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Early screening of thalassemia in pregnant women in northern China by capillary electrophoresis for the determination of hemoglobin electrophoresis

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ABSTRACT

The objective of this study was to analyze the effectiveness of capillary electrophoresis detection of hemoglobin electrophoresis (HE) for the early screening of thalassemia. In the first choice, 974 pregnant women were selected for capillary electrophoresis to detect HE, which showed that 46 of them were abnormal (4.72%), including 16 cases with HbA2<2.5% and 28 cases with HbA2>3.5% and/or HbF≥2.0%. In one case each of HbH and HbBart's abnormal bands was found. The genotype test results showed the presence of thalassemia in 34 cases, using the genotype test results as the gold standard, after calculation it was seen that capillary electrophoresis for HE diagnosis of the occurrence of thalassemia had a sensitivity and specificity of 54.34% and 70.97% (P<0.05). These results suggest that in the screening of thalassemia in northern China, capillary electrophoresis for HE has good application and can be used as one of the routine screening tools, but further confirmation by genotype testing is still needed.

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Introduction

Thalassemia, the most common type of hereditary chronic hemolytic anemia in clinical practice today, is likewise a focus of concern in pregnant women (1). Surveys have shown that the number of pregnant women carrying the potential gene for thalassemia is currently about 10-15% of all pregnant women, and the prevalence of thalassemia is more pronounced in some Middle Eastern, Indian, and Southeast Asian countries (2). As there is no effective treatment for thalassemia, the symptoms of thalassemia can only be relieved by blood transfusion, which imposes a great burden on the patient's family (3, 4). Therefore, timely assessment and prevention of thalassemia during pregnancy can greatly limit the further development of thalassemia (5). The disadvantages of genotypic testing as the gold standard for the diagnosis of thalassemia are its high cost and the relatively long time taken for the test, leading to some limitations in its promotion (6, 7).

For thalassemia, hemoglobin electrophoresis (HE) is of great importance, but traditional methods of detecting abnormal hemoglobin can lead to certain errors in the test results (8), and some pregnancy complications (such as gestational diabetes, gestational hypertension, etc.) affect the missed screening of HE and reduce the diagnostic compliance rate of geodystrophy (9). Therefore, there is an urgent clinical interest in finding a protocol that is more suitable for screening pregnant women for the development of thalassemia. And with the development of technology, there are more accurate methods for HE detection in clinical settings, Such as capillary electrophoresis, etc (10). Among them, capillary electrophoresis is an HE analysis technique developed in recent years, mainly using capillary as the separation channel and high-voltage DC electric field as the driving force, which has the advantages of fast detection and high precision (11).

Thus, this study will analyze the clinical effectiveness of capillary electrophoresis for HE assessment of thalassemia, thus providing a more reliable guarantee for the safety of maternal and neonatal life in future pregnancies.

Materials and Methods

Research subjects

A total of 974 cases of pregnant women who underwent maternity checkups in our hospital from August 2019 to December 2022 were selected for retrospective analysis. The mean age was (28.93±3.98) years, 706 cases had no history of pregnancy and 268 cases had a history of pregnancy. All mothers were examined for HE. The study was approved by the ethics committee of our hospital, and all study subjects signed an informed consent form by themselves.

Inclusion and exclusion criteria

Inclusion criteria: aged 20-35 years; singleton, full-term, cephalic primigravida; complete medical records. Exclusion criteria: those who have a history of smoking,

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alcohol, and drug use; nervousness; Those with autoimmune defects, other cardiovascular and cerebrovascular diseases. Rejection criteria: request for withdrawal during the test; those who provide false information.

Methods

All mothers maintained a normal diet and exercise for 3 d prior to testing and consumed food containing at least 150 g of carbohydrates. Fasting for 10-12 h before the test, peripheral blood samples were collected early the next morning for the test. HE was detected using a fully automated capillary electrophoresis instrument and its supporting reagents. The HE electrophoretic bands of patients were detected according to the standard operating procedures, and the spurious bands appearing in the electrophoretic bands were excluded, and the detection value of each HE band was calculated.

