveys on various regions have found that the incidence rates in Guangdong,

Guangxi, and Hainan are higher than they are in other regions [9, 10]. Therefore, the early screening of pregnant women can effectively help to prevent the births of infants with thalassemia.

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Table 4. Analysis of the mutation types of the genes of 82 couples using genetic testing of the amniotic fluid

Type of gene mutation	Mother	Father
$_{-SEA}/\alpha \alpha$	34	22
$-\alpha^{3.7}/\alpha \alpha$	2	11
$-\alpha^{4.2}/\alpha \alpha$	0	1
$-\alpha^{CS}/\alpha \alpha$	2	2
$-\alpha^{3.7}/-\alpha^{CS}$	1	0
$-\alpha^{3.7}/_{-SEA}$	0	1
$-\alpha^{WS}/\alpha \alpha$	0	1
β^{41-42}/β^N	12	18
β^{-17}/β^N	23	16
$\beta^{IVS-II-654}/\beta^N$	3	7
β^{-28}/β^N	1	0
β^{-90}/β^N	1	0
Chinese G γ + (A γ δ β) O/ β^N	2	0
β^{71-72}/β^N	0	1
$_{-SEA}/\alpha \alpha$ composite $\beta^{IVS-II-119}$, β^{-17}/β^N	0	1
$_{-SEA}/\alpha \alpha$ composite β^{41-42}/β^N	1	0
Total	82	82

Table 5. Analysis of the outcomes of the amniotic fluid genetic testing in the 82 couples

Type of anemia	Genotype	Cases (n)	Percentage (%)
Normal	$\alpha \alpha/\alpha \alpha$	9	10.98
	β^N/β^N	9	10.98
Minor	$-\alpha^{3.7}/\alpha \alpha$	2	2.44
	β^{-17}/β^N	7	8.54
	$_{-SEA}/\alpha \alpha$	12	14.63
	β^{41-42}/β^N	12	14.63
	$\beta^{IVS-654}/\beta^N$	1	1.22
	$\beta^{IVS-II-16}/\beta^N$	1	1.22
Intermedia	$_{-SEA}/\alpha^{4.2}$	1	1.22
	$_{-SEA}/\alpha^{3.7}$	3	3.66
	$_{-SEA}/\alpha^{CS}$	3	3.66
	$_{-SEA}/\alpha^{WS}$	1	1.22
Major	$_{-SEA}/_{-SEA}$	7	8.54
	$\beta^{41-42}/\beta^{-17}$	5	6.10
	$\beta^{41-42}/\beta^{41-42}$	2	2.44
	Chinese G γ + (A γ δ β) O/ $\beta^{IVS-II-654}$	2	2.44
	β^{-17}/β^{-17}	3	3.66
Composite	$\alpha^{3.7}/\alpha^{CS}$	1	1.22
	$_{-SEA}/\alpha \alpha$ composite $\beta^{IVS-II-119}$	1	1.22
Total		82	100

According to the preliminary screening carried out in this study, 752 cases were positive,

accounting for 32.61% of the total (752/2,306). There were 213 cases of thalassemia gene carriers (9.24%, 213/2,306), among which, 144 cases of α -thalassemia (4.94%, 114/2,306), 85 cases of β -thalassemia (3.69%, 85/2,306), and 14 cases of α/β -thalassemia (0.61%, 14/2,306) were found. The above results were higher than the average levels in China, which may be related to the inconsistency of the initial screening standards of different research units and the inconsistency of the disease occurrence in different regions.

The preliminary screening of the pregnant women carrying pathogenic genes found that the proportion of α -thalassemia was higher than β -thalassemia and α/β -thalassemia. Previous studies demonstrated that, the numbers of pregnant women with α -thalassemia and β -thalassemia were 801 and 498 in Guangdong and were 290 and 115 in Guizhou, which agrees with the results of this study [11, 12]. For the α -thalassemia genotype, this study found that $_{-SEA}/\alpha \alpha$ (64.04%) and $-\alpha^{3.7}/\alpha \alpha$ (21.05%) were the most common, which is consistent with the results from a study done in Wuhan ($_{-SEA}/\alpha \alpha$ (78.75%) and $-\alpha^{3.7}/\alpha \alpha$ (15.00%)) [13], but contrary to the results from a study done in Guizhou ($_{-SEA}/\alpha \alpha$ (26.62%) and $-\alpha^{3.7}/\alpha \alpha$ (49.49%)) [12]. For the β -thalassemia genotype, β^{41-42}/β^N (39.28%), $\beta^{IVS-II-654}/\beta^N$ (29.76%) and β^{-17}/β^N (14.28%) were the most common, which is similar to the results from a study done in Wuhan, accounting for 20.30%, 43.61% and 19.55%, respectively [13].

In this study, the spouses also agreed to undergo thalassemia genetic testing if their wife was diagnosed as positive in the genetic testing. Gene paring was adopted to observe any genetic combinations of intermediate to severe thalassemia. And genetic testing in the amniotic fluid was carried out for 82 couples with high-risk gene combinations.

There were 64 positive cases, including 8 cases of the intermediate type, 13 cases of the severe type, and 1 case of the composite type, for a prevalence rate of 0.95% (22/2,306). In a prenatal diagnosis study from Guangxi, 129 pregnant women were diagnosed with the intermediate or severe type through amniotic fluid genetic testing, for a prevalence rate of 3.22% (129/4,000) [14], which is consistent with the results of this study.

Most pregnant women carrying thalassemia genes are often mild or quiescent, so confirming their spouses' genes is an essential part of the prenatal diagnosis, as it can effectively avoid the birth of infants with intermediate to severe thalassemia and reduce the burden on society and families [15, 16]. With the improvement of genetic diagnostic technology, genetic testing in amniotic fluid has high accuracy and low trauma [17, 18]. In areas with a high incidence of thalassemia such as Pakistan, large-scale screening and genetic testing in the amniotic fluid make it possible to find children with severe β -thalassemia earlier, and pregnant women can choose to terminate their pregnancies in advance, greatly reducing the incidence of severe β -thalassemia [19]. Therefore, the publicity of thalassemia genetic testing has been greatly enhanced, and even some legislation has been launched to draw the attention of pregnant women and their families to prenatal diagnosis. Also, medical expenses subsidies and the training of rural medical personnel are now carried out [20, 21]. However, prenatal diagnosis still needs further popularization in China.

This was a single-center and retrospective study with a limited sample size. Therefore, a large multi-center study should be further conducted to clarify the prevalence of thalassemia in pregnant women in order to confirm the significance and accuracy of prenatal diagnosis.

In summary, genetic testing for thalassemia and early prenatal diagnosis can effectively prevent the birth of infants with intermediate and severe thalassemia, which will significantly improve eugenics and postnatal care.

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Disclosure of conflict of interest

None.

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