Review article:

Non invasive prenatal diagnosis of β- Thalassemia, A narrative review study

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Abstract:

β- Thalassemia is major monogenic disorder. A practical way to prevention of Thalassemia is identification of carries couples; genetic counseling and offer prenatal diagnose services for both carrier couples. Routine prenatal diagnose are chorionic villus sampling and amniocentesis, but both of them are invasive method and they can be ended to bleeding and pregnancy loss. Recently non invasive prenatal diagnosis has been done by researchers for early detection of pre-eclampsia, chromosomal aneuploidies, RhD-genotyping. Regarding non invasive prenatal diagnosis of β- Thalassemia, detection of paternally inherited mutation in maternal plasma is possible. If the fetus inherited normal paternal allele the performance of invasive method it is not necessary, so this method can be eliminate 50% performance of routine prenatal diagnosis.

Keywords: Non invasive prenatal diagnosis, Thalassemia, cff DNA, maternal plasma.

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1. Beta Thalassemia

B-Thalassemia is one of major monogenic disorder in the world [1] . About 4% of population IR of Iran (>3000,000 persons) are β-thalassemic carriers' [2].The main treatment of it is repeated blood transfusion and iron chelating, however, bone marrow (stem cell) transplantation is final cure the disease in some patients and gene therapy is not yet a routine procedure. A fraction of β- Thalassemia major patients have milder form of anemia and used to be classified as β-Thalassemia intermediate (β-TI). The new categories of this disease is non transfusion-dependent β-TM and transfusion-dependent β-TM, which is still an arbitrary name and is the subject of debate regarding the attitude of care takers, availability of medical treatment and emergence of growth failure and bone deformities. However, patients who need repeated blood transfusion for prevention severe complications such as heart failure are called transfusion-dependent β-TM [3].

2. The extent problem in Iran

B-thalassemia has been reported in most of the communities that have been screened in Iran. About 3milions carriers are living in IR of Iran [4] . The overall prevalence varies in different areas. The highest incidence of β-Thalassemia carrier rate (11%) is in the south border of Caspian Sea and also alongside of Persian Gulf. Four common β-Thalassemia point mutation in Iranian patients are IVS-II-I (G -A), IVS-I-5 (G-C), FSC 8/9(+G), IVS-I-110 [5] .

3. The need for accurate identification of couples at risk

Classical β-Thalassemia carriers have typically reduced MCV (mean corpuscular volume) less than 80 fl, MCH (mean corpuscular haemoglobin) less than 27 pg, with high RBC counts and elevated HbA_2 levels (>3.5%). However, a few β-thalassemia heterozygote's are termed as silent carriers with normal HbA_2, they doesn't follow recent classification

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On the other hand individuals with borderline HbA_2 levels (3.3-3.9%) need to be assessment carefully to avoid misdiagnosis [6].

A practical way to prevention of Thalassemia is identification of carries couples; genetic counseling and offer prenatal diagnose services for both carrier couples. Abortion of affected fetuses is legal and routine in the country since1991 [7].

4. The most suitable time to screen and genetic counseling

Prevention of the birth the thalassemic baby via prenatal diagnosis (PND) is the major way to control the spread of Thalassemia. Both carrier couples are also under special supervision regarding family planning and their method of contraception [7-8]. They have records in health center and periodic visits are being performed by health workers. PND and abortion of the affected fetuses is available and legal and costs are covered by insurance companies [2, 9-10].

5. Prenatal diagnosis

Prenatal diagnosis designs in two categories: 1- "prenatal screening" for all pregnant women as a routine antenatal care to determine if the fetus is at significant risk of having particular disorder such as sickle cell anemia and Down syndrome. 2- If the fetus is at high risk of having particular disorder "prenatal diagnosis" is offered [11].

The first report of invasive prenatal diagnosis was in the late 1960s. The most common available methods for prenatal diagnosis are amniocentesis (after 15 weeks) and chorionic villus sampling (CVS) (between 11-14 weeks). CVS was first introduced in mid-1970$_s$. In CVS procedure aspiration of placenta tissue requires and in amniocentesis; amniotic fluid aspiration is required. CVS procedure performed via transabdominal, transvaginal or transcervical under the guidance of ultrasound. Of cource the selection between these 3 approaches depends on operator s personal favorite. Although some researcher believes that transvaginal approach has higher risk than the other approaches. Also, this approach needs considerably more skill and experience and it takes time and this method requires various and more frequent insertion and causes vaginal bleeding in about 10% of cases [12].

