Editorial

Noninvasive prenatal diagnosis of β-Thalassaemia

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Thalassaemia is a common autosomal recessive disorder. Globally, 1–5% of the population are carriers of thalassaemia, including an estimated 45–70 million people in South Asian countries.[1,2] It is highly prevalent in "world thalassaemia belt", which includes Southeast Asia, the Indian subcontinent, Mediterranean and Middle Eastern countries.[3,4] Bangladesh is in the thalassaemia belt and 6–12% of the population (about 10–19 million people) are carriers of Thalassaemia.[2] According to World Health Organization (WHO), at least 3% of the population are carriers of β-Thalassaemia in Bangladesh.[5] It is presumed that approximately 6000 thalassaemic children are born each year in Bangladesh.[6]

In all developing countries, the main treatments of β-Thalassemia major patients are repeated blood transfusion and iron chelation. Without regular blood transfusions, it can lead to death in the first year because of profound anaemia in early life. Developing countries, where health facilities are limited, the cost of these treatment is high and greatly exceeds the average family income, especially in case of rural and poor patients.[2] Stem cell transplantation, the final cure of the disease, is not yet available in most developing countries. Therefore, prevention is an utmost necessity for these countries.

To prevent Thalassemia, a number of intervention strategies are implemented in different countries–premarital screening, identify the carrier couples, genetic counseling and offer prenatal diagnosis (PND) services for both carrier couples, with an option for termination of affected pregnancy. Cyprus, Italy, Greece, Turkey and Iran have achieved high level of success (80–100%) in preventing the births of children with thalassaemia.[7]

Globally, prenatal diagnosisis done within the first 2 months of pregnancy with invasive methods like chorionic villus sampling (CVS) and amniocentesis. These procedures are not only invasive but are also expensive and carry a significant risk of bleeding and even also fetal loss up to 1%.[8] In Bangladesh, we have also started to do the invasive prenatal diagnosis (amniocentesis and CVS) at Bangabandhu Sheikh Mujib Medical University (BSMMU) in 2007. After that, gradually few other Govt. and non govt. private labs have also started to do these tests.

Chorionic villus sampling is usually and preferably done in the first trimester of pregnancy but could be done between 11-14 weeks. A small sample of developing placental tissue is collected, because it has the same genetic makeup as the fetus. The tissue is obtained by means of a needle inserted through the abdominal wall under ultrasound guidance. A small amount of chorionic villi material is aspirated. When CVS procedure is done through transvaginal route, it may cause vaginal bleeding in about 10% of cases.[9]

Amniocentesis is normally done after the 16th week of pregnancy. The procedure is aspiration of about 15-20 ml of fluid from the amniotic cavity with the help of a small needle inserted through the abdomen under ultrasound guidance.[9] After collection of the sample, DNA analysis is done to determine whether the fetus is *affected, carrier or normal*. If the fetus is affected (thalassaemic), the choice is with the parents to abort or carry on with the pregnancy.

Most people are worried about the invasive procedure and the risk of miscarriage. Researchers and physicians have tried to develop a procedure which could avoid the

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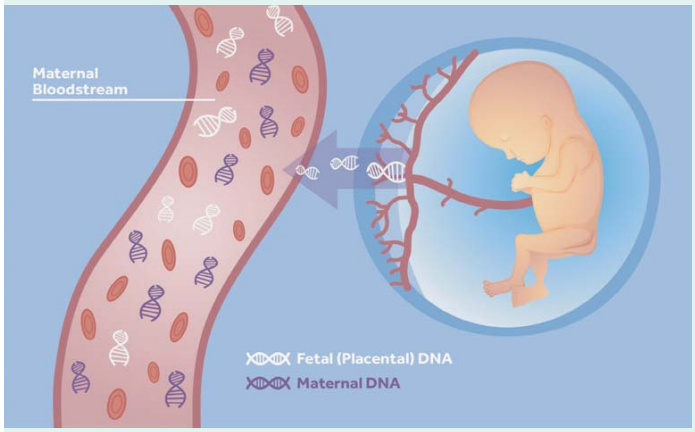
invasive methods and also the unwanted events of the invasive tests. Finally, they were able to introduce non-invasive prenatal test (NIPT).[10] NIPT is a simple procedure that tests the fetal DNA through a maternal blood sample, thus eliminating the risk of miscarriage.[8] The discovery of cell-free fetal DNA (cffDNA) in the maternal plasma opens the possibility to conduct non invasive prenatal diagnosis (NIPD) during pregnancy.[11] This test analyzes small fragments of DNA that are circulating in a pregnant woman's blood and compares it to the maternal and paternal DNA to check for the genetic status.



