CPQ Women and Child Health (2020) 3:2
Research Protocol

CIENT PERIODIQUE

The Mystery in Mother's Blood: An Early Look to the Genetic Inheritance of Fetus

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Received: 12 August 2020

Published: 02 September 2020

Keywords: *Noninvasive Prenatal Diagnosis; Fetal DNA; Thalassemia; High Resolution Melting; Maternal Plasma*

Abstract

The discovery of cell free fetal DNA (cffDNA) in the maternal circulation have opened up new possibilities for noninvasive prenatal diagnosis (NIPD) more than one decade. Analysis of cffDNA from maternal plasma by PCR based technologies and high resolution melting analysis (HRM) offers great potential screening single gene disorders for NIPD. The aim of our study is to screen the paternal alleles derived from father in cffDNA for the risk of alpha and beta thalassemias using HRM assay using cffDNA in maternal blood. Maternal plasma samples obtained from 120 beta and 50 alpha thalassemia carrier pregnancies at risk for beta and alpha thalassemia whose couples carries different mutations. Paternally alleles were detected in 79 of 120 for beta thalassemia and 32 of 50 for alpha thalassemia in cffDNA. The paternal beta thalassemia mutations which found in fetal DNA's were HBB:c.93-21G>A (IVSI-110), HBB:c.93-1G>A (IVSI-130), HBB:c.316-3C>G (IVSII-848), HBB:c.47G>A (Cd15), HBB:c.92+6T>C (IVSI-6), HBB:c.315+1G>A (IVSII-1), HBB:c.25_26delAA (Cd8) and HBB:c.113G>A (Cd37). The paternal alpha thalassemia mutations 20.5kb del, Med I, -3.7kb del and -4.2kb del were also detected in cffDNA. The results confirmed with CVS by sequencing analysis for beta thalassemias and GAP-PCR for deletional alpha thalassemias. HRM analysis is a rapid and useful mutation scanning method in NIPD of

thalassemias to detect paternally derived alleles in cffDNA in the situation of the couples carries different mutations.

Introduction

The discovery of free fetal cells in 1950s, and cell free fetal DNA (cffDNA) in the 1990s, in the maternal circulation early in gestation, has opened up new possibilities for prenatal diagnosis of single gene disorders [1].

Prenatal diagnosis is the only effective way to prevent the birth of a fetus with genetic disorders [2]. The most direct way to provide fetal genetic sample is to obtain fetal tissue through conventional methods such as amniocentesis or chorionic villus sampling (CVS) [2]. These conventional methods are invasive and associated with a risk for fetal miscarriage (0.5-1%) [3]. The detection of fetal DNA sequences is a reality and could reduce the risk of invasive techniques for certain fetal disorders. Several encouraging clinical applications, such as the noninvasive detection of fetal sex and RhD status, have been developed based on the detection of paternally inherited genetic traits in maternal plasma [4,5]. Analyzing paternal mutations in cffDNA can eliminate the risk for a double heterozygote fetus in monogenic disorder [6]. The technique requires no other modification of routine PCR procedures compared with traditional methods.

Thalassemias are the most common single gene disorder deriving from mutations in alpha and beta gene clusters in human, chiefly in the Mediterranean area, Asia and Africa. Approximately %7 of the world population is known as being carrier of globin gene mutation [6-8]. The frequency of the β-thalassemia carrier is 3.7% in the Cukurova region which is in the South part of Turkey and alpha thalassemia has been reported to be 3.6% in Turkey [9,10]. There are lots of approaches (digital PCR, DNA sequencing, real-time PCR, nested PCR, cold PCR) that have been used to screen and determine alpha and beta thalassemia. HRM analysis portrays the next generation of mutation screening and defining technology [8,11,12].

The melting property of the PCR product in the HRM analysis depends on the GC content, length and sequence composition. The change in melting properties occurs in the presence of mutations and heteroduplexes [8].

This is a single center study which aimed to take an earlier look at baby's genetic inheritance with HRM analysis. This is suggested as a noninvasive paternal mutation screening method in cffDNA for thalassemia to reduce the use of invasive prenatal testing in the situation of the fetus not inherited with paternal mutation.

Materials and Methods

The selected study population included 170 pregnant women who were admitted to the University Hospital Gynecology and Obstetrics Clinic and to Medical Biochemistry for prenatal diagnosis of thalassemias. The study protocol was approved by the Ethics Committee of the Faculty of Medicine of Çukurova University and informed consent was obtained from each subject.