Both of these are invasive procedures and pose risks to the mother & fetus e.g., most significantly a risk of miscarriage 1 in 200-400 and 1 in 100-200 respectively [13]. Of course the accuracy of these methods is estimated to be 98-99% [14]. These invasive methods increase risk of fetal loss and other probable risks such as; massive bleeding which may lead to abortion and fetal loss. The fetal loss rate is about 2 -4.5%, and it due to kind of method. Feta loss rate in Trans abdominal is less than transvaginal method. Of course the fetal death can be due to infection following CVS [15]. Iran started the national Thalassemia prevention program since 1996. At the onset this program carrier couples identified before marriage and to offer counseling, then providing them with the opportunity to separate. At that time PND and the option of selective abortion were not wildly available [16].

6. preimplantation genetic testing

Preimplantation genetic testing was first described by Handyside et al in 1990, it consisted all types of genetic testing performed on the embryos obtained from an IVF (in vitro fertilization) cycle. Preimplantation genetic testing is divided into two categories: preimplantation genetic screening (PGS, (it performed on embryo obtained from parents with normal karyotypes)) and preimplantation genetic diagnosis (PGD, (it involves testing the embryos for a specific genetic disorder)). PGD needs prior identification of genetic disorder in the family, and it was initially developed to identify the embryo with serious genetic disorder. Different methods can be used for PGD, such as; FISH, chromosomal microarrays CNVs, DNA sequencing for single disorder with known mutation in parents. The biopsy of the embryo is performed on days 3 or 5 after fertilization, although blastomeric stage biopsy is associated with higher risk of damaging the embryo and misdiagnosis secondary to possible mosaicism. Also, trophectoderm biopsy that was done at blasctocyts stage on fifth day is associated with better results and more perfect diagnosis. In overall the risk of mosaicism at blastocyst stage is lower than blastomeric period. At the moment, even though advanced techniques, the risk of embryo misdiagnoses following PGD is not unlikely. When there is a proven family history of genetic CVS or amniocentesis should be used to rule out genetic diagnosis in the fetus [17].

7. Non invasive prenatal diagnosis (NIPD)

For decades, researchers and physicians introduced NIPD in a world [13]. Now NIPD via free fetal DNA and fetal cell in maternal circulation is performed for determination fetal sex, fetal Rh and genetic disorders.

8. single fetal cell in maternal blood

Fetal cell such as; trophoblasts, erythrocytes and

leukocytes circulated in maternal blood. Among these fetal cells, nucleated erythrocytes are suitable for NIPD because they are uncommon in peripheral blood of normal mother. Fetal erythrocyte can be collected from peripheral blood of pregnant mother during 8-11 weeks [18]. The main problem about fetal cell for NIPD is; the fetal cell will remain in maternal circulation after delivery, it creates a limitation of NIPD just for primigravida.

9. Cell free fetal nucleic acids (DNA)

The history of cell free fetal DNA studies, back to 1940s, Mandel & Metais published the first paper about presence of circulating nucleic acid (CNA). More studies have been done regarding the relation between CNA and diagnose of some complication such as: auto-immune disorder, diabetes, stroke, trauma, inflammation, infection, and cancer. Of course CNA can be detected in maternal circulation along growing the fetus and placenta [19]. At the first time Lo et al. published the first paper about presence of Y chromosome in maternal plasma that carried male fetus[20]. Also, other studies demonstrated the relation between CAN and pre-eclampsia, chromosomal aneuploidies, RhD-genotyping, etc [11, 20].

CNA is in small fragments and the length of it is; 150-200 base pairs. The fragments of CNA is called the fetal fraction, it produced from cells that have undergone cellular apoptosis. The half-life of CAN is 4-30 minutes , with an average 16.3 minutes [14].