Now the question may arise on how the cffDNA comes in to the maternal circulations?

As we know that the Circulating nucleic acids (CNA) are present in small amounts in the plasma of all healthy individuals. However, increased levels of plasma CNA have been reported in a number of clinical disorders like auto immune disorder, cancer etc.[12] The rapidly growing fetus and placenta have 'tumor like' characteristics, therefore, CNA of fetal origin can be expected to be found in maternal circulation. On the other hand, placenta is thought to act as a selectively permeable membrane; therefore, fetal DNA/RNA circulates freely in maternal plasma. Lo et al. demonstrated the presence of male fetal DNA in maternal plasma for the first time.[13]

The concentration of cffDNA originating from placental apoptosis is 10% of total maternal DNA.[14] It appears in maternal plasma from 4-5 weeks of pregnancy and increases with the duration of pregnancy.[15] Fetal DNA clears from the maternal circulation rapidly after birth. Therefore, cffDNA is considered as a pregnancy specific marker.[16,17]

Now NIPT is done for determination of fetal sex, fetal Rh genotyping and genetic disorders, chromosomal aneuploidies etc. This new molecular diagnostic method has been based on PCR techniques (real-time, nested, Mass ARRAY system).

NIPT was introduced in 2011, initially being launched by commercial providers but in course of time, it has been widely adopted into regular health systems. Side by side, already worldwide, many studies had been done on non-invasive prenatal diagnosis (NIPD) of β-Thalassemia by this new diagnostic method.[18-22]

Some studies found that this new technology could differentiate both paternal and maternal mutations in maternal plasma and a genomic-wide genetic map of fetus could be made for parents' haplotypes. Targeted next-generation sequencing with haplotype analysis allowed NIPD for α-Thalassemia as well as β-Thalassemia by paternally inherited mutation in cffDNA found in maternal circulation.[23] American college of Obstetrics and Gynaecology, the Society for Maternal-Fetal Medicine and National Society of Genetic Counselors recommended NIPD only for high risk pregnancy. In the case of it's positive results, a conventional prenatal diagnosis (amniocentesis and CVS) should be done.[15]

Considering the result of some previous studies, reviewers concluded that detection of paternal allele in maternal plasma is feasible but of course, more study needs to be performed for developing and validating this method for the NIPD of β–Thalassemia.[23]

Lazaros et. al found the sensitivity and specificity of this noninvasive method, were 96% and 100% respectively.[22] Another recent meta-analysis on NIPD of β-Thalassemia found that the sensitivity and specificity of the test was 99% and

99%, respectively. This study also concluded that detection of paternally inherited mutation of thalassemia using analysis of cell-free fetal DNA is highly accurate.[24] Therefore, it is expected that this method could be replaced conventional and invasive methods.

Some reviewers are more optimistic and hope that the easy, affordable and faster techniques can make NIPT a routine biochemical laboratory investigation in future. And it can also eliminate the conventional and expensive prenatal diagnostic tests like CVS and amniocentesis.[12]

Optimistically, we also hope that this diagnostic test would become more efficient, precise, and reliable assay for the NIPD of β-Thalassemia. Moreover, that it will be available globally very soon. We dream of a Thalassemia free world and where this test will begin a new era.

(All photos : source : Internet)

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