BLOOD RESEARCH
VOLUME 54 · NUMBER 1
March 2019
REVIEW ARTICLE

Recent progress in laboratory diagnosis of thalassemia and hemoglobinopathy: a study by the Korean Red Blood Cell Disorder Working Party of the Korean Society of Hematology

Young Kyung Lee[1], Hee-Jin Kim[2], Kyunghoon Lee[3], Sang Hyuk Park[4], Sang Hoon Song[5], Moon-Woo Seong[5], Myungshin Kim[6], Jin Yeong Han[7]

[1]Department of Laboratory Medicine, Hallym University College of Medicine, Anyang, [2]Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Department of Laboratory Medicine, [3]Seoul National University Bundang Hospital, Seongnam, [4]University of Ulsan College of Medicine, Ulsan University Hospital, Ulsan, [5]Seoul National University Hospital and College of Medicine, [6]College of Medicine, The Catholic University of Korea, Seoul, [7]Dong-A University College of Medicine, Busan, Korea

p-ISSN 2287-979X / e-ISSN 2288-0011
https://doi.org/10.5045/br.2019.54.1.17
Blood Res 2019;54:17-22.

Received on December 28, 2018
Accepted on January 3, 2019

Correspondence to
Jin Yeong Han, M.D., Ph.D.
Department of Laboratory Medicine,
Dong-A University College of Medicine, 26 Daesingongwon-ro, Seo-gu, Busan 49201, Korea
E-mail: jyhan@dau.ac.kr

Abstract

Genetic hemoglobin disorders are caused by mutations and/or deletions in the α-globin or β-globin genes. Thalassemia is caused by quantitative defects and hemoglobinopathies by structural defect of hemoglobin. The incidence of thalassemia and hemoglobinopathy is increased in Korea with rapid influx of people from endemic areas. Thus, the awareness of the disease is needed. α-thalassemias are caused by deletions in α-globin gene, while β-thalassemias are associated with decreased synthesis of β-globin due to β-globin gene mutations. Hemoglobinopathies involve structural defects in hemoglobin due to altered amino acid sequence in the α- or β-globin chains. When the patient is suspected with thalassemia/hemoglobinopathy from abnormal complete blood count findings and/or family history, the next step is detecting hemoglobin abnormality using electrophoresis methods including high performance liquid chromatography and mass spectrometry. The development of novel molecular genetic technologies, such as massively parallel sequencing, facilitates a more precise molecular diagnosis of thalassemia/hemoglobinopathy. Moreover, prenatal diagnosis using genetic testing enables the prevention of thalassemia birth and pregnancy complications. We aimed to review the spectrum and classification of thalassemia/hemoglobinopathy diseases and the diagnostic strategies including screening tests, molecular genetic tests, and prenatal diagnosis.

Key Words Thalassemia, Hemoglobinopathies, Anemia, Diagnosis, Genetic testing

INTRODUCTION

Red blood cells (RBCs) are biconcave disk-shaped cells without nuclei and are the most common cells found in the blood. RBCs deliver oxygen to peripheral tissues and thus play a critical role in maintaining the life of organisms. The oxygen delivery is carried out by hemoglobin molecules in the cytoplasm of RBCs, which are highly specialized machinery that bind, carry, and release oxygen. A hemoglobin molecule consists of 4 globin polypeptide chains (2α and 2β), each harboring a heme molecule inside that binds with oxygen. The quantitative balance among the globin chains and their structural stability are important to the hemoglobin molecules and RBCs. When the genes encoding the globin chains (*HBA* for α-globins and *HBB* for β-globins) have defects (pathogenic variants) leading to the changes in the quantity or structure of globins, hemolytic anemia occurs. Thalassemia is the most common form of hemoglobinopathy in humans and is caused by pathogenic variants leading to the defective synthesis of protein, thus resulting in the quantitative imbalance in globin chains [1, 2]. Other pathogenic variants that result in structural abnormalities in globin chains cause hemoglobinopathies such as sickle cell disease [3].

The diagnosis of thalassemia/hemoglobinopathy begins with suspicion of the disease in anemic patients based on phenotype, family history, and relevant laboratory screening

test results. Molecular genetic confirmation by detecting pathogenic variants establishes the diagnosis. In the past, molecular genetic diagnosis was complicated by the genetic heterogeneity of the disease and the mutations that were missed by conventional sequence analyses. Recently, however, the introduction of novel molecular genetic technologies such as dosage mutation tests to detect large deletion/duplication mutations and multiple gene panel tests by massively parallel sequencing facilitated a more precise molecular diagnosis of hereditary hemolytic anemia and a better understanding of the genetic/genomic mechanisms of the disease [4, 5].

