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Rapid Aneuploidy Detection (RAD) Techniques for Prenatal Diagnosis

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Introduction

Chromosomal abnormalities, both numerical and structural, are known to occur in approximately 1 in 200 live births. The diagnosis for chromosomal abnormalities in the antenatal period is usually done by conducting cytogenetic analysis of amniotic, chorionic or fetal blood cells obtained by invasive procedures. Karyotyping is a well-established cytogenetic technique which has been extensively used as a diagnostic tool for pregnant women undergoing these procedures. The technique is considered 100% sensitive and specific and the gold standard for the detection of autosomal trisomies and sex chromosome aneuploidies, against which all other techniques are compared. In addition to chromosomal aneuploidies, structural rearrangements and triploidy can also be detected with a resolution of 10 million DNA base pairs. The various indications for fetal cytogenetic testing include: 1) abnormal ultrasound scan, 2) abnormal maternal serum biochemical results, 3) advanced maternal age (≥35 years at the expected time of confinement) 4) family history of chromosomal aberrations or other genetic disorders.

The rapid rise in the number of pregnancies undergoing maternal biochemical screening and targeted anomaly scans has led to increase in the number of invasive prenatal diagnostic procedures as well. Rapid diagnosis of aneuploidies in these cases is warranted for appropriate management of pregnancy as well as to relieve anxiety for the family. The legal limit of 20 weeks for pregnancy termination in India is a unique legal circumstance which calls for early detection of these abnormalities.

The autosomal trisomies constitute 80% of the chromosomal aberrations. Hence, Rapid Aneuploidy Detection (RAD) methods, which are targeted methods for the diagnosis of common autosomal trisomies (13, 18, 21) and sex chromosome aneuploidies are offered in cases of invasive prenatal testing. Three methods i.e. Fluorescent In Situ Hybridization (FISH), Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR), and Multiplex Ligation Dependent Probe Amplification (MLPA) have been validated for use in prenatal diagnosis (Mann et al., 2004).

- Fluorescent In Situ Hybridization (FISH) technique: It is usually performed on uncultured interphase cells with probes designed specifically for chromosome 13, 18, 21, X and Y. The number of fluorescent signals per cell gives the number of copies of the targeted chromosome (Mann et al., 2004). The technique is known to be almost 100% sensitive and specific for detection of aneuploidies (Grimshaw et al., 2003). Another advantage is the capacity to detect triploidy where an extra set of chromosomes is present in the cell. However, the FISH technique is non-automated, time consuming and necessitates a skilled technician Mann et al., 2004; Grimshaw et al., 2003).

- Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR): This assay has been widely used for the past 20 years for rapid aneuploidy detection. It is a PCR based molecular method which uses fluorescent labeled primers to amplify specific DNA markers which are polymorphic (STRs) and unique for chromosomes 13, 18, 21, X and Y. The amplified products are separated through capillary electrophoresis. The copy number of a specific sequence of each chromosome is determined based on the intensity of the fluorescent signal. The sensitivity and specificity of the assay is in the range of 95% -100% (Grimshaw et al.,

2003; Cirigliano et al., 2009). There are several commercially available QF-PCR kits (Aneufast TM, Chromoquant aneuploidy detection kit) (Allingham-Hawkins et al., 2011). Detection of maternal cell contamination, triploidy and mosaicism as low as 15% are important advantages of these techniques (Mann et al., 2004; Cirigliano et al., 2009). However, extensive blood staining of specimens interferes with the results and interpretation of this test.

- Multiplex Ligation Dependent Probe Amplification (MLPA): It is also a PCR based method. It is relatively cheaper and less labor intensive than the FISH technique. The technique involves the use of two fluorescent labeled probes which are hybridized to specific DNA sequences and are then joined by the enzyme DNA ligase. The free ends of the ligated probes are complementary to the primer which enables the amplification of target sites. The amplified products are separated based on size using capillary electrophoresis. Each peak is considered to be the amplified product of a specific probe. The technique has a capacity to quantify up to 40-50 different target sequences in one reaction. The commercially available kit MLPA P095 kit is useful for the detection of chromosome 13, 18, 21, X and Y chromosome aneuploidies. For detection of aneuploidies, a sensitivity and specificity of 100% is attained by employing this technique (Van Opstal et al., 2009). One of the major drawbacks of this technique is the failure to detect triploidies especially in a female fetus. It is a completely automated method, and is being increasingly used as a method for RAD especially where large scale testing of samples is required.

Additional newer techniques are available such as the chromosomal microarray technique (CMA) which has the capacity to detect targeted submicroscopic deletions and duplications other than aneuploidies in the prenatal samples.

Non–invasive prenatal testing (NIPT)

In addition to invasive procedures there are non-invasive screening methods which are gaining importance in recent times. Non-invasive prenatal testing (NIPT) has been widely accepted as a part of routine care for pregnant women in many countries. The testing involves analysis of cell-free DNA fragments which circulate in the blood of the pregnant women. It can be offered after 10 weeks of gestation. This technique has a sensitivity of 99% and a specificity of 99.92% for trisomy 21. For trisomy 18, the test has 96.8% sensitivity and 99.85% specificity and for trisomy 13, 92.1% and 99.80% respectively (Gil et al., 2014). The positive predictive value of this technique is approximately 45% (10 times better than the other maternal biochemical screening tests) for low risk women. The obvious advantage of this technique is that it provides an alternative for invasive testing and the related complications. However, currently NIPT is still considered a screening test and the aneuploidy should be confirmed by an invasive definitive test. It can be used as a first tier test in the first trimester or after abnormal results of the biochemical screening tests for women unwilling to opt for invasive procedures. However, there are numerous factors including twin/triplet pregnancies, inadequate fetal fraction of DNA, higher maternal body mass index, gestational age of less than 10 weeks etc. which can result in either failure or inaccurate results. Also, the test is unable to detect triploidy in the fetus. To date this testing is available in the US, Europe and some Asian countries including India. High cost and availability are also limitations for the use of this technique.