Genetic testing

Whole blood DNA was extracted by whole blood genomic DNA extraction kit, and three common deletion types of α-thalassemia genes, -SEA/ααα, -α3.7/αα, -4.2/αα, were detected by the spanning PCR technique (Gap-PCR). Detection of non-deficient α-thalassemia and β-thalassemia genes: Using reverse speckle hybridization, three α-thalassemia mutations were detected as wild type and mutant, including Hb CS, Hb WS, and Hb QS; 17 β-thalassemia point mutations were detected by RDB method, including -28, -29, -30, -32, CD14-15, CD17, CD26 (βE), CD27-28, CD31, CD41-42, CD43, CD71-72, IVS-I-1, IVS-I-5, IVS-II-654, CAP+1, and initiation condon. The operation procedure was carried out strictly according to the kit instructions.

Diagnostic performance calculation

Genotype identification is the gold standard and HE is the observation protocol (12). The gold standard and the observation program were both judged to be true positive for thalassemia; the gold standard and the observation program were both judged to be true negative for non-thalassemia. The gold standard was determined as thalassemia and the observation program was determined as non-thalassemia as false negative; the gold standard was determined as non-thalassemia and the observation program was determined as thalassemia as false positive. Sensitivity = true positive/ (true positive + false negative) ×100%. Specificity = true negative/ (true negative + false positive) ×100%. Accuracy = (true positive + true negative)/total number×100%.

Statistical analysis

SPSS 22.0 software was used for statistical analysis and counting data such as family history and place of residence was expressed as (%), The measurement data such as age were expressed as ($\chi\pm s$). Statistical significance was declared if the P value was < 0.05. The consistency of the two diagnostic methods was tested by McNemar's test, and the consistency was evaluated by the Kappa coefficient, with Kappa≥0.75, 0.4<Kappa<0.75, and Kappa≤0.4 suggesting good, medium, and poor consistency, respectively.

Results

Basic information of the study subjects

The subjects included in this study were on average (28.93±3.98) years old, mostly urban population. 706 cases had no history of pregnancy, 268 cases had a history of pregnancy and only 10 patients had a family history of geodeprivation. The distribution was more even in terms of dietary preferences and exercise habits, and the sleep status was more normal (Table 1).

Diagnostic value of HE detection by capillary electrophoresis for thalassemia

Among the 974 patients, 46 cases (4.72%) had abnormal HE electrophoresis detection. Among them, there were

Table 1. Basic information about the study subjects.

	n	Percentage (%)
Age	28.93±3.98	-
Family history of illness		
have	10	1.03
none	964	98.97
Place of residence		
city	684	70.23
rural	290	29.77
Exercise Habits		
have	509	52.26
none	465	47.74
Dietary preferences		
light food	581	59.65
heavy taste food	393	40.35
Sleep state		
good	642	65.91
not good	332	34.09
Pregnancy history		
have	268	27.52
none	706	72.48

16 cases with HbA2<2.5% and 28 cases with HbA2>3.5% and/or HbF≥2.0%. 1 case each had abnormal bands of HbH and HbBart's.

Genotype identification results

Genotype identification manifested that a total of 34 cases of pregnant women had thalassemia, including 21 cases of α-thalassemia and 13 cases of β-thalassemia. Most of the α-thalassemia were α-thalassemia gene SEA deletion heterozygotes, followed by α-thalassemia gene 3.7 deletion heterozygotes. In the case of β-thalassemia, they were mostly heterozygous for mutations at the 41/42 locus of the β-thalassemia gene, followed by heterozygous for mutations at the 28 locus of the β-thalassemia gene (Table 2).

Diagnostic accuracy of capillary electrophoresis for the detection of HE in the diagnosis of geodystrophy

The diagnostic sensitivity of the capillary electrophoresis was 54.34%, the specificity was 70.97% and the accuracy was 61.04% compared to the genotype identification results (Table 3). The results were generally consistent with the genotype test (Kappa=0.704, P<0.05), which had a more excellent diagnostic efficacy.

Discussion

Severe forms such as α-thalassemia can result in stillbirths and enlarged fetuses, while β-thalassemia patients tend to have growth arrest and require lifelong blood transfusions and iron removal therapy (13, 14). Therefore, prenatal screening for thalassemia is an essential part of pregnancy and delivery (15, 16).