Another study reported that, fetal DNA are fragmented and the size of them are 150 bp(range from 50-400bp), compared other DNAs with 800 bp (range 100-1600 bp)[21].

The mechanisms of presence the cell – free DNA in maternal circulation are: direct transfer of DNA, placenta, and hematopoietic cells [19] . the concentration of cell-free DNA is 10% of total maternal DNA [22], it presented in maternal plasma from 4-5 weeks of pregnancy[17] , and it increases as during pregnancy [23]. Fetal DNA clears rapidly after birth [24], it means that cffDNA is a pregnancy specific marker [25].

Of course the low concentration of all cell-free DNA in maternal circulation, different amount of cell-free DNA between mothers, Molecules of cffDNA are outnumbered, and Contamination with maternal DNA ; are some problems for detection of cffDNA in maternal circulation[11].

The most common technique used for amplification of cffDNA is real –time PCR, nested PCR, Mass ARRAY system. These techniques have high sensitivity and minimizing the risk of contamination[11,26 ,14].

Many studies published regarding NIPD of β-Thalassemia; Lam et al. believe that RHDO analysis (Relative Haplotype Dosage Analysis), had demonstrated the entire fetal genome in circulation of mother. They studied on 2 families that couples were at risk for having affected baby. DNA that was extracted from parents and CVS were genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0 system. The results following these two methods were confirmed with conventional PND. They believe that sequencing method might not be cost-effective approach and the clinically relevant genomic regions appear only a minor fraction of sequencing data [27].

Li guang-hua. Investigated the clinical feasibility of cff-DNA – BASED NIPD of β-thalassemia. They compared the results of nine samples of amniocentesis with their method. They reported 2 cases of false negative result. The language of this paper was Chinese; we just studied the English abstract [28].

Warunee et al. established NIPD for identifying Hb E-β-thalassemia. They had considered 3 most common β-thalassemia mutations by analysis of cffDNA in maternal plasma by using combined conventional PCR and real-time PCR. The participants had different mutations. The fetal $\beta^{41/42}$ and β^{17} mutations were detected in 6 of 12 and 4 of 9 specimens and these results were concordance with the results obtained by conventional PND. Complete concordance results between cffDNA and conventional procedure were obtained for all mutations by using combined conventional PCR and RT-ASPCR (real –time – allele –specific polymerase chain reaction) analysis. Also they believe that correct diagnosis of HbE could be obtained in early gestational age (in 7 week) and if the initial result is negative for presence of paternal β^0 thalassemia or Hb E – mutation, the test can repeated in second trimester, before conventional PND [29].

Regarding NIPD of Thalassemia, in couples with different mutations, the absence of the paternal mutation in maternal plasma can prevent the possibility of the affected fetus, on the other hand detection of paternal mutant allele increase the risk of affected fetus from 25% to 50%. Some studies published regarding detection paternally mutation in maternal circulation. Chiu et al. Published a similar study for detecting paternal mutation in maternal plasma. Eight carrier couples with different mutations entered in this study before performance of conventional PND. Among 8 fetus, 6 cases has been inherited the paternal mutation. The results on

conventional method showed that 4 fetuses were affected by thalassemia major. They believed that this method had high sensitivity for detection of inherited paternally mutation [30].

Ding et al. reported detection of paternal inherited mutation in maternal plasma with two protocols; Mass ARRAY assays and SYBER (single allele base extension reaction) methods. The result of these two methods compared to fetal genotyping that determined with amniocentesis, chorionic villus sample, and fetal blood sample. They reported two false negative results in SABER method [31].

Li et al. reported detection of paternally inherited fetal point mutations for β-thalassemia using size – fractionated cell-free DNA in maternal plasma. Fetal DNA extracted from maternal plasma of 32 pregnant women who had referred for conventional prenatal diagnosis. The mutations of parents were different and they focused on four common β-globin gene mutations. They believe that, the size of fetal DNA (< 300 base pairs (bp)) is smaller than maternal DNA (> 500 bp). Also they evaluated the difference between the results by analysis of size – fraction circulating DNA and by the analysis of total circulatory DNA. The result of this study showed only one false positive, sensitivity 100%, and specificity 93.8%. The result of 3 cases were uncertain; probably the low concentration of the fetal DNA in maternal plasma reduced the accuracy of analysis [32].