We aimed to review the spectrum and classification of thalassemia/hemoglobinopathy diseases and the diagnostic strategies including screening tests, molecular genetic tests, and prenatal diagnosis, sharing the data obtained from Korea.

CLASSIFICATION

Hemoglobinopathies are genetic hemoglobin disorders caused by mutations and/or deletions in the α-globulin or β-globulin genes. These are divided into 2 main categories: thalassemias and structural hemoglobin variants. The synthesis defects of hemoglobin chains cause thalassemias, classified as α- or β-thalassemia depending on the involved α- or β-globin chain, respectively. Structural hemoglobin variants including those involved in sickle cell disease, hemoglobin C (HbC) disease, and hemoglobin E (HbE) disease are produced by gene defects that change the hemoglobin structure. In addition, there are many mixed forms that combine the features of both thalassemia and structural variants (Table 1) [6, 7].

α-thalassemias almost result from partial deletions (α^+) or complete deletions (α^0) of α-globin gene. Individuals with gene more deletions present more severe clinical symptoms. Fatal hemoglobin Bart's hydrops fetalis, related to homozygous α^0-thalassemia, is generally characterized by severe hemolytic anemia, hydrops, and ascites in utero or just after birth, which can lead to fetal death [8, 9].

β-thalassemias are associated with insufficient (β^+–β^{++}) or absent (β^0) synthesis of β-globin due to β-globin gene mutations. The severity of symptoms is related to the extent of absent production of β-globin chain [10].

Contrary to thalassemias, abnormal hemoglobins have structural defects due to altered amino acid sequence in the α- or β-globin chains. The common hemoglobin abnormalities include hemoglobin S (HbS), HbC, and HbE. HbS is the most dangerous of all hemoglobinopathies. Vascular obliterations and infarctions in main organs could result from the sickle cells [11]. HbC disease course is similar to progression of sickle cell disease but is less fatal. However, HbE disease is like β-thalassemias. There are other abnormal hemoglobins with structural detects.

COMPLETE BLOOD COUNT

For screening of thalassemia, mean corpuscular volume (MCV) of less than 80 fL and/or mean corpuscular hemoglobin (MCH) value of less than 27 pg can be generally used as cutoff levels for a positive screening result [12]. These cutoff levels are derived from 2 standard deviations of the normal distribution of MCV and MCH from the normal

Table 1. Classification of major hemoglobinopathies.

Type		Diagnosis	Gene type
Thalassemia	α-thalassemias	Heterozygous α^+-thalassemia	−α/αα
		Homozygous α^+-thalassemia	−α/−α
		Heterozygous α^0-thalassemia	−−/αα
		Mixed heterozygosity, α^+/α^0-thalassemia	−−/−α
		Homozygous α^0-thalassemia	−−/−−
	β-thalassemias	Heterozygous β-thalassemia	β^{++}/β, β^+/β, β^0/β
		Mild homozygous or compound heterozygous β-thalassemia	β^+/β^+, β^+/β^{++}, β^+/β^0, β^0/β^0
		Homozygous β-thalassemia	β^+/β^+, β^0/β^0
		Compound heterozygous β-thalassemia	β^+/β^0
Structural variants	HbS	HbS heterozygosity	HbAS
		Sicklecell disease	HbSS
	HbC	HbC heterozygosity	HbAC
		HbC disease	HbCC
	HbE	HbE heterozygosity	HbAE
		HbE disease	HbEE
Mixed variants	β-thalassemias+HbS or HbE	Sickle cell β^+-thalassemia	HbS β^+-thalassemia
		Sicklecell β^0-thalassemia	HbS β^0-thalassemia
		HbE β^+-thalassemia	HbE β^+-thalassemia
		HbE β^0-thalassemia	HbE β^0-thalassemia
	HbS+HbC	HbSC	HbSC disease

population. The advantage of the thalassemia screening by MCV and MCH is achievement of rapid, cost effective, reproducible, and accurate results from automated hematology analyzers. However, microcytic anemias such as iron deficiency anemia (IDA) can also induce low MCV; in contrast with MCH, which seems to be consistent among different automated hematology analyzers, variation in the MCV from different automated blood cell counters had also been reported [13]. In addition, a low MCV is not suitable for screening HbE carriers and individuals with single α-globin gene deletion ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or nondeletional α-globin gene mutations [i.e., Hb Constant Spring (Hb CS) and Hb Quong Sze] [14]. Moreover, the interaction of heterozygous β-thalassemia with α-thalassemia trait alone or with glucose-6-phosphate dehydrogenase deficiency may lead to normal MCV and a false-negative result during thalassemia screening [15]. Therefore, it would be more appropriate to screen thalassemia using both MCV and MCH than using MCV only; it would be very important to determine their cutoff levels using the automated hematology analyzer utilized in each laboratory.

