NATIONAL NARCHI PERIODICAL

Volume 1 Issue 2, November 2024

Theme:
Fetal Medicine and Genetics: Beyond the Silos

National President
Dr Achla Batra

Editor-in Chief
Dr Monika Gupta

Guest Editor
Dr Sumitra Bachani

National NARCHI Secretariat
Dept of Obstetrics & Gynaecology,
25-B, C.I.T. Road, Entally, Kolkata – 700 014

NATIONAL NARCHI OFFICE BEARERS

PATRONS


Dr S.N. Mukherjee


Dr Chandravati


Dr Manjula Rohatgi

NATIONAL PRESIDENT


Dr Achla Batra

SECRETARY GENERAL


Dr Subroto Dawn

TREASURER


Dr Dolon Dawn

TASK FORCE MEMBERS (2022-24)

EDITOR-IN-CHIEF


Dr Monika Gupta

ANEMIA ERADICATION


Dr Manju Puri

PUBLIC AWARENESS


Dr Anita Sabarwal

ICMCH OFFICE BEARERS

CHAIRPERSON GOVERNING COUNCIL


Dr Veena Acharya

DEAN OBSTETRICS


Dr Sushma Chawla
Dean Obstetrics & Gynaecology

DEAN PAEDIATRICS


Dr V. P. Dandge

REGISTRAR


Dr Manju Shukla

NARCHI STATE BRANCHES OFFICE BEARERS

AHMEDABAD (GUJARAT)
Dr. Jignesh Shah (President)

AMRAVATI (MAHARASHTRA)
Dr. Shobha Potode (President)

AURANGABAD (MAHARASHTRA)
Dr. A. G. Bagdia (President)

BULANDSHAHR (U. P.)
Dr. Pramila Mittal (President)

DELHI
Dr. Manju Puri (President)
Dr. Sharda Patra (Secretary)

DURGAPUR (WEST BENGAL)
Dr. Tapas Kr Bhowmik (President)

IMPHAL (MANIPUR)
Dr. Kh Thomba Singh (President)
Dr. Siddhartha Khoiram (Secretary)

JAIPUR (RAJASTHAN)
Dr. Deepa L. Masand (President)
Dr. Sushila Saini (General Secretary)

JALANDHAR (PUNJAB)
Dr. Sushma Chawla (President)
Dean Obstetrics & Gynaecology

LUCKNOW (U. P.)
Dr. Chandravati (President)

NAGPUR (MAHARASHTRA)
Dr. Alka Kumar (President)
Dr. Neetu Singh (Secretary)

PALAYAMKOTTAI (TAMIL NADU)
Dr. L.N. Radha (President)
Dr. (Mrs) Gigi Selvan (Secretary)

RAJKOT (GUJARAT)
Dr. Nitaben Thakkar (President)
Dr. Krushna Chag (Secretary)

Content

Message from National President NARCHI

Dear All,

Warm greetings to all NARCHI members!

It gives me great pleasure to present to you all, the second issue of 'National NARCHI Periodical'. The first issue has been very well received in all parts of the country. During my Presidential tenure I have attempted to focus on academic, social and community health initiatives aimed at improving the profile of women and children in our country. This periodical is another step in the academic continuum

This issue **"Fetal Medicine and Genetics: Beyond the Silos"** is dedicated to highlight the latest evidence-based and novel topics which are relevant in our daily practice of Obstetrics & Gynaecology and Fetal Medicine. It focuses on screening, diagnosis, monitoring and treatment of various important health conditions concerning the pregnant woman and the unborn baby.

I congratulate Dr Sumitra Bachani, the worthy guest editor for this all-important issue on Fetal Medicine, for her contribution in helping to plan, write, collate, and edit the articles. Faculty of national repute have contributed to this periodical

My gratitude to Prof. Monika Gupta, editor-in-chief, National NARCHI periodical for her sincere efforts to shape up my vision in the form of this periodical.

Happy reading

Dr. Achla Batra
National President NARCHI

From the Editor-in-Chief's Desk

Dear NARCHI Members,

It is with great pleasure and pride that we introduce to you the second issue of **National NARCHI Periodical**. As Editor-in Chief of this periodical, it is heartening to share that the first issue dedicated on anaemia was well appreciated by everyone all over the NARCHI state chapters.

I humbly express my sincere thanks to **Dr Sumitra Bachani** for guest editorship for this dedicated issue with the theme of **"Fetal Medicine and Genetics : Beyond the Silos"**. She has been instrumental in establishing the Fetal Medicine and Genetic Clinic at Safdarjung Hospital, which is one of the largest tertiary referral institute of India. She has also been a dedicated faculty for the High risk Maternal and Fetal medicine Fellowship at Safdarjung Hospital.

The topics of this issue have been meticulously chosen and authors have compiled evidence-based guidelines and management approaches to address the unique challenges encountered in their day-to-day practice.

We wish to bring a stimulating dedicated issue in every quarter for our members. I am hopeful that these periodicals will serve as an invaluable resource for practitioners and academicians for important issues in reproductive and child health.

Happy reading to all !

Prof. Monika Gupta
MD, DNB, MNAMS, ACME, FICOG, FICMCH
Fellow (UK) Urogynaecology & Pelvic Reconstructive Surgery
Professor, Dept of Obstetrics & Gynaecology,
In-Charge Urogynaecology Speciality Clinic,
VMMC & Safdarjung Hospital, N Delhi. India
Chairperson AOGD Urogynaecology Committee (2023-25)
Secretary AOGD (2021-22)
Secretary NARCHI-Delhi (2018-20)
Editor ICOG (2017-18)

Guest Editorial

Greetings from Fetal Medicine & Genetic Clinic Safdarjung Hospital

The NARCHI periodical, a brainchild of Prof Achla Batra National President and shaped up by Prof Monika Gupta, Editor in chief is a great initiative by them.When I was asked to design and edit the second periodical with a theme related to Fetal medicine I took this as an opportunity to bring together most recent and novel topics which are relevant in our daily practice of Obstetrics,Gynaecology and Fetal Medicine. It amalgamates well with the core goal of improving the lives of women and neonates. I have thought of breaking the silos and exploring hitherto unique role of genetics in various maternal fetal conditions such as Infertility,Primary Amenorrhoea, Recurrent pregnancy loss and Unexplained stillbirth. Optimising quality of life in Turner syndrome and Genetic association with male infertility will be very useful for practicing Obstetricians and IVF specialists.

Fetal medicine and Genetics go hand in hand and the latter plays an important role in counselling the couples, offering the most appropriate management strategy tailored to the couples needs. There has been many recent developments in these fields from expanding the Non invasive prenatal tests to delving into fetal diagnosis, molecular genetics and diagnosing fetal morbidities such as infections.There still exists a disparity in access to these services in our country and hence such National periodicals can be an important step to reduce the gap. The articles have been written by eminent national faculty who are teachers in their institutions and they have given thought to the practical application and management concerns of their topics. Looking at the final copy it certainly is a collectors item! I hope you will find it very useful in your practice and it will arouse the desire to learn more and more about Fetal medicine and Genetics. Cheers ! and warm regards.

Dr. Sumitra Bachani
MD MNAMS FICOG FICMCH
Dip Expert Imaging Maternal Fetal Medicine (Barcelona Spain)
Fellow (MFM) AIIMS Delhi
Professor & Sr specialist
Faculty Fetal Medicine & Genetic Clinic
Dept of Obstetrics &Gynaecology
VMMC& Safdarjung Hospital.

