Implementation of Newborn Screening for Hemoglobin H Disease in Mainland China

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Abstract Hemoglobin H disease is the most severe non-fatal form of α -thalassemia syndrome characterized by pronounced microcytic hypochromic hemolytic anemia. It is predominantly seen in Southeast Asia, the Middle East and the Mediterranean. Studies suggest that hemoglobin H disease is not as benign a disorder as previously thought. Newborn screening for hemoglobin H disease is especially appealing because the screening test is based on the detection of hemoglobin Bart's (γ_4) that is only possible within the newborn period. In this study, we reported on a 4-year period of newborn screening program at a mainland Chinese hospital, which detected 35 babies with hemoglobin H disease in a total of 26 152 newborns. The overall prevalence for hemoglobin H disease among all newborns in southern China is ~ 1 in 1,000. These children need appropriate follow-up and potential comprehensive care during their growth and development.

Keywords α -Thalassemia · Hemoglobin H disease · Newborn screening

Introduction

Hemoglobin H disease is the most severe non-fatal form of α -thalassemia syndrome characterized by pronounced

microcytic hypochromic hemolytic anemia. It is predominantly seen in Southeast Asia, the Middle East and the Mediterranean [1]. Clinical features of patients with hemoglobin H disease are highly variable and generally develop in the first years of life but may not develop until adulthood in some patients [2]. Studies suggest that hemoglobin H disease is not as benign a disorder as previously thought [3]. It can bring about growth retardation during childhood and iron overload in adults even in the absence of transfusion, leading to hepatic, cardiac, and endocrine dysfunction.

Unlike Hb Bart's hydrops fetalis and β -thalassemia major, hemoglobin H disease is usually not the target disorder in prenatal screening and control programs in countries with a high prevalence of thalassemia. One reason is that most individuals with hemoglobin H disease can live long and healthy lives. Hemoglobin H disease alone is not an indication for prenatal diagnosis. Another reason is that there is no effective strategy for prenatal control of hemoglobin H disease. The cutoff of $MCV < 80$ fL is commonly used as the first step in thalassemia screening. Although this cutoff can almost screen all carriers of α^0 -thalassemia, it is not able to detect around 75 % of subjects carrying α^+ -thalassemia [4]. Most of these carriers usually have normal hematologic parameters and lack the hallmarks of microcytosis and hypochromia in thalassemia trait. This means that detection of α^+ -thalassemia is ineffective using red blood cell parameters. In other words, hemoglobin H disease would be overlooked during prenatal screening for thalassemia.

Therefore, newborn screening, instead of prenatal screening, is currently the main approach to identify hemoglobin H disease in many regions. In this study, we reported our experience and results of newborn screening for hemoglobin H disease in mainland China.

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Fig. 1 Hemoglobin analysis of a normal newborn (a) and a newborn with hemoglobin H disease (b)

Material and Method

During the period from January 2010 to December 2013 a newborn screening program for hemoglobin H disease was conducted at Guangzhou Maternal and Neonatal Hospital, Guangdong, China. After obtaining informed consent from the parents, the blood samples of the newborns were collected and sent for the screening. Shortly after the birth of babies, the umbilical cord is clamped and cut. Two ml blood was obtained by syringing out through the umbilical vein detached. The blood samples in EDTA were kept at 4 °C until analysis within 24 h.

Hemoglobin analysis was performed at the Thalassemia Screening Laboratory using an automated capillary electrophoresis system (the Capillarys 2) (Sebia, Paris, France). The Sebia Capillarys 2 system, software version 6.2, uses the principle of capillary electrophoresis in which charged molecules are separated at alkaline pH by their electrophoretic mobility, electrolyte pH and electroosmotic flow. Each peak of hemoglobin components appears in a specific

zone and the relative concentration of variants can be determined automatically (Fig. 1).

