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Cell-Free DNA for Prenatal Screening

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Cell Fee DNA (cf DNA) in maternal circulation is derived from both mother and fetus.Prenatal screening for aneuploidies can be performed using these cfDNA in maternal blood. It is new noninvasive technique which provides highly accurate results but it is still screening test due to very infrequent false positive& false negative results.

CfDNA is highly fragmented hemopoitic cells. Each fragment is between 50 to 200 base pairs. There is clear pattern to fragmentation size and patterns differ between maternal &fetalcfDNA. Maternal clearance of fetalcfDNA occurs in 2 days after delivery. Half life of cfDNA is approximately one hour.

When this Prenatal Test should be done

Although fetalcfDNA can be isolated as early as 5 weeks but fraction is lower prior to 10 weeks. So it is better to do testing after 10 weeks.

Fetal Fraction

Feta cfDNA comprises about 13% of total cfDNA in late first trimester to about 50% up to time. It increases by 0.1% per week from 10-20 weeks and 1% thereafter .Minimum 4% fraction is required for testing.

Detection by cfDNA

Detection of aneuploidies Trisomy 21, 18, 13 and sexchromosome (Turner's syndrome) is performed by cfDNA. Some laboratories have begun offering for selected micro deletion & duplication syndrome. Detection of other aneuploidies is technically possible but not recommended as many of them are lethal and other may not be phenotypically normal.

Methods of Testing

Shotgun Sequencing

Random sequencing of upto 10 million mapped cfDNA fragments are required to obtain reliabie results.

Targeted Sequencing

Targeted sequencing for chromosomes 21,18,13,X,Y is done where only one million fragments can give reliable results.

SNP Genotyping

Thousands of highly polymorphic single nucleotide polymorphism located on chromosome on interest is used for testing. It can not be used in presence of additional confounding chromosome like donors oocyte .

Natera: Targeted microdetection panels are also available.

Test Performance

Over all detection rate is 97% with false positive

rate of 1.25%. Although performance varies by Trisomy because of different fetal fraction of cfDNA in different Trisomy. Detection rate of Trisomy 21 is better than others due to higher fraction of cfDNAin Trisomy 21, 15% vs. 9% in Trisomy 18.

Test Results

Report of 'No Result'

Test failure rates are 1-5% .Reason may be low fetal fraction (<4%)

Causes of low fraction may be -

- Early gestation age
- Suboptimal sample collection
- Obesity
- Type of fetalkaryotype. It is lower in Trisomy 18, 13 turners, Triploid fetus have extremely low fraction.

What to do if no Results

- Repeat test : It is successful in about 2/3 cases
- Standard markers& USG screening if patient is low risk
- Invasive diagnostic test if patient is high risk

Tests may be False Positive & False Negative

False Positive

- Confined placental mosaicism
- Demised twin
- Maternal mosaicism
- Maternal cancer, transplant recipient
- Technical issue
- Recent blood transfusion

False Negative

- Confined placental mosaicism
- Borderline low fetal fraction
- Technical issue

Screening Performance

Anomaly	Detection Rate	False Positive Rate	False Negative Rate
Trisomy 21	98.6%	1%	1.4%
Trisomy 18	94.9%	0.14%	5.1%
Trisomy 13	91.3%	0.14%	8.7%
Turners	90.3%	0.23%	-
47xxy , xxx	93%	0.14%	-
Overall	97%	1.25%	-

Screening Methods

Primary Screening Test

Use of cfDNA as primary screening test is limited universally due to its cost and lack of consensus for follow up procedures.

Secondary Screening Test

cfDNA is used as secondary screening test for trisomy 21,18,13. In secondary testing there are very few false positives (0.1-0.2%) so it prevents unnecessary invasive testing.

Overall Indications

1. It can be offered as a follow up non diagnostic test in previous screen positive, not willing for invasive test or high risk for invasive testing, repeated abortions,IVF 2. 2, 2.conception etc
3. Abnormal USG findings
4. Maternal age > 35 years
5. Familyhistoryor previous aneuploidy
6. Balanced translocation in parents.

Time Taken

Overall takes 7 days, but it may take more in Indiaif sample is processed abroad.

Limitations

- Use in twin pregnancy is controversial.
- In obese chances of failure is high.
- Invasive testing is required in positive results.

It is highly accurate test yet a screening test. It should be offered as secondary screening test to obviate the need of unnecessary invasive testing.