Using both MCV and MCH, the interpretation of peripheral blood smear would be considered as an important screening method for thalassemia. Typical RBC morphology in thalassemia disease consists of microcytosis, hypochromia, and anisopoikilocytosis. Microcytes can be evaluated by comparing the size of RBC with those of nucleus of small lymphocytes, and hypochromic RBCs are defined as having an increase in the diameter of central pallor of RBCs, that is, more than one-third of their diameter. Anisopoikilocytosis results from various abnormal RBC morphologies including schistocytes, microspherocytes, target cells, polychromasia, and nucleated RBCs. However, peripheral blood smear results might only suggest certain types of thalassemias from other causes of anemia, such as IDA or anemia of inflammation, and it is not possible to define a specific type of thalassemia disease based on RBC morphology only.

The red cell distribution width (RDW) is a measure of the degree of variations in red cell size, and some causes of microcytic anemia, most notably IDA, are characterized by an increase in RDW. Although thalassemia produces uniform microcytic red cells without a concomitant increase in RDW, this observation is variable among the thalassemia syndromes, including notable increases in RDW in the Hb H disease and β-thalassemia minor [16]. Therefore, the RDW may provide information that might be used as an adjunct to diagnosis but is not useful as single screening indicator [16]. The RBC count is also useful as a diagnostic adjunct because thalassemia produces a microcytic anemia with increase in the RBC number, but IDA and anemia of chronic disease are typically associated with a decrease in the RBC number that is proportional to the degree of anemia. However, the RBC count should not be used as a sole screening tool for thalassemia and hemoglobinopathies.

Given all this, various indices utilizing complete blood count (CBC) components have been developed for the screening of thalassemia and hemoglobinopathies, but none exceed the value of the combination of MCV and MCH in selecting cases for subsequent investigations.

ELECTROPHORESIS INCLUDING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In 1978, the International Committee for Standardization in Haematology has recommended laboratory tests for 3 types of laboratories [17]. In that guideline, screening laboratory should be able to perform alkaline electrophoresis. The reference laboratory had to perform very difficult tests like citrate agar electrophoresis and globin electrophoresis. Those electrophoresis techniques required manual steps throughout hemoglobin analysis from reagent preparation, electrophoresis, and data analysis, and thus experience of the laboratory professional was a key to successful identification. Recent development of laboratory techniques and increased knowledge on thalassemia and hemoglobinopathy has driven the publication of updated guidelines [18]. The British Committee for Standards in Haematology recommends presumptive identification of hemoglobins on a minimum of 2 techniques and regards definitive identification as that based on DNA analysis, mass spectrometry, or protein sequencing.

High performance liquid chromatography

High performance liquid chromatography (HPLC) technique is a method used to separate compounds or molecules based on their chemical characteristics. Many separation principles such as size, affinity, and partition are available; for hemoglobin, ion-exchange chromatography is the most efficient and most widely used. The method can be also manually operated, but recently fully automated systems are available. Those systems may be dedicated to hemoglobin analysis; however, in low prevalence areas, the systems that can switch between glycated hemoglobin analysis for diabetes and variant hemoglobin analysis for thalassemia and hemoglobin variants may be more feasible to use.

It is known to be useful for the diagnosis of β-thalassemia trait because HbA2 can be accurately quantitated [18]. Similar to other HPLC techniques, careful control of analytical conditions such as column temperature, flow rate, and buffer conditions is essential.

Electrophoresis

Electrophoresis is a technique used to separate molecules or compounds based on their migration pattern in a gel and electrical field. It is still widely used in clinical laboratories for protein electrophoresis and differentiation of some isoenzymes. Manual preparation of gel and electrophoresis is rarely used in developed countries as more advanced and automated techniques such as capillary electrophoresis are available.

Cellulose acetate electrophoresis is a representative custom electrophoresis technique. It is known to enable identification of Hb A, F, S/G/D, C/E, and H and other variants

[18]. Automated capillary electrophoresis is widely used and showed benefits in the identification of some variants indistinguishable in many automated HPLC systems [19].

