Prevention of Genetic Disorders: Guidelines for India

Prenatal testing is the best strategy for reducing the burden of genetic disorders and birth defects that cause significant postnatal functional impairment. Universal prenatal screening is advisable for common genetic disorders such as Down syndrome, beta thalassemia, neural tube defects. A number of prenatal screening tests are now available for Down syndrome, but knowledge about the appropriate timing of the test and the need for pre-test and post-test counseling is often limited among the primary care physicians. There is also a considerable degree of confusion regarding the prenatal screening test to be chosen in each case, due to the recent advent of a number of new and advanced screening techniques. At present there is no nation-wide consensus as to the nature and timing of these prenatal screening protocols. Due to the absence of any definite guidelines and the additional lacunae in the knowledge and awareness regarding prenatal screening amongst primary obstetric care providers in the country, the optimum benefits of these screening protocols are not reaching the population.

Beta thalassemia (homozygous or compound homozygous with other hemoglobinopathies like hemoglobin E, C, S, etc), Down syndrome (Trisomy 21) and Neural Tube Defects (NTD) are the disorders to be included for a population-based preventive program in India.

Concept of screening test vs confirmatory test

Principles of population-based screening tests are different from those for the tests done in individual patients for specific reasons like high risk of a particular genetic disease. Screening tests for population-based prevention are offered to all families irrespective of the risk and

hence have to be easy to do, cost effective (cost for the patient and time for the doctor or laboratory doing the test to be considered) and need to be offered with appropriate pretest counseling.

Suggested screening protocol for Beta thalassemia, NTD and Trisomy 21 in the Indian scenario

Test	**Gestational age (in weeks)**	**Next Step / Advantages**	**Confirmatory Test**	**Comments**
Hb, RBC indices (MCV, MCH, MCHC) & Hb HPLC (for HbF, HbA2 levels & abnormal Hb variant)	Preferably pre-pregnancy OR at first antenatal visit in the first trimester -can be done for one spouse (husband or wife) first	1. If one of the couple is found to be carrier of Beta Thalassemia or hemoglobin variant, the spouse should be tested at the earliest. 2. If both of them are found to be carriers, DNA testing of both for Beta globin (HBB) gene mutations, genetic counseling & prenatal diagnosis should be offered.	DNA based mutation testing on fetal sample (Chorionic villi or amniotic fluid) *All the steps including screening of both, mutation testing & prenatal testing should be done in time bound fashion; so as the final result are available before 20 weeks of gestation.*	1. DNA testing is essential for doing prenatal testing and may need sending patient or sample to specialized genetics centre or laboratory and hence, screening should be done pre-pregnancy or in the first trimester. 2. Due to cost constraints if only red cell indices are used for screening then some of the beta thalassemia carriers (who may have normal MCV during pregnancy) or other clinically important hemoglobinopathies may be missed.
USG with dating, NT measurement (nasal bone if possible)	12 Weeks to 14 weeks	USG is an important part of obstetric management. i] Confirmation of gestational age	CVS / placental biopsy (< 16 weeks) or amniocentesis (16 weeks & later) for karyotype if	1. Preferable mode of screening if first visit is in the first trimester 2. NT measurement & scan for nasal bone is time consuming

biochemical screening by PAPP-A & fb hCG		ii] Major malformation may be detected (not to be considered as an essential part of USG) iii] Chorionicity of twins	high risk of Down syndrome is detected on the combined biochemical and USG test. QF PCR or FISH for common aneuploidies may be offered for quick diagnosis.	many a times & need expertise 3. Those needing invasive testing may be offered CMA in addition to traditional karyotyping 4. It should be noted that using this strategy, misses the opportunity of simultaneously screening for NTD by msAFP which is included in second trimester screening by quadruple test
AFP + hCG +uE3 + InhA & simultaneous anomaly scan	16 to 18 weeks [If strict time line is followed for testing and reporting and the family knows them, then this is the best option. It covers Down syndrome, NTD screening & malformation scan in one visit]	i] Malformation scan and Quadruple screening in one visit ii] Some chromosomally abnormal fetuses get spontaneously aborted during first trimester iii] Amniocentesis is a simpler procedure, with a very low abortion rate. iv] USG visibility is appropriate & most malformations are picked up around 18 weeks. It avoids the need of repeat USG.	Amniocentesis (16 weeks & later) for karyotype. QF PCR or FISH for common aneuploidies may be offered for quick diagnosis.	1. Limited time in view of the MTP act that allows termination till 20 weeks only for a fetal abnormality 2. The cases needing invasive test can be offered CMA on prenatal sample to look for submicroscopic imbalances. It will detect 1% more chromosomal abnormalities which can cause intellectual disability. 3. Biochemical screening result need to be available by 17 to 18 weeks. For screen positive cases, results of confirmatory tests should be available by 20 weeks.