Prenatal diagnosis of genetic disorders

Deepti Saxena
Associate Professor, Department of Medical Genetics
Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow

Introduction

Congenital malformations are seen in 3-5% of all pregnancies, most commonly cardiac, central nervous system, skeletal and urogenital malformations. Prenatal diagnosis consists of all the tests done in antenatal period to gain information regarding the health of the fetus. The purpose of prenatal diagnosis to reassure the prospective couple in case of normal results. If any fetal abnormality is detected, parents may choose termination of pregnancy or they may prepare themselves for the birth of a child with special needs. Prenatal diagnosis of genetic disorders is to test whether a specific genetic condition is present in the fetus. Invasive testing is required to obtain fetal/ placental sample for genetic testing. The type of test done on the prenatal sample depends upon the indication of invasive testing in a particular family. Prenatal testing for chromosomal abnormalities is required before undergoing fetal surgery e.g. for congenital diaphragmatic hernia and neutral tube defects.

Indications

1. Advanced maternal age
2. Positive result of serum aneuploidy screening test
3. Presence of any structural malformation on antenatal ultrasound
4. Presence of multiple soft markers on antenatal ultrasound
5. Previous child with chromosomal abnormality/ monogenic disorder
6. One of the partners is a carrier of chromosomal rearrangement such as balanced translocation or inversion
7. Both partners are carrier for any single gene disorder e.g. Thalassemia

Collection of Fetal Sample

Invasive procedures are required to collect fetal cells or tissues for further genetic testing as per the indication.

- **Pre-procedure counselling:** Before doing any invasive testing, the nature and consequence of the disorder for which prenatal testing has been advised, should be explained. The risk of abortion associated with the procedure and the possibility of failure to obtain sufficient sample and need of repeat sampling in such cases should also be explained. The possibility of fetus being affected should be provided.
- There are various methods for taking fetal sample, such as amniocentesis, chorionic villus sampling, fetal blood sampling and fetal biopsy. After obtaining fetal sample using any one of these methods, we can test the sample for chromosomal abnormalities or for single gene disorder according to the indication of invasive testing.
  - **Amniocentesis:** It can be performed after 15 weeks of gestational age. In this technique, a 20/22-G spinal needle is inserted through the maternal abdomen and uterine wall into the amniotic cavity under ultrasound guidance and 15-20 ml of amniotic fluid is aspirated. Increased incidence of talipes equinovarus is seen if amniocentesis is done before 16 weeks. Risk of abortion associated with this procedure is 0.1-0.2%. The other complications can be leakage of amniotic fluid, needle-injury to the fetus,maternal orfetal infection, maternal cell contamination of the sample, and failure of growth of cultured cells in 0.2-0.4% of cases.
  - **Chorionic villus sampling:** It can be done between 10-12 weeks of gestational age. The two commonly used approaches for chorionic villus sampling (CVS) are transcervical and transabdominal. In transabdominal approach, after applying local anesthesia using Inj. Lignocaine, under ultrasound guidance, an 18-G spinal needle is inserted into the placenta, negative pressure is applied using a 20 ml syringe and placental tissue is collected by to and fro movement of the needle. In the transcervical approach, a thin flexible tube is inserted through the vagina and cervix and sample of placenta is taken as in the transabdominal approach. The primary advantage of CVS is that it can be performed at earlier gestational age as compared to amniocentesis. The risk of abortion associated with this procedure is 0.2%. The chance of failure of growth of cultured cells is 0.5-1.0% and that of maternal contamination is 1-2%. Confined placental mosaicism can also be seen in case of chorionic villus sampling.In such a case a repeat fetal sample is obtained by Amniocentesis after

16 weeks gestation.

- **Placental biopsy:** A sample of placental tissue is taken in later part of pregnancy. The steps to obtain a sample are similar to transabdominal chorionic villus sampling.
- **Cordocentesis:** In this procedure, under ultrasound guidance, an 18-G spinal needle is inserted through the maternal abdomen and uterine wall into the umbilical vein, preferably at the site of placental insertion and 1-2 ml of fetal blood is aspirated into a 5-ml syringe. The commonest indication is to look for fetal anemia in case of hematological disorders, parvovirus B19 infections, and Rh isoimmunization. It can be used for rapid karyotyping or other molecular tests. It is also used for fetal therapy e.g. administration of any medications to the fetus or intrauterine transfusion of blood in case of fetal anemia. There is risk of premature delivery, transient fetal bradycardia, bleeding and fetal loss in 1-2% of cases.
- **Fetoscopy:** It is an endoscopic procedure during pregnancy and is mainly used for fetal surgical interventions and for laser occlusion of blood vessels as in twin-twin transfusion syndrome. It can also be used to obtain skin sample for inherited disorders of skin or liver biopsy in case of metabolic disorders, however with the widespread availability of molecular tests, utility of fetoscopy for these indications is limited.

Laboratory Tests for Chromosomal Abnormalities

- **Karyotyping:** It can detect major chromosomal abnormalities in the number and structure of chromosomes such as aneuploidy, translocations and large deletions and duplications. The cells obtained by any of the methods mentioned above are cultured in a culture medium and the cultured cells in metaphase are used for chromosomal analysis. (Figure 1) The resolution of karyotyping is around 5-10 Mb. It can detect polyploidy, aneuploidy, translocations, large deletions/ duplications, and presence of any extra chromosome known as marker chromosome. It will not detect smaller deletions/ duplications, complex rearrangements, origin of supernumerary marker chromosome and subtle telomeric rearrangements. The reporting time for karyotype is around two weeks. Also, as the analysis is subjective, so it is operator-dependent. The risk of failure to culture amniotic fluid cells is 0.2 to 0.4% and for chorionic villi, it is 0.5 to 1%. The results are available in 2-3 weeks.

Figure 1: Karyotype showing three copies of chromosome 21 i.e. Trisomy 21

Fluorescent in Situ Hybridization (FISH)

In this technique, fluorescently labeled probes bind to the target sequence on the chromosomes in metaphase/ interphase nuclei and analysis is done by detecting the fluorescence signal. It can detect aneuploidy, translocations, and microdeletion/ duplication according to the probes used. In normal individuals, two signals will be seen for each of the probe used, however, an extra signal will be seen in case of Trisomy and only one signal will be seen in case of monosomy of the chromosome/ segment of chromosome tested. It cannot detect maternal contamination. The reporting time is 1-2 days as cell culture is not required .

Quantitative Fluorescent – Polymerase Chain Reaction

QF-PCR is based on amplification of specific DNA sequences known as STR (short tandem repeats) markers. The STR markers are polymorphic in length between various individuals. Using fluorescent primers, the amplified segments can be visualized and quantified as peak areas by fragment analysis using capillary electrophoresis. In normal individuals, there will be two peaks (ratio 1:1) corresponding to each of the DNA sequence tested. In monosomy, only one peak corresponding to the marker on that chromosome will be seen, whereas in case of Trisomy, there will be either three peaks or the ratio between two peaks will be 2:1. In can detect aneuploidy, polyploidy, and maternal contamination. QF-PCR is quicker, less costly, and less labor intensive than conventional karyotyping. The result is available in 2-3 days.

Multiplex Ligation Dependent Probe Amplification (MLPA)

In this technique pairs of oligonucleotide pairs are used

that can hybridize to a specific genomic sequence and multiple such probes (up to 50) can be multiplexed in the same reaction.(Figure 2) After binding to the target sequence the probes are amplified and the amount of amplification product is proportional to the amount of target sequence present in the sample. In case of deletion, the amplification product will be reduced in comparison to the reference and it will be increased in case of duplication of the target sequence. It can be used to detect specific chromosomal deletions/ duplications according to the clinical indication. The reporting time is 2-3 days .

