Cell Free Fetal DNA: Marker for Predicting Pregnancy Outcomes

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Healthy mother and healthy baby are the expected outcome in every pregnancy. However, maternal mortality and morbidity are major challenging issues. Preterm labour, intrauterine growth restriction (IUGR), intrauterine fetal death, fetal congenital anomalies etc. often complicate a normal pregnancy. Hypertensive disorder in pregnancy is the leading cause of maternal death in developed countries and its incidence is increasing. Approximately 830 pregnant women die every day from preventable causes worldwide, which accounts for maternal mortality ratio (MMR) 239 per 100,000 live births in developing countries and 12 per 100,000 live births in developed countries [1]. WHO has demonstrated that 85% maternal death is contributed by the African and South-east Asian countries [1]. The MMR in India was 174 in 2015 and it contributed up to 20% maternal death worldwide [2]. Sustainable development goals (SDG) after millennium development goals (MDG) have been aimed to reduce MMR below 70 by the year 2030 [3]. Screening during antenatal period is necessary for taking preventive measures and act accordingly.

Conventional invasive prenatal testing such as amniocentesis or chorionic villous sampling for the diagnosis of

fetal aneuploidies like Down syndrome (trisomy 21), Edward syndrome (trisomy 18) and Patau syndrome (trisomy 13), have procedure related risk of fetal miscarriage, limb-reduction defects, craniofacial malformations, contamination with the maternal cell and wait up to second trimester. With the advent of cell free fetal DNA (cffDNA) in the first trimester it can be used as a non-invasive prenatal diagnostic tool (NIPT) for predicting and diagnosing fetal abnormalities in utero.

cffDNA originates from the trophoblastic cells and enters into the maternal circulation invading the fetoplacental barrier. cffDNA can be detected as early as 5 weeks of gestation and constitute about 10% of cell free (cf) DNA, which is cleared rapidly from maternal circulation after delivery [4]. It offers potential source of prenatal diagnosis for various genetic conditions, such as achondroplasia, autosomal recessive disorders, fetal thalassemia, aneuploidy, RhD genotyping.

cffDNA is typically fragmented hypermethylated DNA of about 150–200 bp [5]. Maternal blood collected in the EDTA vial is centrifuged at 4 °C to remove cells. It is then isolated by any of the following methods, such as: salting out method, guanidium isothiocyanate based method, triton X-100/heat/phenol chloroform method (THP); and also by using commercial kits. However, apart from cffDNA, maternal cfDNA will also be present in the samples. Promoter of RASSF1A is hypermethylated in trophoblast resulting in resistant to digestion by methylation sensitive restriction endonuclease HhaI, HpaII, BstI. On the contrary, maternal RASSF1A promoter is hypomethylated and sensitive to digestion by the above restriction endonucleases. Therefore, purified cfDNA is digested with the above restriction enzymes, and subsequent PCR amplification with specific primers would detect the fetal DNA [6].

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This gender-independent detection of cell free fetal DNA in maternal plasma using RASSF1A/beta-actin has curtailed off a new dimension regarding its utility to predict the adverse pregnancy outcomes. Recent studies showed the utility of cffDNA using the methylation-dependent DSCR3 and RASSF1A markers along with total cf-DNA in maternal serum by HYP2 marker are useful in predicting preeclampsia, intrauterine growth restriction (IUGR) [7]. However, variation of standardized protocol is a major issue for meta-analysis report to opine the efficacy of cffDNA in predicting pregnancy outcomes.

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Compliance with Ethical Standards

Conflict of interest Dr. Subir Kumar Das, and Dr. Mrignaka Mouli Saha declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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