Mass spectrometry

Mass spectrometry is a technique to identify molecules based on their mass (molecular weight) to charge ratio. The strong advantage of the technique is that it uses minimal specific binding reagent for the molecules of interest. The simple analytical principle enables less interference and more accurate identification. The analysis of hemoglobin with mass spectrometry is not simple because the laboratory should have both technical expertise for analysis of proteins and very expensive instrument. Besides identification of hemoglobin based on intact molecule's molecular weight, it can also analyze the sequence of amino acids in some degree. It is useful for the identification of new variants and confirmation of DNA sequencing [20].

MOLECULAR CHARACTERIZATION

α-thalassemia is caused by gene deletion in over 90% of cases. A minority of α-thalassemia cases are due to sequence variations like single nucleotide substitution, insertion, or short insertion/deletion. α gene cluster is composed of highly homologous genes as well as 2 *HBA* genes encoding identical proteins. Gene deletion is likely due to unequal crossing between these homologous regions during meiosis. Several breakpoints including the most common deletion of 3.7 kb have been reported thus far [21].

Over 90% of β-thalassemia cases, as compared with α-thalassemia cases, are caused by sequence variations. More than 280 sequence variants are reported to be associated with β-thalassemia [21]. Some β-thalassemia is due to gene deletion encompassing the *HBB* gene.

Many different molecular techniques are used to detect globin gene mutations. Molecular techniques can be grouped by mutation type to be targeted as follows: 1) detection methods for structural variations such as gene deletion, duplication, or triplication and 2) detection methods for sequence variations such as nucleotide substitution, insertion, or short insertion/deletions.

Known gene deletions can be detected by gap polymerase chain reaction (PCR) specifically designed for the deletion concerned. Southern blotting using labeled complementary gene probes may be used for unknown gene deletions. The multiplex ligation-dependent probe amplification (MLPA) method can detect both known and unknown gene deletions. MLPA is widely used because it is highly sensitive, is easy to use, and can detect various deletions.

Common sequence variations can be detected in a cost-effective manner for some ethnic populations using techniques such as allele-specific PCR, reverse dot blotting, denaturing gradient gel electrophoresis, and amplification refractory mutation system. Rapid improvement and cost reduction of sequencing technology made it possible to sequence the globin gene including promoter, 3′ UTR, exon-intron boundaries, and deep introns in many laboratories. In particular, massively parallel sequencing technology can be applied to targeted genes, exomes, or even genomes.

PRENATAL DIAGNOSIS

Prenatal diagnosis includes carrier screening, genetic counseling, and prenatal genetic studies. To date, the prenatal diagnosis of thalassemia and hemoglobinopathy represent 1 of the most frequent genetic analyses performed worldwide. Because of population migration, hemoglobinopathies are common in many immigration countries as well as endemic regions [22-25]. The purpose of the prenatal diagnosis is to identify and counsel asymptomatic individuals whose offspring are at risk of an inherited hemoglobinopathy and to monitor the pregnancy for complications. The clinical types of hemoglobinopathies that are targets of prenatal diagnosis are associated with potentially severe sequelae and intervene, such as sickle cell disease, β-thalassemia major resulting from homozygosity of β-thalassemia, and hemoglobin Bart's nonimmune hydrops fetalis caused by deletion or dysfunction of all 4 α-globin genes [26]. Despite the early lethality of hydrops fetalis, prenatal diagnosis is useful in this condition because a significant number of women carrying fetuses with this abnormality develop severe toxemia and severe postpartum hemorrhage [27]. Therefore, a profound understanding of genotype-phenotype correlation, the effect of genetic modifiers, and rare cases including dominantly inherited β-thalassemia, uniparental isodisomy, and de novo mutation is warranted [28].

The prenatal diagnosis involves the study of fetal material from chorionic villi, amniotic fluid, cord blood, and fetal DNA in maternal circulation. Invasive prenatal diagnosis involves the performance of chorionic villus sampling at the first trimester and amniocentesis or cordocentesis at the second trimester. Although analysis of fetal hemoglobin types is successfully performed by automated HPLC, it is assessable through analysis of fetal blood obtained by cordocentesis and the procedure is prone to errors due to contamination by maternal tissue [29]. DNA analysis is valuable especially in prenatal diagnosis because abnormal hematologic findings are detected and samples can be more easily obtained postnatally. Advances in molecular testing have facilitated the diagnosis of complex thalassemias and hemoglobinopathies observed in ethnically diverse populations. Universal screening programs aimed at detecting carriers and offering prenatal diagnosis in pregnancies at risk for thalassemia have been adopted in Canada and European countries [30, 31]. The parental screening is not invasive and can be performed without increasing risks to the fetus. The recent development of noninvasive prenatal diagnostic testing using cell-free fetal DNA from maternal plasma allows active investigation of genetic analysis of fetus avoiding invasive procedure [32]. Another advantage of this approach is that fetal DNA can be isolated from maternal blood earlier than using invasive