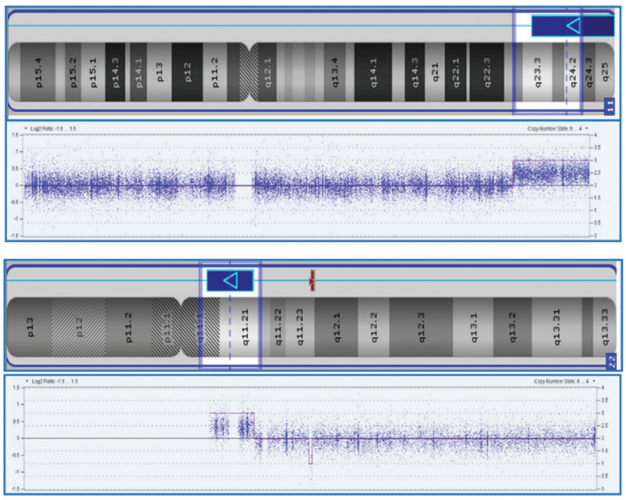


Figure 2: MLPA showing deletion at 22q11.2 region suggestive of DiGeorge syndrome

Cytogenetic Microarray

It has been recommended by various societies such as American College of Obstetrician and Gynecologists that cytogenetic microarray (CMA) should be available to all women undergoing invasive diagnostic tests for any indication. It can detect submicroscopic chromosomal abnormalities and it has been estimated that 1.7% of those with a normal ultrasound examination will have pathogenic/ likely pathogenic copy number variation which is further increased to 6-8% in case of presence of structural malformation in the fetus. (Figure3) There are various types of microarrays such as "Targeted microarrays" developed to look for copy number changes at specific regions of genome and "Whole genome microarrays" used to look for copy number changes in the entire genome.

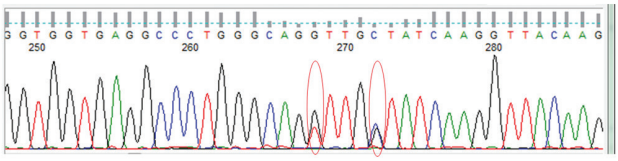


Figure 3: Chromosomal microarray showing gain of 18 Mb at 11q23.3 and 3.8 Mb gain at 22q11.1

It can also be classified based on the type of probe used, such as oligonucleotide microarray where probe is composed of a small DNA sequence known as oligonucleotide and SNP array where both types of probes – the oligonucleotide probes as well probes targeting single nucleotide polymorphisms (SNPs) are used. It has very high resolution, fast and report is available in 7-10 days. DNA can be extracted directly from fetal cells or from cultured cells.

The advantages of using CMA include detection of submicroscopic deletions and duplications. SNP array can also detect uniparental disomy (isodisomy), mosaicism and polyploidy. The limitations of CMA include inability to detect balanced rearrangements, single nucleotide variants and low-level mosaicism (<30%), imprinting disorders, and interpretation of variants of uncertain significance.

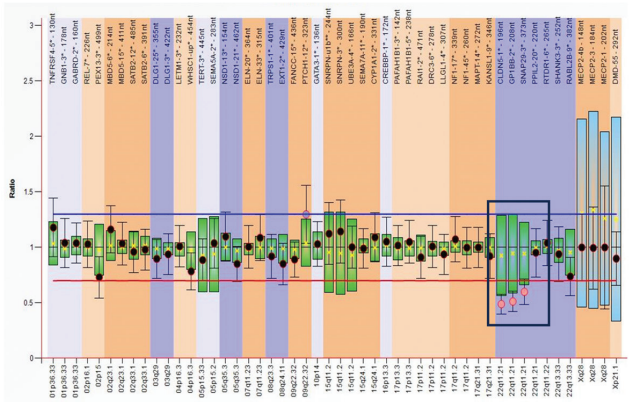


Figure 4: Sequence chromatogram showing nucleotide change, c.92+1G>T and c.92+5G>C in HBB gene

Tests for Single Gene Disorders

- Sanger sequencing: In the families where, previous child is affected with a single gene disorder such as thalassemia, targeted mutation analysis of the variant present in the affected child can be done by Sanger sequencing. (Figure 4)
- Multiplex Ligation dependent probe amplification (MLPA): For single gene disorders also, MLPA can be used to detect exon level deletions/ duplications such as for Duchenne muscular dystrophy and Spinal muscular atrophy.
- Exome sequencing: In case of fetal malformations, even after doing fetal karyotype and cytogenetic microarray, around 65% of the cases remain unsolved. As some of them are due to single gene disorders, exome sequencing can be done and it has been found to detect the underlying etiology in around 10-50% of cases with fetal malformations. The diagnostic yield is more in case of multiple malformations and depends upon the organ system affected.

- Future prospects: In a family with a child affected with any genetic disorder, the risk of birth of another child having same disorder is increased and prenatal diagnosis is required to assist the reproductive decision making. At present, fetal sample is taken by invasive techniques for further genetic testing. However, with the discovery of cell free-fetal DNA and its widespread use in screening for chromosomal abnormalities, in future cell free fetal DNA can be used in prenatal diagnosis of monogenic disorders also.

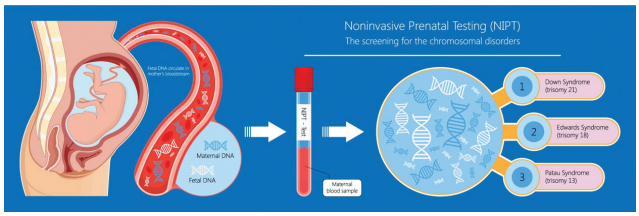
Suggested Reading:

1. Stembalska A, Slezak R, Pesz K, Gil J, Sasiadek M. Prenatal diagnosis--principles of diagnostic procedures and genetic counseling. Folia Histochem Cytobiol. 2007;45 Suppl 1:S11-6. PMID: 18292841.
2. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol2015;45:16–26.
3. Xiao H, Yang YL, Zhang CY, Liao EJ, Zhao HR, Liao SX. Karyotype analysis with amniotic fluid in 12365 pregnant women with indications for genetic amniocentesis and strategies of prenatal diagnosis. J Obstet Gynaecol. 2016;36(3):293-6. doi: 10.3109/01443615.2015.1041889.
4. Tepperberg J, Pettenati MJ, Rao PN, Lese CM, Rita D, Wyandt H, Gersen S et al. Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH): 2-year multi-center retrospective study and review of the literature. Prenat Diagn. 2001 Apr;21(4):293-301. doi: 10.1002/pd.57.
5. Mann K, Petek E, Pertl B. Prenatal detection of chromosome aneuploidy by quantitative fluorescence PCR. Methods Mol Biol. 2008;444:71-94. doi: 10.1007/978-1-59745-066-9_6. PMID: 18425473.
6. Willis AS, van den Veyver I, Eng CM. Multiplex ligation-dependent probe amplification (MLPA) and prenatal diagnosis. Prenat Diagn. 2012 Apr;32(4):315-20. doi: 10.1002/pd.3860. PMID: 22467161.
7. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018 Feb;109(2):201-212. doi: 10.1016/j.fertnstert.2018.01.005.
8. Van den Veyver IB, Chandler N, Wilkins-Haug LE, Wapner RJ, Chitty LS; ISPD Board of Directors. International Society for Prenatal Diagnosis Updated Position Statement on the use of genome-wide sequencing for prenatal diagnosis. Prenat Diagn. 2022 May;42(6):796-803. doi: 10.1002/pd.6157. PMID: 35583085

Non Invasive Pre-Natal Screening : Recent Advancements

Alka Mukherjee[1], Apurva Mukherjee[2]
[1]DGO FICOG FICMCH PGDMLS PGDCR, [2]MS OBGYN FRM Dip Cosgyn,
Consultant Mukherjee Multi speciality Hospital, Nagpur

Non-Invasive Prenatal Screening (NIPS) is a test that analyses cell-free fetal Deoxyribonucleic acid (cfDNA) in the mother's bloodstream to detect potential chromosomal abnormalities in the foetus.



https://www.advancedwomensimaging.com.au/nips

Figure 1: NIPS Diagrammatic representation Current guidelines recommend to use NIPS for aneuploidy screening and RhD genotyping.