Maternal blood was rapidly centrifuged and fetal DNA was extracted from plasma using previous methods [9]. The mutation of each parent had been identified previously by conventional PCR (ARMS, RFLP, GAP-

PCR) and Sanger sequencing [2,8,11]. For detect cffDNA DYS14 gene expression with the housekeeping gene (beta-actin) as internal control examined with qRT-PCR using specific primers and probes [9].

For HRM analysis, the portion of the mutation region of interest is initially amplified by RT-PCR. In the HRM process, the amplicon DNA temperature rises from about 50°C to 95°C. During this time, the two strands of DNA are separated from each other at the melting temperature of the amplicon [13]. Double-stranded DNA is identified by fluorescent staining according to its structural properties and changes in its melting temperature (Tm) [9,13]. The paternally mutations were detected in cffDNA by HRM analysis with real-time PCR using related primers for beta globin, and alpha globin genes [9].

Results

The samples obtained from the South and East part of Turkish population who underwent prenatal diagnosis of thalassemias. The median gestational age of CVSs was 13 weeks (range: 6 weeks to 21 weeks). The paternal beta thalassemia mutations of fetal DNA's were detected with HRM were HBB:c.93-21G>A (n=32), HBB:c.93-1G>A (n=1), HBB:c.316-3C>G (n=5), HBB:c.47G>A (n=2), HBB:c.92+6T>C (n=15), HBB:c.315+1G>A (n=13), HBB:c.25_26delAA (n=10), HBB:c.113G>A (n=1). The paternal alpha thalassemia mutations 20.5kb del (n=1), Med I (n=8) and -3.7kb del (n=21) and -4.2kb del (n=2) were also detected in cffDNA. The results confirmed by sequencing analysis for beta thalassemia mutations and GAP-PCR for alpha thalassemia mutations using CVS.

Discussions

The detection of fetal DNA sequences is a reality and could reduce the risk of invasive techniques for certain fetal disorders [14]. The detection of cffDNA in maternal plasma and serum has led to clinical applications for the identification of fetal aneuploidies, pre-eclamptic pregnancies, noninvasive diagnosis of fetal Rhesus D genotype and single gene disorders [15,16]. Several molecular diagnostic methods has been developed for genotyping single gene disorders such as thalassemias based on PCR techniques (ARMS, RFLP, GAP-PCR, VNTR, etc.) and high-throughput technologies (Gene expression, HRMA, MicroArray, MLPA, NGS, etc.) [17-19]. The noninvasive tests using cell-free DNA (cfDNA) from a maternal blood sample is also an alternative method, thus eliminating the risk of miscarriage for fetus. To detect paternal mutation in cffDNA extracted from maternal plasma is possible, only the couples carries different mutations [9,12].

This method is time consuming and cost effective when compared to conventional PCR techniques. While it costs nearly $ 100 per run, HRM analysis decreases it approximately %80 [6]. In addition to this advantage, it also provide to pass the post-PCR phase of the conventional PCR methods, including gel electrophoresis, enzymatic cleavage. HRM assay has a low risk for PCR contamination compared with the routine molecular diagnosis PCR based approaches [9].

Conclusions and Future Perspectives

In conclusion, this should be admitted that cffDNA is the Mystery in mother's blood which is the mirror of the fetus. This noninvasive material is useful for diagnosis several gene disorders such as thalassemias in the early stage of pregnancy. Comparing with conventional PCR methods, HRM analysis is an efficient, rapid

and also useful in verifying of other methods for genotyping inherited paternal thalassemia mutations for cffDNA for laboratories that have real-time PCR.

Studies on NIPD show an increasing momentum day by day with the development of technology. Today the commercial kits are available for chromosomal aneuploidies and fetal RHD status in routine use but there is promising advanced methods have been achieved, give rise to studies to detect maternally inherited mutations and fetal whole-genome sequencing [17-20].

Acknowledgements

We would like to thank Professor Cuneyt Evruke, Professor Cansun Demir, Professor Selim Büyükkurt at the University of Cukurova, Faculty of Medicine Department of Obstetrics and Gynecology & Perinatology for taking CVS.

Conflicts of Interests

There is no conflict of interest between authors.

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