procedures [33]. Various techniques have been applied to identify fetal hemoglobinopathies such as mass spectrometry, next-generation sequencing, and genotyping assay [34-36]. The techniques are still challenging; therefore, more studies are needed to develop and validate them and ultimately lead to efficient, precise, and reliable noninvasive prenatal diagnosis of thalassemia and hemoglobinopathies [37].

CONCLUSION

Thalassemia and hemoglobinopathies are no longer uncommon in Korea, and the incidence increases according to the migration of people from endemic areas. The physicians should carefully review CBC, especially MCV and MCH, in addition to family history to suspect thalassemia and hemoglobinopathies in anemic patients. Quantitative and structural abnormalities of hemoglobin can be more sensitively and accurately detected by using advanced electrophoresis techniques involving HPLC and mass spectrometry. Various molecular tests are used for molecular diagnosis of thalassemia and hemoglobinopathy, and the recent improvement in sequencing technology such as massively parallel sequencing technology enables more precise genetic diagnosis. We should also focus on prenatal diagnosis and genetic counseling on thalassemia and hemoglobinopathy to prevent thalassemia births and pregnancy complication. Suspicion of disease by carefully reviewing the results of screening tests and advanced molecular testing can assist in the early diagnosis and intervention of the disease. Furthermore we expect the development of treatment modality through the understanding of molecular and protein characteristics of thalassemia and hemoglobinopathies.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Adam MP, Ardinger HH, Pagon RA, et al. GeneReviews: Beta-thalassemia. Seattle, WA: University of Washington, 2018. (Accesed December 2, 2018, at https://www.ncbi.nlm.nih.gov/books/NBK1116/).
2. Adam MP, Ardinger HH, Pagon RA, et al. GeneReviews: Alpha-thalassemia. Seattle, WA: University of Washington, 2016. (Accesed December 2, 2018, at https://www.ncbi.nlm.nih.gov/books/NBK1435/).
3. Adam MP, Ardinger HH, Pagon RA, et al. GeneReviews: Sickle cell disease. Seattle, WA: University of Washington, 2017. (Accesed December 2, 2018, at https://www.ncbi.nlm.nih.gov/books/NBK1377/).
4. Ferrão J, Silva M, Gonçalves L, et al. Widening the spectrum of deletions and molecular mechanisms underlying alpha-thalassemia. Ann Hematol 2017;96:1921-9.
5. Russo R, Andolfo I, Manna F, et al. Multi-gene panel testing improves diagnosis and management of patients with hereditary anemias. Am J Hematol 2018;93:672-82.
6. Kohne E. Hemoglobinopathies: clinical manifestations, diagnosis, and treatment. Dtsch Arztebl Int 2011;108:532-40.
7. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet 2018;391:155-67.
8. Higgs DR, Weatherall DJ. The alpha thalassaemias. Cell Mol Life Sci 2009;66:1154-62.
9. Harteveld CL, Higgs DR. Alpha-thalassaemia. Orphanet J Rare Dis 2010;5:13.
10. Olivieri NF. The beta-thalassemias. N Engl J Med 1999;341:99-109.
11. Steinberg MH. Sickle cell anemia, the first molecular disease: overview of molecular etiology, pathophysiology, and therapeutic approaches. ScientificWorldJournal 2008;8:1295-324.
12. Viprakasit V, Ekwattanakit S. Clinical classification, screening and diagnosis for thalassemia. Hematol Oncol Clin North Am 2018;32:193-211.
13. Chaitraiphop C, Sanchaisuriya K, Inthavong S, et al. Thalassemia screening using different automated blood cell counters: consideration of appropriate cutoff values. Clin Lab 2016;62:545-52.
14. Chan LC, Ma SK, Chan AY, et al. Should we screen for globin gene mutations in blood samples with mean corpuscular volume (MCV) greater than 80 fL in areas with a high prevalence of thalassaemia? J Clin Pathol 2001;54:317-20.
15. Viprakasit V, Limwongse C, Sukpanichnant S, et al. Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand. Clin Chem Lab Med 2013;51:1605-14.
16. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem 2000;46:1284-90.
17. Recommendations of a system for identifying abnormal hemoglobins. By the International Committee for Standardization in Hematology. Blood 1978;52:1065-7.
18. Ryan K, Bain BJ, Worthington D, et al. Significant haemoglobinopathies: guidelines for screening and diagnosis. Br J Haematol 2010;149:35-49.
19. Yun YM, Ji M, Ko DH, et al. Hb variants in Korea: effect on HbA1c using five routine methods. Clin Chem Lab Med 2017;55:1234-42.
20. Kim SY, Lee SH, Cho SI, et al. Molecular identification of the novel Gγ-β hybrid hemoglobin: Hb Gγ-β Ulsan (Gγ through 13; β from 19). Blood Cells Mol Dis 2010;45:276-9.
21. Huisman T, Carver M, Baysal E, Efremov G. A Database of Human Hemoglobin Variants and Thalassemias. State College, PA: The Pennsylvania State University, 2013. (Accesed December, 8, 2018, at http://globin.cse.psu.edu/cgi-bin/hbvar/query_vars3).
22. Sayani FA, Kwiatkowski JL. Increasing prevalence of thalassemia in America: implications for primary care. Ann Med 2015;47:592-604.
23. Anwar WA, Khyatti M, Hemminki K. Consanguinity and genetic diseases in North Africa and immigrants to Europe. Eur J Public Health 2014;24:57-63.
24. Jahng J, Yoon KH. A family with a hemoglobin E variant including