- Trisomy 21 (Down syndrome)
- Trisomy 18 (Edwards syndrome)
- Trisomy 13 (Patau syndrome)
- Sex chromosomal aneuploidy (e.g., Turner syndrome, Klinefelter syndrome)
- Fetal Rhesus (Rh) D genotype

Other tests are in research setting in India such as:

- Single gene disorders/ Monogenic disorders
- Sex Chromosome related disorders
- Micro-deletions

Who is Eligible

1. Pregnant women of all ages.
2. Women with a previous child affected with aneuploidy
3. Women with ultrasound findings of soft markers.
4. Women with biochemical screening/combined first trimester screening having intermediate risk.

When to perform NIPS: As early as 9-10 weeks of gestation, ideally to be performed after first trimester screening ultrasound (NT/NB scan) and can be performed till delivery.

NIPS Methodology

Currently, three main techniques are used commercially: massively parallel shotgun sequencing (MPSS), targeted massively parallel sequencing (t-MPS), and single nucleotide polymorphism (SNP)-based methodology. Several other approaches of NIPT have been investigated, including DNA methylation-based assay, cell free foetal RNA assay, and enrichment and isolation of foetal cells in maternal circulation.

Here we shall discuss the above mentioned three techniques in detail :

1. MPSS

The MPSS method is the commonest among published studies. This approach is based on the observation that the whole human genome is equally represented in the plasma cffDNA of pregnant women. With MPSS, all maternal and foetal DNA fragments are sequenced and mapped to the human genome, and the ratio of the number of fragments from the chromosome of interest (such as chromosome 21) to that from a reference chromosome that is expected to be disomic is calculated. The presence of foetal aneuploidies can be detected by calculating chromosomal sequence read deviations from a reference dataset (a Z score) and the proportion of 1 Mb bp (in percent) of a given chromosome that has a Z score > 3. Since maternal and fetal DNAs are not differentiated by MPSS, a positive result could be due to abnormalities originating from the pregnant woman instead of the foetus such as a case of maternal neoplasm, and maternal Turner mosaic.

2. t-MPS

The t-MPS approach is basically the same as the MPSS approach, except that only DNA fragments from the chromosome of interests are being captured from the cell free DNA in the maternal plasma (e.g. chromosome 21, 18, 13 and sex chromosomes). Thus the cost could be minimized because only the chromosomes of interest are captured and sequenced rather than the whole genome.

3. SNP

SNPs are the most common genetic variation among individuals. In the SNP-based method, thousands of SNPs for each chromosome of interest are captured from the cell free DNA and examined by

next generation sequencing. Foetal- and maternal-derived SNP profiles are produced by testing cell-free DNA and maternal white cells, and the relative amount of the paternal-specific alleles in maternal plasma is determined and compared with the hypothetical model in which the foetus is assumed to be trisomic, disomic, monosomic or has triploidy. This approach enables study on foetal genetic information alone, and avoids the possibility of a false call due to maternal abnormalities. It also has the theoretical advantage of detecting triploidies, zygosity of twin pregnancies, maternal mosaicism and uniparental disomy, which were not detectable by MPSS or t-MPS method.

How does NIPS Work?

During pregnancy, the placenta releases cfDNA into the mother's bloodstream through apoptosis. A bioinformatics analysis is performed on a cell-free DNA (cfDNA) sample extracted from the mother's placenta to determine the fetal fraction (FF). This FF measurement is vital for quality control and ensuring statistical confidence in the test results. The fetal fraction should be 4% or more for a valid result. When the cell free DNA is fragmented, the maternal component having 166 base-pair can be differentiated from fetal DNA component having 143 base-pair.

Non-Invasive Prenatal Testing (NIPS) is NOT recommended for:

1. Previous Pregnancy with Chromosomal Abnormality excluding aneuploidy.
3. Family History of Monogenic disorder
4. Thickened Fetal nuchal translucency
5. Higher order Pregnancies (Triplets and above)
6. History of Recurrent Miscarriage
7. Previous Child with Birth Defect
8. Women with cancer, transplant recipients.

Table1: Advantages and Limitations of NIPS.

Advantages	Limitations
Non-invasive (no risk of miscarriage)	Screening test, Not diagnostic
High accuracy (>99% for trisomies)	False positives and false negative can occcur
Early detection (as early as 9-10 weeks)	Limited detection of chromosomal abnormalities
Reduced need for invasive testing	May not detect all genetic conditions
Provides assurance in low risk pregnancy	False positive can result in unnecessary anxiety
Simple blood test	Over-testing: May lead to unnecessary follow-up testing or procedures
Turn around time: 10-14 days	Lack of regulation: NIPT is not regulated by the FDA, which raises concerns about quality and accuracy
	Counselling challenges: Requires skilled counselling to interpret results and discuss limitations
	Potential for misuse: May be used for sex selection or other non-medical purposes

Submicroscopic microdeletions or duplications less than 5 Mb are generally not detectable by NIPT except one or few microdeletions that have been specifically optimized for their detection. It follows logically that if further chromosomal investigation is required in foetuses with structural abnormalities, NIPS is an inappropriate test. An invasive test with Chromosomal Microaarray (CMA) study should be offered, this echoes the professional guideline and recommendations issued by American college of medical geneticists (ACMG).

Steps of NIPS

Step 1: Blood Sample Collection: A healthcare professional collects 10 ml of maternal blood in the "Cell free DNA blood collection tube." It contains a preservative reagent that limits the release of genomic DNA allowing the isolation of high quality cell-free DNA.

Step 2: Blood Processing

The blood sample is sent to a laboratory where it is processed to extract the cell-free DNA (cfDNA) from the plasma.

Step 3: cfDNA Analysis

The extracted cfDNA is then analysed using advanced genetic sequencing technologies, such as Next-Generation Sequencing (NGS) or Massively Parallel Sequencing (MPS).

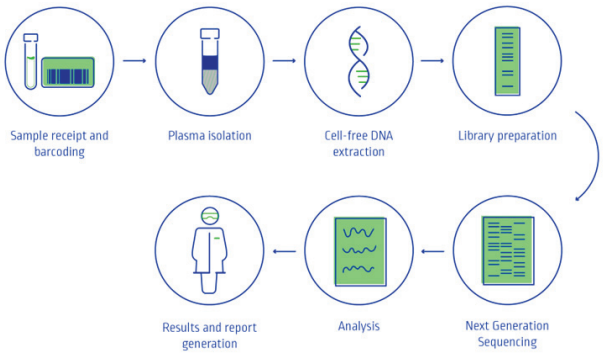
Step 4: Chromosomal Analysis

The genetic sequencing data is then analysed to detect any chromosomal abnormalities, such as trisomies (e.g., Down syndrome) or sex chromosomal abnormalities.

Step 5: Results Interpretation

The results are interpreted by a doctor or a genetic

counsellor, who will discuss the findings with the expectant parents.



https://nextbio.co.za/divisions/specialists/genetics/triscreen

Figure 2: Steps of NIPS Procedure

Approach after a positive NIPS report:

Post-test counselling is of utmost importance. Points to be discussed after a positive test result are;

- The sensitivity and false positive rate for particular aneuploidy detected
- Need to perform a confirmatory test/ Invasive test and its yield, cost, turn around time and complications like risk of miscarriage
- Timing of performing invasive testing to be decided depending on period of gestation, availability of genetic sonogram

False Positive NIPS - What to Expect :

It is increasingly realized that false positive NIPS results may not be simply a technical failure, but could be due to foetal mosaicism, confined placental mosaicism, interference from a vanished twin, maternal mosaicism, maternal transplantation, or maternal cancer. Since the major source of cffDNA is from the placenta, NIPS can be considered as equivalent to direct preparation studies on chorionic villus samples. Confined placental mosaicism is a significant cause of false positive of NIPS, in particular when is used to study chromosomal abnormalities with high miscarriage rate in the first trimester, such as trisomy 18 or trisomy 22.