Lazaros et al. was done a study regarding detection of paternal beta-globin gene mutations and polymorphisms as predictors of thalassemia major diagnosis by CVS sample. They studied 97 couples and their parents for identification of beta-globin gene mutations and haplotypes. Also, they identified the haplotypes of 100 control non heterozygote couples. 37 of couples had different mutations (the father was carrier of IVSI-110, and mother had another common mutations), the sensitivity and specificity of this method were 96% and 100% respectively [33].

Galbiati et al. performed a study for identification of paternally inherited mutations in maternal plasma. The results obtained with COLD-PCR in 35 cases were in concordance with conventional PND [34].

Phylipsen et al. used two combined methods; PAP (pyrophosphorolysis-activated polymerization) and MCA (Melting Curve Analysis) for NIPD of β-thalassemia major and sickle cell disease. In all cases, they were able to detect paternally inherited allele in maternal plasma. PAP results were confirmed by direct sequencing analysis after birth [35].

Prajantasen et al. was done a non invasive prenatal diagnosis of β-thalassemia and hemoglobin E gene by DHPLC (Denaturing High Performance Liquid Chromatography) method. They enrolled 42 couples at risk of having Hb E-β offspring. They obtained diagnostic result of 100% concordance with result obtained by ARMS-PCR method and fetal blood specimen was analyzed by capillaries 2 automated capillary electrophoresis [36].

New technologies can differentiate both paternal & maternal mutations in maternal plasma, and a genomic-wide genetic map of fetus has been made for parent's haplotypes. Targeted next-generation sequencing with haplotype analysis allowed NIPD for α and β Thalassemia by paternally inherited mutation in maternal circulation. Regarding the limitations of NIPD; for as much as sequence information is derived from placenta, the possibility of false-positive result can be increased, because of placenta mysticism. Professional organizations e.g. American college of OBS & GYN, the Society for Maternal-Fetal Medicine and National Society of Genetic Counselors recommended NIPD by cffDNA only for high risk pregnancy. In the case of positive result a conventional prenatal diagnosis should be done [17].

Of course some studies indicated about Parents inherited SNP in maternal circulation and its application for NIPD of Thalassemia.

Papasavva et al. Assayed NIPD by detection of paternally inherited SNPs (single nucleotide polymorphisms) with using APEX (arrayed primer extension) method. Eleven SNPs of β-globin gene that have high degree of heterozygisity were selected in 34 families (β-thalassemia carrier couples). Among seven families were informative for the SNP rs 10837631, three families were negative and four families were positive for the paternal allele. These results were in accordance with CVS analysis in all samples except one in which they failed detecting the paternal allele [37].

Chan et al. performed a study on 20 carrier couples one week prior to the conventional PND. They consider four common mutations and SNPs in couples. The results of new method were most useful when absence of the paternal mutation was corroborated by the absence of SNPs linked to the paternal β^T allele, and it needs to performance the conventional PND [38] .

Papasavva et al. had studied on advantage of the allele specificity and sensitivity of the AS-PCR

(Allele-specific polymerase chain reaction) as NIPD for detection of paternally inherited SNPS (single nucleotide polymorphisms) as well as β-thalassemia. The AS-PCR approach detected paternally inherited allele in maternal plasma. In this study, 3-4 ng/μL fetal DNA were extracted and AS-PCR proves to be sensitive and specificity assay for detection of fetal DNA in maternal plasma even at lowest concentration [39].

Papasavva et al. performed a study to assay high heterozygouse SNPs were examined in 101 β-thalassemia carrier couples for NIDP. The results of this study revealed that, 72.28% of couples eligible for qualitative SNPs based NIDP, 92% are quantitative detection. They believe that this method is sensitive and specific for detection of paternally inherited mutation in maternal plasma [40].

10. Conclusion:

The conclusion of this study is; Detection of paternal allele in maternal plasma is feasible. Of course, more study needs to be performed for developing and validating methods into efficient, precise, and reliable assays for the NIPD of β-Thalassemia.

Conflict of interest:

None declared.

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