a Thai immigrant woman in Korea. Ann Lab Med 2017;37:71-3.
25. Lee HS, Lee DY, Kim HJ, Lee IS. Two cases of alpha-thalassemia in Korean children from multicultural family. Clin Pediatr Hematol Oncol 2011;18:136-9.
26. Li DZ, Yang YD. Invasive prenatal diagnosis of fetal thalassemia. Best Pract Res Clin Obstet Gynaecol 2017;39:41-52.
27. Seale TW, Rennert OM. Prenatal diagnosis of thalassemias and hemoglobinopathies. Ann Clin Lab Sci 1980;10:383-94.
28. Shang X, Xu X. Update in the genetics of thalassemia: what clinicians need to know. Best Pract Res Clin Obstet Gynaecol 2017;39:3-15.
29. Sanguansermsri T, Thanaratanakorn P, Steger HF, et al. Prenatal diagnosis of hemoglobin Bart's hydrops fetalis by HPLC analysis of hemoglobin in fetal blood samples. Southeast Asian J Trop Med Public Health 2001;32:180-5.
30. Cousens NE, Gaff CL, Metcalfe SA, Delatycki MB. Carrier screening for beta-thalassaemia: a review of international practice. Eur J Hum Genet 2010;18:1077-83.
31. Langlois S, Ford JC, Chitayat D; CCMG Prenatal Diagnosis Committee; SOGC Genetics Committee. Carrier screening for thalassemia and hemoglobinopathies in Canada. J Obstet Gynaecol Can 2008;30:950-9.
32. Li Y, Hahn S, Holzgreve W. Recent developments in the detection of fetal single gene differences in maternal plasma and the role of size fractionation. Ann N Y Acad Sci 2006;1092:285-92.
33. Norwitz ER, Levy B. Noninvasive prenatal testing: the future is now. Rev Obstet Gynecol 2013;6:48-62.
34. Papasavva T, van Ijcken WF, Kockx CE, et al. Next generation sequencing of SNPs for non-invasive prenatal diagnosis: challenges and feasibility as illustrated by an application to β-thalassaemia. Eur J Hum Genet 2013;21:1403-10.
35. Li Y, Di Naro E, Vitucci A, et al. Size fractionation of cell-free DNA in maternal plasma improves the detection of a paternally inherited beta-thalassemia point mutation by MALDI-TOF mass spectrometry. Fetal Diagn Ther 2009;25:246-9.
36. Breveglieri G, Travan A, D'Aversa E, et al. Postnatal and non-invasive prenatal detection of β-thalassemia mutations based on Taqman genotyping assays. PLoS One 2017;12:e0172756.
37. Zafari M, Kosaryan M, Gill P, et al. Non-invasive prenatal diagnosis of β-thalassemia by detection of the cell-free fetal DNA in maternal circulation: a systematic review and meta-analysis. Ann Hematol 2016;95:1341-50.