Approach after a "No Call" or "Intermediate" Report

It occurs because of low fetal fraction which may be due to performing the test before 9 weeks, maternal obesity or multiple pregnancy. If NIPS is repeated there is 60-70% yield. But in selected cases performing an invasive testing is more appropriate. All couples should be offered a first trimester screening ultrasound (NT/NB scan) and second-trimester anomaly ultrasound since these may occur with or without foetal aneuploidy. Women with a positive screening test result for foetal aneuploidy should undergo genetic counselling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results.

❖ NIPS in TWIN Pregnancy

Twin pregnancies are now increasing due to wide use of assisted reproductive techniques and associated with higher pregnancy complications and adverse outcomes. Prenatal clinical management is intensive and has been hampered by inferior screening and less acceptable invasive testing. For aneuploidy screening, meta-analyses shows that non-invasive prenatal screening (NIPS) through analysis of cell-free DNA (cf-DNA) is superior to serum and ultrasound-based tests. The sensitivity to screen for aneuploidy particularly Trisomy 21 is comparable to singleton pregnancy. Individual foetal fractions in dizygotic twin pregnancies can be measured. Zygosity can be established using NIPS and this can be particularly useful when there are concerns about chorionicity or determining whether one versus two foetuses are affected.Vanishing twins can be detected through NIPS and this testing could be considered for some apparently singleton pregnancies with complications. The SNP-based NIPS will identify a vanishing dizygotic twin (with or without aneuploidy) provided the maternal plasma contains sufficient fetal cf-DNA.Analysis of single nucleotide polymorphisms (SNPs) in cf-DNA allows distinction between maternal and foetal sequences. In dizygotic twin pregnancies, SNPs can also allow an assessment of the cf-DNA from each separate foetus. This can be done as a separate analytic component of a counting-based NIPS or integrated into the overall assessment of zygosity and aneuploidy. Still it will not be able to specify which twin is affected with the abnormal results which remains a drawback.

Most laboratories do not offer cf-DNA screening for sex chromosome abnormalities or limit the testing to monosomy-X in multifetal pregnancies. Many sex chromosome abnormalities are mosaic and not confirmed after initially being detected in trophoblasts. Additionally, results for X-chromosome aneuploidies need to be interpreted cautiously when using methods that do not distinguish between maternal and foetal genotypes (i.e., counting based NIPS) because maternal-age related gain and loss of an X-chromosome in maternal tissues is a recognized cause for false-positive NIPS results.

❖ **NIPD in Rh Isoimmunization**

- Non-invasive Rh typing can reliably be done from 11 weeks gestation onwards, on circulating cell free foetal DNA, which is present in low and variable amounts in plasma of pregnant women.
- Indications for non-invasive prenatal Rh typing are
  (a) to target antenatal prophylaxis
  (b) to guide antenatal and postnatal prophylaxis
  (c) to identify cases at risk in Rh-alloimmunized women.
- In a diagnostic setting in allo-immunized women, presently droplet digital PCR (ddPCR) is the method of choice and allows the inclusion of controls for the presence of foetal DNA, such as methylated RASSF1a.
- The major clinical benefit of implementing foetal RHD typing is that approximately 95% of the D-negative women carrying a D-negative foetus can avoid the unnecessary administration of Rh-Ig.
- In Rh isoimmunised pregnancies with history of previously affected/hydropic fetus, RhD genotyping can avoid unnecessary interventions like serial MCA-PSV measurements, administration of Immunoglobulin, Intra-uterine transfusion

❖ **NIPS for Sex Chromosome aneuploidies**

NIPS for the sex chromosome aneuploidies (45,X, 47,XXY, 47,XXX, and 47,XYY) differs significantly from that for the autosomal aneuploidies (trisomy 13, 18, and 21).

As a group, sex chromosome aneuploidies occur more commonly (1/400) than any one isolated autosomal aneuploidy, the phenotypic variation is greater, the role of mosaicism more challenging, and the positive predictive value of a high-risk NIPS result is substantially lower. These considerations should be identified during pretest counselling, the inclusion of sex chromosome testing offered separately, and the differences from autosomal aneuploidy NIPS clearly delineated.

❖ **NIPS for Monogenic Disorders**

The NIPS market has expanded beyond testing for common aneuploidy of chromosomes 21, 18, and 13 to include other sub-chromosomal copy number variants (CNVs), SCAs, and rare autosomal trisomies (RAT). This expanded NIPS field was created through screening technology advancements that broadened the testing offering to cover more conditions, overcoming the limitations of traditional screening methods. For example, the development of high throughput molecular counting methods with single base pair resolution from companies such as Billion to One and Medicover Genetics has enabled the clinical implementation of NIPS for monogenic disorders such as sickle cell disease, cystic fibrosis, hemoglobinopathies, and spinal muscular atrophy.

Unlike carrier screening, single-gene NIPS does not require paternal DNA samples, which previously hindered testing sensitivity due to poor uptake. In addition, long-read sequencing platforms have allowed researchers to explore long cfDNA molecules which monogenic diseases and detection/monitoring of pregnancy-associated disorders such as preeclampsia. More recently, researchers have combined cfDNA screening with artificial intelligence to widen the scope of disease testing, helping detect epigenetic aberrations, such as foetal congenital heart defects.

❖ **NIPS in Pre Implantation genetic Diagnosis – NiPGT**

The recent discovery of DNA within the blastocoele fluid (BF) of blastocysts and in spent embryo culture media (SCM) has led to interest in the development of non-invasive methods of PGT (niPGT). The limitations of niPGT-A are a lower quantity and lesser quality of the cell-free genetic material, and its unknown origin.

❖ **NIPS in RAT :**

The term 'rare autosomal trisomy' (RAT) refers to a trisomy for any autosome other than 13, 18 and 21. Constitutional forms of these aneuploidies are almost invariably lethal, and hence the overwhelming majority of cases represent mosaicism which may be confined to placental tissue. Trisomy 7 is the most commonly detected RAT, while trisomies 15, 16 and 22 are more frequently detected via NIPS than previous studies using chorionic villous sampling data. Constitutional RATs are usually associated with spontaneous miscarriage, but mosaic RATs may be associated with a range of adverse outcomes such as placental insufficiency, low birth weight, miscarriage and structural anomalies due to foetal mosaicism.

Suggested Reading

1. Perrot, A., Horn, R. The ethical landscape(s) of non-invasive prenatal testing in England, France and Germany: findings from a comparative literature review. Eur J Hum Genet 30, 676–681 (2022). https://doi.org/10.1038/s41431-021-00970-2

2. Moise KJ Jr, Gandhi M, Boring NH, et al. Circulating Cell-Free DNA to Determine the Fetal RHD Status in All Three Trimesters of Pregnancy. Obstet Gynecol 2016;128:1340–6.

3. Grati FR. Implications of fetoplacental mosaicism on cell-free DNA testing: a review of a common biological phenomenon. Ultrasound Obstet Gynecol. 2016;48:415–23.

4. Samura O, Sekizawa A, Suzumori N et al. Current status of non-invasive prenatal testing in Japan. J Obstet Gynaecol Res 2017; 43: 1245–1255.

5. Non-invasive prenatal testing in the context of IVF and PGT-A, Best Practice & Research Clinical Obstetrics & Gynaecology,Volume 70, 2021,Pages 51-62.ISSN 1521-6934, https://doi.org/10.1016/j.bpobgyn.2020.07.004.