At birth, hemoglobin composition in the normal newborns consists of ~70–80 % hemoglobin F ($\alpha_2\gamma_2$) and 20–30 % hemoglobin A ($\alpha_2\beta_2$). The absence or reduced synthesis of α -globin chain can lead to the production of abnormal hemoglobin Bart's (γ_4) in the fetus. The Bart's was automatically positioned with regard to the hemoglobin A fraction in zone 12. According to a retrospective study of our primary screening program [5], in which newborns with hemoglobin H disease had a mean Bart's value of 20.2 % (range 17.5–22.5 %), hemoglobin Bart's levels above 10 % were used as a cut-off range for hemoglobin H disease diagnosis in newborns.

If a sample was screened positive, it would be subjected a DNA analysis for the confirmation of α -thalassemia. Genomic DNA was extracted from cord blood using phenol and salting out protocols. Gap-polymerase chain reaction (PCR) was used to detect the four common deletion α -thalassemia defects: Southeast-Asian ($-\alpha^{SEA}$), Thailand ($-\alpha^{THAI}$), 3.7 kb ($-\alpha^{3.7}$) and 4.2 kb ($-\alpha^{4.2}$) deletions [6]; the reverse dot-blot hybridization was used to detect the two nondeletional α -thalassemias: hemoglobin Constant Spring and hemoglobin Quong Sze [7]. Otherwise, direct DNA sequencing was performed to identify rare point mutations of α -globin gene using the ABI PRISMTM 310 genetic analyzer. Ethical approval was obtained from the Biomedical Research Ethics Committee at the Guangzhou Women and Children's Medical Center.

Results

During the study period, a total of 26 152 newborns were screened for hemoglobin H disease. The automated capillary electrophoresis detected various amounts of hemoglobin Bart's in 1,389 (5.3 %) samples; among these, 35 had a Hb Bart's level above 10 %, and were designated as hemoglobin H disease. Deletional hemoglobin H disease was confirmed in all of these cases (Table 1). The prevalence rate of deletional hemoglobin H disease was 0.1 % in this population. All infants with hemoglobin H disease were normal at birth, and all of their mothers had an uneventful pregnancy.

The average levels of hemoglobin Bart's were 20.9 ± 3.8 % (range 15.6–32.3 %) in newborns with hemoglobin H disease. Most of these cases had a very small amount of hemoglobin H (0.4 ± 0.2 %) (range 0–1.0 %) in the cord blood. The average levels of hemoglobin Bart's, F, A and A₂ were (1.75 ± 0.6 %) (range 0.1–8.6 %), (75 ± 9.6 %) (range 71.0–86.2 %), (23.1 ± 5.2 %) (range 12.3–27.1 %) and (0.4 ± 0.1 %) (range 0–1.1 %), respectively, in the remaining 1,354 newborns who had detectable <10 % Bart's.

Table 1 Hemoglobin patterns in cord blood of 35 newborns with deletional hemoglobin H disease