Table 1 summarizes the principles, techniques, advantages and limitations of the important rapid aneuploidy detection tests. Prior to ordering any of these tests, appropriate pretest counseling is essential.

Conclusion

There are different rapid prenatal tests and options which can be offered to pregnant women. However, even with different options available, a rapid prenatal aneuploidy test should meet certain important criteria: (1) vastly accurate with less number of false-negative results; (2) no false-positive results because certain important irreversible decisions such as pregnancy termination may be taken as a result of an abnormal result; (3) robust with minimum failure rates and ambiguous results; (4) rapid with high specimen throughput; (5) cost effective, as the rapid test is likely to be conducted in addition to full karyotype analysis; (6) efficient in detection in specimens of low quality and quantity and (7) sensitive to detect MCC and mosaicism (Mann et al., 2004). Counseling, both pre and post test, should accompany the above mentioned testing to facilitate informed decision-making for the family.

Description	QF-PCR	FISH	MLPA	NIPT
Principle of the technique	Selective amplification of genomic DNA regions (STRs) by binding of fluorescently labeled primers to the target sequences. The products are then separated by size	Binding of a fluorescently labeled probe specific for a DNA sequence and visualized using a microscope	Two probes of unique length hybridized to target DNA sequences and joined by DNA ligase. The amplified target sites separated by size	Sequencing of cell free fetal DNA present in the maternal plasma
False result findings	No false-positive, minimal false negative results	False-positive rate of less than 1 in 30,000 cases and false-negative rate of less than 1 in 4000 (Tepperberg et al., 2001)	–	False-positive rates 0.1%-0.2% (Bianchi et al., 2014)
Sensitivity	98.9%* (Allingham-Hawkins et al., 2011) 95.65% (Cirigliano et al., 2009)	100%	100%	99% for trisomy 21, 96.8% for trisomy 18, 92.1% for trisomy 13 (Gil et al., 2014)
Specificity	100%* (Allingham-Hawkins et al., 2011) 99.97% (Cirigliano et al., 2009)	100%	100%	99.92% for trisomy 21, 99.85% for trisomy 18, 99.80% for trisomy 13 (Gil et al., 2014)
Mosaicism	Can detect as low as 15% (Mann et al., 2004)	Standard practice is to score 100 cells to exclude mosaicism at a level of greater than 10% to 15%, a level similar to that of full karyotype	Unknown sensitivity for mosaicism. Further detection has to be done by employing the FISH technique	Interpretation can be altered by presence of mosaicism
Maternal cell contamination	Results cannot be obtained in heavily blood stained samples (1%) due to the presence of MCC (Cirigliano et al., 2009)	MCC can rarely interfere with interpretation. Although it is less sensitive to MCC, in female fetus MCC goes undetectable	Female fetus detection of MCC not possible. In male fetuses the evidence of MCC is examined from the results of probes located on X chromosome	–

* Aneufast TM kit used

Table 1 Comparison of different techniques used for rapid aneuploidy detection.

Table 1 continued...

Cost	Due to automated methods, this technique is relatively cheaper than the FISH method. However commercial kits can increase the cost of diagnosis per sample	Comparatively more expensive than QF-PCR	Cost comparable to QF-PCR	Current cost is high
Turnaround Time	Average is 30.5 hours, Median is 25.1 hours (Allingham - Hawkins et al., 2011)	Reported within 24-72 hours	30 hours (Van Opstal et al., 2009)	Mean turnaround time is 5.1 business days (Bianchi et al., 2014)
Advantages	Reliable, automated, detects triploidy and mosaicism. MCC problems minimized	Reliable for detection of targeted aneuploidies. Detects triploidy and MCC does not interfere with interpretation	Low cost and amplification of different markers in one tube	Rapid method and accurate for detection of Down syndrome
Disadvantages	Commercial kits may increase the cost. Cannot detect structural chromosomal aberrations	Non-automated, requires skilled technician, labour intensive, intact cells, considerable time, fails to detect balanced rearrangements and imbalanced aberrations of chromosomal segments	Cannot detect all cases of triploidy, and sensitivity to mosaicism is unknown. MCC detection not possible in female fetus. Cannot detect structural chromosomal aberrations	Need to confirm aberrant findings using invasive methods. Relatively high percentages of test failures rates (approx 5%) (Bianchi et al., 2014)
Kits used	Aneufast TM and Chromoquant (Cirigliano et al., 2009; Allingham - Hawkins et al., 2011)	AneuVysion Assay kit	P095 detection kit (Van Opstal et al., 2009)	Sequencing libraries-Illumina True seq kit v2.5 (Bianchi et al., 2014), STR analysis: Ampfl STR minifiler kit

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