6. Lannoo, L., van Straaten, K., Breckpot, J. et al. Rare autosomal trisomies detected by non-invasive prenatal testing: an overview of current knowledge. Eur J Hum Genet 30, 1323–1330 (2022). https://doi.org/10.1038/s41431-022-01147-1

Approach to Primary Amenorrhoea: A Gynaecologist's Perspective

Seema Thakur[1], Mayank Nilay[2]

[1]Director, Medical Genetics, Fortis Hospital, AA Block, Shalimar Bagh, New Delhi, Fortis Hospital, Vasant Kunj, New Delhi, Rainbow Children Hospital, Malviya Nagar, New Delhi, [2]Assistant Professor, Department of Medical Genetics, Post Graduate Institute of Child Health, Sector-30, Noida, U.P.

Introduction

Menarche is an important milestone in a woman's life. Attainment of menarche signifies feminity and first step in achieving fertility. Feminity and motherhood are two wonderful assets bestowed upon women.

A normal menstrual cycle can occur only in the presence of normal functioning:

- hypothalamus–pituitary axis
- Ovaries
- Outflow tract

Any dysfunction in any of these systems can result in absence or cessation of menses and is broadly classified as Premature ovarian failure (POI). It is defined by the following association of one clinical criterion plus one biochemical criterion:

Clinical - Primary amenorrhoea or secondary amenorrhea of >4 months.

Biochemical- FSH elevation >25 IU/L on two assays at >4 weeks' interval, with low estradiol.

Primary amenorrhoea/ secondary amenorrhoea are a type of Premature ovarian insufficiency (POI)

Primary amenorrhoea is defined as

1. Absence of menses by 15 years of age in the presence of secondary sex characters or
2. Absence of menses by 13 years of age if no secondary sex characters.

It affects approximately 0.01% of girls.

Secondary amenorrhea is defined as absence of previously regular menses for three months in a previously menstruating women of reproductive age.

Patients meeting the criteria for either primary or secondary amenorrhea warrant an evaluation.

Causes of Primary Amenorrhoea

The causes are due to abnormalities in

- Hypothalamic pituitary axis- 10%
- Gonadal lesions- 30%
- Outflow tract abnormalities- 10%
- Idiopathic- 50%

In clinical practice, classification of amenorrhoea is done on the basis of raised, normal or low FSH as mentioned below:

I: Hypogonadotrophic hypogonadism- FSH <5 IU/ml

II: Hypergonadotrophic hypogonadism- FSH >25 IU/ml

III. Eugonadotrophic hypogonadism- FSH 5-25 IU/ml

I. Hypogonadotrophic hypogonadism

Congenital hypogonadotropic hypogonadism	Acquired hypogonadotropic hypogonadism
Kalman syndrome	Brain tumor
Prader–Willi syndrome	Chemo-radiotherapy
Septo-optic dysplasia	Hyperprolactinemia
	Functional hypothalamic amenorrhea

II. Hypergonadotrophic hypogonadism

A. Gonadal dysgenesis	B. Enzyme deficiency	Premature ovarian failure
1. Abnormal karyotype a. Turner syndrome -45, X b. Mosaicism 2. Normal karyotype a. Pure gonadal dysgenesis i. 46, XX ii. 46, XY (Swyer syndrome)	1. 17a - Hydroxylase deficiency 2. 17, 20 - Lyase deficiency 3. Aromatase deficiency	1. Idiopathic 2. Injury a. Chemotherapy b. Radiation 3. Resistant ovary syndrome

III. Eugonadotrophic hypogonadism

Mullerian agenesis (Mayer-Rokitansky- Kuster-Hauser syndrome)
Complete androgen resistance (testicular feminization)
- Intrauterine synechiae (Asherman syndrome)
Imperforate hymen
Transverse vaginal septum
Cervical agenesis—isolated
Cervical stenosis—iatrogenic
Vaginal agenesis—isolated
Endometrial hypoplasia or aplasia—congenital

Approach to a case with Primary amenorrhoea (Fig 1)

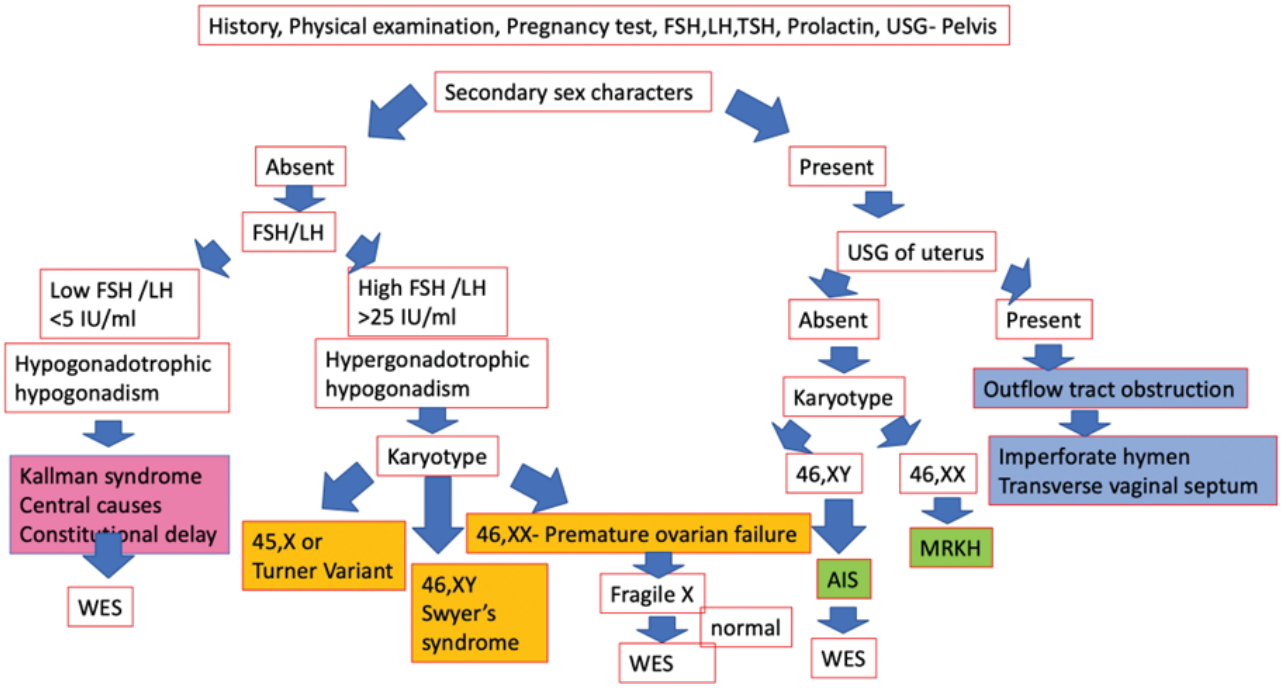


Fig 1: Approach to a case with Primary amenorrhoea

The basic requirements for normal menstrual function include four anatomically and functionally distinct structural components:

- Hypothalamus,
- Anterior pituitary gland,
- Ovary, and the
- Genital outflow tract composed of the uterus/ endometrium, cervix, and vagina.

History:

- Hypothalamus and pituitary lesions- History of anosmia, headache, visual problems, galactorrhoea
- History of head trauma/ surgery.
- Ovary- Features of hypogonadism such as delayed puberty, secondary sexual characters
- Short stature
- History of radiotherapy/ chemotherapy.
- Cyclical pain abdomen.

Physical examination

Physical examination for features of Turner stigmata and examination of secondary sexual characters which includes examination of breast, pubic and axillary hairs and their stages should be noted. Signs of hyperadrogenemia such as virilisation, hirsuitism should be looked for.