As detected by capillary electrophoresis, 46 patients with thalassemia were present in 974 pregnant women, including 16 cases with HbA2<2.5% and 28 cases with HbA2>3.5% and/or HbF≥2.0%. Compared with previous studies (17-19), the number of thalassemia was significantly lower in this study, which is presumably related to the current regional differences in the incidence of thalassemia in China. In a previous epidemiological survey about thalassemia in a northern Chinese city, we found the incidence of thalassemia to be below 1% (20, 21). In the present study, a certain increase in the incidence of thalassemia was observed, which is presumed to be caused by population migration from the north to the south in recent years. Subsequently, 34 of 46 maternal cases of pregnant women were identified as thalassemia by genotype identification. Among them, 20 cases were α- and 13 cases were β- thalassemia. Its prevalence is consistent with previous epidemiological studies of thalassemia (17). By comparing the results with the genotype test, we found that the diagnostic sensitivity, specificity and accuracy of capillary electrophoresis detection of HE for thalassemia were 54.34%, 70.97% and 61.04%, respectively, with good agreement (Kappa=0.704), which indicates that capillary electrophoresis for HE can also be used as an early screening protocol for thalassemia. However, during capillary electrophoresis examination, there are many stray bands in addition to the desired HE band, and these stray bands can affect the electropherogram, even to the extent that the A_2 band is shifted (22). In this case, the bands need to be manually trimmed, and the influence of subjective consciousness and testing experience of human intervention is large. It is easy to ignore some slightly abnormal bands, thus causing errors in the judgment of the zones and resulting in lower diagnostic accuracy of α-thalassemia (8). And this is the main drawback of capillary electrophoresis. If this problem can be solved in the future, it may be more significant to improve the early diagnosis of thalassemia by capillary electrophoresis. The advantage of capillary electrophoresis is that it can accurately identify various normal and abnormal HBs and distinguish between bands such as HbA, HbA2, HbF, HbH, HbBart's, and HbCS (23). In several studies, cellular and molecular aspects of pregnant women have been reported (24-27).

The accuracy of capillary electrophoresis in screening for abnormal HB is as high as 80-90% (28), which also

Table 2. Genotype identification results.

	n	Percentage (%)
α-thalassemia gene 3.7 deletion heterozygotes	4	11.76
α-thalassemia gene 4.2 deletion heterozygotes	1	2.94
α-thalassemia gene SEA deletion heterozygotes	15	44.12
α-thalassemia gene SEA deletion heterozygotes with β-thalassemia gene 654 deletion heterozygotes	1	2.94
β-thalassemia gene 28 deletion heterozygotes	3	8.82
β-thalassemia gene 17, 41/42 deletion heterozygotes	1	2.94
β-thalassemia gene 27/28, 654 deletion heterozygotes	1	2.94
β-thalassemia gene 41/42 deletion heterozygotes	5	14.71
β-thalassemia gene 654 deletion heterozygotes	2	5.88
β-thalassemia gene 71/72 deletion heterozygotes	1	2.94

Table 3. Diagnostic value of capillary electrophoresis in the diagnosis of thalassemia.

		Capillary electrophoresis		Total	Kappa	P
		(+)	(-)			
HE	Strips abnormalities	25	9	34	0.704	0.028
	Strips normal	21	22	43		
	Total	46	31			

demonstrates the extremely excellent results of capillary electrophoresis for the detection of abnormal HB disease. This is of paramount importance for maternal screening for HB. Although capillary electrophoresis examination has some limitations for the diagnosis of thalassemia to be improved, it can accurately assess HB abnormalities, which is extremely reliable and safe for the early assessment of thalassemia in the case of pregnant women. We believe that in the future, HE examination by capillary electrophoresis can be used to initially identify pregnant women who may have thalassemia, and then further examination can be performed to improve the diagnosis rate of thalassemia.

Nevertheless, the present study has many limitations. For example, the test results of capillary electrophoresis, which we mentioned above, may be influenced by operational experience. Hence, we also need to start training for test personnel on capillary electrophoresis operation to improve the accuracy of the test. Besides, we should subsequently add the assessment of the diagnostic efficacy of combined capillary electrophoresis and blood cell testing for the early development of thalassemia in pregnant women. There is also a need to expand the sample size of the study to obtain more comprehensive results for clinical reference.

The incidence of thalassemia in northern China has increased in recent years, and capillary electrophoresis for HE has a good initial diagnostic effect on the occurrence of thalassemia in pregnant women. In the future, the early assessment of thalassemia by capillary electrophoresis HE test and further genotype testing of pregnant women with abnormal results can improve the early detection rate of thalassemia and protect maternal and fetal health and life safety.

Conflict interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

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