Case	Hb H (%)	Hb Bart's (%)	Hb A (%)	Hb F (%)	Hb A ₂ (%)	Genotype
1	0.5	18.6	22.8	57.3	0	–SEA/– $\alpha^{4.2}$
2	0.2	21.8	19.0	57.9	0.3	–SEA/– $\alpha^{3.7}$
3	0.2	18.4	29.6	51.5	0.3	–SEA/– $\alpha^{3.7}$
4	0.5	21.5	35.5	41.9	0	–SEA/– $\alpha^{3.7}$
5	0.5	23.1	30.6	44.8	0	–SEA/– $\alpha^{4.2}$
6	0.3	17.3	20.8	60.5	0.3	–SEA/– $\alpha^{3.7}$
7	0.3	17.8	35.6	45.6	0	–SEA/– $\alpha^{3.7}$
8	0.6	19.2	30.3	48.8	0.1	–SEA/– $\alpha^{3.7}$
9	0.5	16.6	21.0	61.9	0	–SEA/– $\alpha^{3.7}$
10	0.5	20.9	15.4	61.7	0.5	–SEA/– $\alpha^{4.2}$
11	0	15.9	25.4	58.5	0.2	–SEA/– $\alpha^{3.7}$
12	0.4	20.0	35.4	43.2	0.2	–SEA/– $\alpha^{4.2}$
13	1.0	18.1	20.0	59.5	0	–SEA/– $\alpha^{4.2}$
14	0.2	20.8	30.1	48.1	0	–SEA/– $\alpha^{3.7}$
15	0.4	17.5	29.2	52.2	0	–SEA/– $\alpha^{3.7}$
16	0.6	32.3	12.6	53.3	0.4	–SEA/– $\alpha^{3.7}$
17	0.8	15.6	24.1	57.6	0	–SEA/– $\alpha^{3.7}$
18	0	17.8	34.4	46.9	0.1	–SEA/– $\alpha^{3.7}$
19	0.4	19.1	26.7	53.1	0	–SEA/– $\alpha^{4.2}$
20	0.4	29.4	35.5	33.8	0.2	–SEA/– $\alpha^{3.7}$
21	0.6	21.6	21.3	55.4	0.2	–SEA/– $\alpha^{3.7}$
22	0.3	21.1	27.3	50.3	0.2	–SEA/– $\alpha^{3.7}$
23	0.5	22.4	17.8	58.3	0	–SEA/– $\alpha^{3.7}$
24	0	26.2	20.2	53.0	0	–SEA/– $\alpha^{3.7}$
25	0.4	18.2	20.8	60.0	0	–SEA/– $\alpha^{3.7}$
26	0.4	21.3	26.2	51.2	0.1	–SEA/– $\alpha^{4.2}$
27	0.6	22.5	24.1	52.0	0	–SEA/– $\alpha^{4.2}$
28	0.5	23.3	24.8	51.4	0	–SEA/– $\alpha^{3.7}$
29	0.5	20.6	32.1	45.7	0.1	–SEA/– $\alpha^{3.7}$
30	0	21.7	26.3	51.9	0.1	–SEA/– $\alpha^{3.7}$
31	0.6	18.6	37.8	42.1	0	–SEA/– $\alpha^{3.7}$
32	0.6	29.9	37.8	30.7	0.2	–SEA/– $\alpha^{3.7}$
33	0	19.3	14.3	65.6	0.1	–SEA/– $\alpha^{4.2}$
34	0.6	22.4	35.7	40.4	0.1	–SEA/– $\alpha^{3.7}$
35	0.4	22.3	29.9	46.6	0	–SEA/– $\alpha^{3.7}$

The average levels of hemoglobin F, A and A₂ were (77 ± 9.2 %) (range 56.0–88.2 %), (20.1 ± 4.7 %) (range 11.3–43.2 %) and (0.4 ± 0.1 %) (range 0–1.3 %), respectively, in the 24 763 neonates who tested clearly negative for Bart's. No nondeletional hemoglobin H disease or hemoglobin Bart's hydrops fetalis was found in newborns during this study.

The 70 parents of the 35 newborns with hemoglobin H disease were invited to participate in this study. Their hematological parameters and genotype of the α -globin genes were characterized. Thirty-five parents with

α^0 -thalassemia of the 70 family members had the classical hematological figures of microcytosis and hypochromia. Of the remaining 35 parents with α^+ -thalassemia, 2 coinherited β -thalassemia and had a microcytic hypochromic phenotype, and 33 were the pure α^+ -thalassemia carriers and presented a normal red blood cell indices.

The parents of the 35 newborns with hemoglobin H disease were referred to Hematology Center at our hospital, where this disorder was discussed with the specialists and detailed guidelines for follow-up care for the children were given.

Discussion

Hemoglobin H disease will not cause a child to get sick more frequently than other children. However, illnesses may last longer or make a child more sick than others. These affected individuals generally have a persistent stable state of anemia, which may be accentuated by increased hemolysis during viral infections and by exposure to oxidant medications, chemicals and some kinds of foods. Also, during times of illness, they may require a blood transfusion. Some children with hemoglobin H have other complications, including gallstones and a larger than expected spleen [8]. Therefore, these children need to be followed more closely by their physicians.