- Height- A short stature in a girl with primary amenorrhoea is highly suggestive of Turner syndrome
- Weight- Both underweight and overweight can be associated with Primary amenorrhoea
- Turner stigmata- webbed neck, low hair line, increased carrying angle, short fourth metacarpal, wide spaced nipples.
- Blood Pressure. – Hypertension indicates Cushing syndrome/ 17 alpha hydroxylase deficiency.
- Focal neurological deficits and vision assessment for (Hypothalmo-pituitary ovarian)HPO axis lesions.
- Secondary sex characters – like examination of breast, axillary hair, pubic hair.
- Features of androgen excess include hirsutism, excessive acne, and virilization (i.e., enlarged clitoris, deepening of the voice).
- Heart and lung exam abnormalities may indicate a chronic disease or illness.
- Abdomen should be examined for any palpable masses and signs of chronic disease (e.g., hepatomegaly), palpable uterus due to haematocolpus or ovarian neoplasms.
- Pelvic examination should include
  - The maturation of the external genitalia, presence of pubic hair and clitoromegaly.

- o An imperforate hymen is diagnosed by a bulging membrane without hymenal fringe near the introitus.
- o A shortened vagina without a palpable cervix suggests müllerian or vaginal agenesis, a transverse vaginal septum, or complete androgen insensitivity syndrome.

Investigations in Primary Amenorrhoea

First Visit	Second Visit	Third Visit
Urine pregnancy test	Karyotype	Fragile X analysis in 46, XX females
Pelvic ultrasound (typically transabdominal)	Absent uterus (Hypergonadotrophic hypogonadism)	
Serum FSH, LH, and estradiol	MRI/CT scan brain (Hypogonadotrophic hypogonadism)	
Serum prolactin		
Serum thyroid - stimulating hormone (TSH)		
In case of features of virilisation- 17 hydroxyprogesterone		
Dihydroepiandrostein-dione sulfate (DHEAS)		

Genetic Analysis in Primary Amenorrhoea(Whole exome sequencing)*

1. Family history of Primary amenorrhoea
2. Consanguinity
3. 46, XX gonadal dysgenesis
4. 46, XY with androgen synthesis defects or androgen resistance defects e.g. Androgen insensitivity syndrome or 5 alpha reductase deficiency

*Monogenic causes of premature ovarian insufficiency are highly heterogeneous and most of them may present with ovarian dysgenesis and primary amenorrhea (PA). The variable expressivity in clinical presentation is typical in such cases and may be due to multifactorial or oligogenic defects. In this era of easy availability of whole exome sequencing, certain monogenic causes of primary amenorrhea are evident and need to be considered if cytogenetic test results are negative.

Single gene disorders causing primary amenorrhoea can be syndromic or nonsyndromic.

Syndromic conditions with single gene disorders

1. 46XY sex reversal due to mutation or deletion on the sex determination region of the chromosome Y (SRY gene) SRY mutation is present in 10-20% cases of Swyer syndrome
2. Late onset congenital adrenal hyperplasia (CAH): Late-onset CAH due to 21-hydroxylase deficiency manifest as hyperandrogenic amenorrhea due to disease causing variations in CYP21A2 gene;
3. Galactosemia is a hereditary disorder of galactose metabolism caused by deficiency of galactose-1-phosphatase uridyltransferase (GALT). Proper galactose utilization is required for normal ovarian function. Around 80%-90% female patients with GALT mutations that partially or completely abolish GALT activity exhibit PA. Due to ineffective metabolism galactose accumulates in the ovary to toxic levels and follicles suffer atresia.
4. Ataxia telangiectasia: an autosomal-recessive disorder characterized by cerebellar changes, oculomotor dysfunction, immunodeficiency, increased risk for cancer, and gonadal abnormalities. ATM (Ataxia Telangiectasia Mutated) gene encodes a protein which is involved in the cellular response to DNA damage and the processing of the DNA strand breaks. Females with ATM mutations have primary amenorrhea owing to ovarian dysgenesis and defects in primordial germ cells.
5. Kallmann syndrome is an X-linked disorder, which is characterized by delayed puberty and anosmia and some females may present with primary amenorrhea. It results from mutations that cause a defect in migration of the GnRH neurons and the olfactory neurons. The genes most commonly implicated in this syndrome include ANOS1, CHD7, FGF8, FGFR1, and PROK2.
6. Certain receptor abnormalities and enzyme deficiencies also result in primary amenorrhea and include Androgen insensitivity syndrome (AR gene), 5-Alpha-reductase deficiency (SRD5A2), 17-Alpha-hydroxylase/17, 20-lyase deficiency (CYP17A1) and P450 oxidoreductase deficiency (POR).

Non syndromic causes of Premature ovarian failure

Mechanism	Genes
Germ Cell development	NANOS3, EIF4ENIF1, NOTCH2
Oogenesis and folliculogenesis	FSHR, LHCG, GJA4
Steroidogenesis	NR5A1, STAR, ALOX12B
Hormone signalling	BMP15, BMPR2, BMPR1A, BMPR1B, GDF9, SOHLH1, SOHLH2, FIGLA, LHX8, NOBOX, ATG7, ATG9A POLR3A, POLR3B

Meiosis and DNA repair	MSH4, MSH5, SPIDR, FANCM, FANCL, BNC1, WDR62, BRCA2, TP63, MCM8, MCM9, STAG3, PSMC3IP, HFM1, NUP107, SYCE1, SYCP2L

Genetic tests such as exome sequencing in those with idiopathic premature ovarian insufficiency have a diagnostic yield of 25-30% with ~ 70% of cases the cause is still unknown.

Common cases of Primary amenorrhoea in gynaecological practice

1. Imperforate hymen
2. Meyer Rokitansky Kuster Hauser syndrome
3. Turner syndrome
4. Swyers syndrome
5. Androgen insensitivity syndrome
6. Hypogonadotrophic hypogonadism or Kallman syndrome

1. Imperforate hymen

Patients present with cyclical pain abdomen and a palpable abdominal mass. An imperforate hymen usually presents as a bluish bulging mass due to hematocolpos at the introitus. The treatment is simple incision of hymen to allow for drainage of menstrual blood.

2. Mayer Rokitansky KüsterHauser (MRKH) syndrome

MRKH syndrome is a common cause of primary amenorrhoea and accounts for 20% of causes of primary amenorrhea. It is associated with mullerian agenesis. Patient will have primary amenorrhoea with normal secondary sex characters and normal FSH, LH and oestradiol and ultrasound shows absent uterus. Patients can also have renal and skeletal anomalies.

Treatment by hormones is not indicated here. In cases of associated vaginal agenesis, vaginoplasty can be done. Pregnancy can be achieved by surrogacy using patient's ovum.

3. Turner syndrome (TS) and gonadal dysgenesis

Gonadal dysgenesis refers to incomplete or defective formation of the gonads, which is the most common cause of primary amenorrhea (30%–40%).These women have decreased estradiol and an elevated FSH level. There are many types of gonadal dysgenesis: pure, partial, and mixed.

A. Pure gonadal dysgenesis patients have streak gonads with karyotypes such as 45, XO (Turner syndrome), 46,XX, or 46,XY (Swyer syndrome).

B. Patients with partial gonadal dysgenesis have bilateral dysgenic gonads, and

C. Patients with mixed gonadal dysgenesis present with one streak and one dysgenetic gonad.

Patients with Turner syndrome have pure gonadal dysgenesis.

About two thirds of cases of gonadal dysgenesis are caused by Turner syndrome.