cffDNA (NIPT)	10 to 12 weeks, (so that early report is available). Can be done later as well. Should be done after NT scan. If NT > 3.5 mm (99th centile), then invasive confirmatory test is better than NIPT.	i] Should be offered to precious pregnancies who wish to screen for Down syndrome and wish to avoid invasive testing. ii] Very high sensitivity & specificity	It is also a screening test and rarely false positive and false negative results are reported. (Confirmation by karyotyping is needed before termination)	1. Though very costly, it can detect only aneuploidies of chromosomes 21, 18, 13 and this needs to be clearly explained to the patient**. 2. Failure rate of 2 to 3% needs to be conveyed to the family. 3. USG at 12 weeks and around 18 weeks for malformation scanning is necessary. 4. At less than 20% of the cost of NIPT, more than 80% fetuses with Trisomy 21 can be detected using biochemical screening tests like double marker(first trimester) or quadruple test (quadruple marker) 5. At almost the same cost, CMA in amniotic fluid can detect 1% additional chromosomal anomalies
Genetic sonogram	First & second trimester	Counseling & confirmatory test for chromosomal anomalies	CVS / placental biopsy (< 16 weeks) or amniocentesis (16 weeks & later) for karyotype. QF PCR or FISH for common aneuploidies may be offered for quick	Though very essential & useful for detecting major malformations, it is not the good choice for screening for chromosomal anomalies. However, it is very important in cases where the family wishes to

			diagnosis.	avoid invasive testing (precious pregnancies) & twin pregnancies.

Note: USG-ultrasonography, NTD-neural tube defects, NT-nuchal translucency, PAPP-A- pregnancy-associated plasma protein A, AFP- alpha fetoprotein, hCG - human chorionic gonadotropin, fb hCG - free beta subunit of human chorionic gonadotropin, uE3 – unconjugated estriol, InhA –inhibin A, CMA – chromosomal microarray, QF-PCR – quantitative fluorescent polymerase chain reaction, FISH- fluorescence in situ hybridization, MTP – medical termination of pregnancy

* Rapid detection tests like QF-PCR, MLPA or FISH help in providing results quickly. However, they can identify only aneuploidies of 21, 18, 13, X & Y. If only rapid test is done, the family should be counseled that the abnormalities of other chromosomes will not be detected and the risk of such abnormalities is 1 in 160 (0.62%) after negative result of rapid aneuploidy detection test. Hence, preferably karyotyping or CMA also should be ordered.

** The sex is not identified unless there is a sex chromosomal abnormality

Special Considerations

Certain special situations in antenatal care require special management. These include pregnancies from assisted reproductive techniques, twin pregnancies, and pregnancies occurring after previous recurrent pregnancy loss. Such families wish to avoid invasive testing and biochemical screening tests may have limitations. In such situations, USG based screening and NIPT are helpful. However, some families with such precious pregnancies do not wish to do any type of screening and are willing to take the small risk of trisomy 21.

Key points to be kept in mind for the success of a prenatal screening program:

i. The aim of screening programs is to provide information to the would-be parents about how to avoid the birth of a child with a serious genetic disorder or birth defect and help them to take decisions that suit their socioeconomic, family and emotional situation.
ii. If both spouses are carriers of beta thalassemia, they are counseled for prenatal testing in each pregnancy, irrespective of the result of testing in the previous conception.
iii. Understand the advantages and limitations of the available antenatal screening and diagnostic tests.
iv. Explain the available options to the family and let the family decide.
v. Pretest counseling for screening for Down syndrome is essential. Many individuals fail to understand the concept of screening and probability and one needs to give time to make it clear to them.
vi. These screening tests do not evaluate for all genetic disorders
vii. A negative screening test or normal amniotic fluid karyotype/CMA does not guarantee a normal baby.
viii. The counseling should be non-directive. Some families may not wish to take the screening test or might not want to proceed with an invasive test after a positive screen result.
ix. The tests or protocols being reported under research should not be applied to patient care till they are verified and accepted by the medical community.
x. A detailed family history should be obtained and minimum three-generation pedigree must be drawn to identify families at risk for other genetic disorders.
xi. High-risk pregnancies should be preferably identified in the preconception period, in order to evaluate the proband to confirm the genetic disorder, offer carrier testing as relevant, for periconceptional folic acid supplementation, to control maternal disorders like diabetes mellitus, to counsel about the teratogenic effects of anticonvulsants/ anticoagulants/ any other drugs that the woman might be taking, and to impart education and awareness for prevention of birth defects.
xii. Surveillance for other pregnancy related complications should not be forgotten.

Hence, the counseling should be non-directive and the caring physician should be supportive of the family's decision. It should be clarified that USG alone cannot rule out trisomy 21 or chromosomal disorders. In case of dizygotic twins the possibility of both twins being trisomy 21 is extremely rare. If amniocentesis detects one twin having trisomy 21 and other to be normal disomic, selective termination has risks for the normal fetus and also may be technically difficult at an advanced gestational age. These issues should be discussed in detail before embarking on a screening strategy. If the first trimester USG detects cystic hygroma or other major malformations like anencephaly, then appropriate decisions like CVS and karyotyping and / or termination of pregnancy need to be considered.

Technological revolutions like incorporation of microdeletion syndromes in NIPT and whole genome sequencing from a single fetal cell will soon pose many more prenatal screening options and challenging situations to the families and obstetricians that will need to be addressed on a timed basis.

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