The purpose of newborn screening is to test or examine for the presence of a disease before symptoms develop so that an intervention that alters the natural history of the disease might be instituted before significant morbidity or early mortality occurs. This is especially appealing for hemoglobin H disease because the screening test is based on the detection of hemoglobin Bart's which is only possible within the newborn period, before γ -globin production switches to β -globin [9]. The presence of hemoglobin Bart's on the newborn screen almost always indicates that one or more of the baby's α -globin genes are deleted. A recent excellent study of the natural history of hemoglobin H disease, particularly during infancy and childhood, has shown that all forms of this disease are manageable and there is a decreased rate of morbidity if diagnosed at birth [10]. These results make a strong case for newborn screening for hemoglobin H disease, especially in areas with high prevalence of α -thalassemia [11].

A hospital-based program for prenatal screening for α - and β -thalassemia has been widely carried out in mainland China [12]. Initially, only α^0 -thalassemia and β -thalassemia trait were the focus of screening. A couple at-risk of conceiving an offspring with hemoglobin Bart's hydrops fetalis or β -thalassemia major were offered genetic counseling services and prenatal diagnose. Later, an investigation for common nondeletional α -thalassemias has been

considered a step in the flow-chart for detection of couples at-risk for α -thalassemia in prenatal screening program. Because of a relatively high frequency of hemoglobin Constant Spring and hemoglobin Quong Sze in Chinese population [13], these two nondeletional alleles were the target ones, especially in individuals whose partners carried α^0 -thalassemia. These were the reasons why only deletion hemoglobin H disease was detected, and no non-deletional hemoglobin H disease or hemoglobin Bart's hydrops fetalis was encountered in newborns in this study.

The prevalence rate of α -thalassemia is high in Guangdong province, south China, with a carrier rate of 4.1 % for α^0 -thalassemia and a similar rate for the total of two common α^+ -thalassemias ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) [14]. The number of births annually in this province is approximately one million. Based on this data, it can be calculated that the number of pregnancies at risk for hemoglobin H disease is $0.041 \times 0.041 \times 1,000,000 = 1,681$ per year. The number of babies born with this disease should be one-fourth of the pregnancies at risk, i.e. 420 annually for hemoglobin H disease. Because it was not a priority in thalassemia prevention and control programs, hemoglobin H disease had seldom been attached great importance in China. Our newborn screening program may have significant clinical implications. It provides not only early diagnosis of hemoglobinopathies but also early intervention. Increased education of primary care providers and families regarding these disorders is an important part of testing and follow-up. Most individuals with hemoglobin H disease can lead relatively normal lives with proper treatment.

Newborn screening for hemoglobin H disease is now being done at many centers. For example, the state of California in the United States mandates newborn screening and follow-up for thalassemic disorders as well as sickle cell disease. This state has incorporated testing for hemoglobinopathies into its existing programs for metabolic and other inherited disorders. However, newborn screening for thalassemia is not mandatory in our local province, and has not been covered by the traditional newborn metabolic screening system. This screening test is selected at birth by the parents after informed consent is obtained. Indeed, the number of babies screened for the hemoglobin H disease only accounted for half of the total newborns in our hospital. In this study, we used an automated capillary electrophoresis system to determine the hemoglobin Bart's levels. Indeed, the Sebia Capillarys 2 system is a sensitive device for the detection of hemoglobin Bart's in cord blood, as also evidenced in other studies [15, 16]. High performance liquid chromatography (HPLC), however, is the traditional common technique currently employed in the majority of laboratories. HPLC is effective in neonatal screening for hemoglobinopathies [17–20]. There were studies comparing diagnostic performance between the Sebia Capillarys 2

system and HPLC, and found that both techniques were suitable for the identification and quantification of both common and unusual hemoglobin variants, producing comparable results [21, 22].

In conclusion, we first operated a newborn screening program for hemoglobin H disease in mainland China. This led to an identification of 35 newborns with hemoglobin H disease at birth. These children need appropriate follow-up and potential comprehensive care during their growth and development. However, like any population screening programs, newborn screening also implicates important concerns about individual autonomy and the interest of the patients. Considering the heterogeneity of hemoglobin H disease, this screening program can be effective and beneficial to some children, while can be ineffective or even harmful to others. When there is such a potential benefit, parents should decide whether their children will undergo testing.

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