- TS occurs in 1 out of 2,000–4,000 females,
- Most common karyotype is 45,X
- TS presents with short stature, primary amenorrhoea due to ovarian dysgenesis, cardiac and renal anomalies,

46,XX gonadal dysgenesis and 46, XY gonadal dysgenesis are other forms of gonadal dysgenesis. 46,XX gonadal dysgenesis is caused by defects in autosomal genes. Girls with 46,XX gonadal dysgenesis present with amenorrhea and absent secondary sex characteristics and raised gonadotrophins.

4. Swyer's syndrome

46,XY gonadal dysgenesis is also called Swyer syndrome. About 10% to 20% of these cases are caused by *SRY* gene mutation. Patients have streak gonads or non-functional testicular tissue. The gonads in 46,XY patients produce neither produce Anti mullein hormone(AMH) nor testosterone. As a result, they have normal female internal and external genitalia. They are phenotypically female, and present with primary amenorrhea and delayed secondary sex characteristics. Gonadectomy is required due to a high risk of malignant transformation.

Treatment of all forms of gonadal dysgenesis is hormone replacement therapy (HRT) with oestrogen and cyclic progesterone is required. Pregnancy is possible with appropriate HRT and assisted reproductive technology is advised in patients with Turner syndrome mosaics or Swyer syndrome.

5. Androgen insensitivity syndrome

Androgen insensitivity syndrome (AIS) is caused by defective function of the androgen receptor resulting in the end organ insensitivity to androgens. AIS can be complete or incomplete. Complete AIS is characterized by XY sex reversal with a normal female phenotype. With residual androgen receptor activity, partial AIS results in a

variable phenotype. Mullerian structures are absent as Anti Müllerian hormone (AMH) is normally secreted. Breast development occurs due to peripheral conversion of androgens to oestrogen. These patients present with primary amenorrhoea and normal breasts and absent axillary or pubic hairs.

Management involves gonadectomy (after puberty) as there is a risk of gonadoblastoma. HRT is indicated after gonadectomy and unopposed oestrogen therapy is given post surgery as uterus is absent.

6. Kallmann's syndrome

Kallmann Syndrome (KS) is a hypogonadotropic hypogonadism (HH) hypogonadism (HH) that manifests with hypo or anosmia. The disorder is due to failure of secretion of GnRh from hypothalamus. Causative genes include: KAL1 (Xp22.32), in the X-linked recessive form, FGFR1 (8p12), FGF8 (10q25-q26), CHD7 (8q12.2) and SOX10 (22q13.1) in the AD form, and PROKR2 (20p12.3) and PROK2 (3p21.1), in both the AR and oligogenic forms.

Treatment involves life-long hormone replacement therapy both to induce puberty and fertility.

Key points:

1. Primary amenorrhoea affects 0.01% of population.
2. Absence of menses by 15 years of age needs evaluation and management.
3. FSH/LH, and oestrdiol and ultrasound pelvis is the first line investigation.
4. Clinical classification into hypergonadotrophic hypogonadism,hypogonadotrophichypogonadism and eugonadotrophic hypogonadism is most useful for evaluation and management.
5. Karyotype is indicated in hypergonadotrophic hypogonadism and women with absent uterus.
6. HRT is the mainstay of treatment in both hypergonadotrophic and hypogonadotrophic hypogonadism.
7. Turner syndrome, Meyer Rokitansky Kuster Hauser syndrome, Kallman syndrome, Androgen insensitivity syndrome, and Swyer syndrome are common causes as seen in the clinical practice.

Suggested Readings

1. Gaspari, L.; Paris, F.; Kalfa, N.; Sultan, C. Primary Amenorrhea in Adolescents: Approach to Diagnosis and Management. Endocrines 2023, 4, 536–547. https://doi.org/10.3390/ endocrines4030038

1. Rossetti R, Ferrari I, Bonomi M, Persani L. Genetics of primary ovarian insufficiency. Clinical Genetics. 2016 Dec 12;91(2):183–98

2. Karska P, Matonog A, Sieradzka P, Kowalczyk K, Madej P. Fresh insights into premature ovarian insufficiency. Ginekologia Polska 2021; 92, 7: 518–524

Recurrent Pregnancy Loss: Role of Genetics and Management

Divya Pandey*, Meenakshi Rajput**
*Professor, **CMO NFSG
Department of Obstetrics and Gynaecology, VMMC and Safdarjung Hospital, New Delhi-110029

Definition

Recurrent pregnancy loss (RPL) has been defined in varied ways by different academic bodies. Federation of Obstetricians and Gynaecologists of India (FOGSI), defines it as, "loss of 3 or more consecutive pregnancies before 20 weeks". American Society of Reproductive Medicine (ASRM) defines it as "loss of 2 or more pregnancies."European Society of Human Reproduction and Embryology (ESHRE) states "loss of 2 or more pregnancies before 24 weeks spontaneous or assisted conception, excludes ectopic pregnancy, molar pregnancy, and chemical pregnancy" as its definition. Royal College of Obstetricians and Gynaecologists (RCOG) has defined it as, "loss of 3 or more consecutive pregnancies (includes chemical pregnancies).

Genetic causes: In over half of first-trimester losses, chromosomal abnormalities are an important etiological factor.Their contribution may be there but less common in second- and third-trimester miscarriages.

Chromosomal abnormalities encompass two main categories: Numerical and Structural. Numerical abnormalities, also known as aneuploidy, arise from non-disjunction during meiosis and represent the predominant cause of chromosomal abnormalities resulting in pregnancy loss (PL). This category encompasses autosomal trisomy (30-60%), Monosomy X (10-15%), triploidy (11-13%), and tetraploidy(about 9%).

In contrast, structural chromosome rearrangements (2%–6%) and mosaicism (8%) contribute to smaller number of PL cases. These include translocations, deletions, duplications, inversion, insertions of a chromosomal segment, and copy number variants.

Genetic polymorphisms and mutations also comprise important causes. It involves embryonic or fetal development-related genes. Besides, there are genetic causes associated with male partners which can have potential adverse effects leading to pregnancy losses. Older parental age is an important and established reason behind chromosomal abnormalities due to errors in chromosomal division. In women with extremes of reproductive age (<20,≥40 years) there is an increased risk of genetic defects. There are 69% higher chances of PL in males >40 years compared to the younger age group. In addition, a specific faulty gene or a group of genes involved in DNA repair, cell proliferation, and regulation of meiosis can also adversely impact gametes and subsequently the embryos. Furthermore, epigenetics i.e. the abnormal external effect on chromosomes also affects gene expression in majority of chromosome abnormalities in sporadic miscarriages seem to occur spontaneously.

The result of the above discrepancies is in either or both gametes and results in a genetically abnormal embryo incompatible with life, often resulting in pregnancy loss.

Genetic Analysis in RPL

Genetic causes account for 3-5% of RPL cases. So once all non-genetic causes are ruled out, clinicians should go for the genetic evaluation. Products of Conception (POC) testing is recommended by various guidelines and depending on the result, parental Karyotyping (KT) can be offered.

RCOG(2023) recommends genetic analysis of products of conception of the third and subsequent consecutive miscarriages (level D recommendation). Parental peripheral blood karyotype (KT) of both partners should be performed in couples with RPL in case of structural chromosomal abnormality in products of conception (level D recommendation).However, ESHRE recommends that the genetic testing of pregnancy tissue should not be routinely performed but could be useful for explanatory purposes (conditional recommendation). Parental KT is not routinely recommended in couples of RPL. It should be carried out after individual assessment of risk. Couples should be informed that despite chromosomal abnormalities, the cumulative live birth rates are good.

Genetic Tests Available

Certain genetic tests can help in the identification of chromosomal and other genetic irregularities leading to PL.

Karyotyping(KT)-Conventionally this has been a microscopy-based (cytogenetic test) used to detect chromosomal imbalances leading to RPL. Parental blood or product of conception (POC) are sampled, and

subjected to cell culture, followed by Chromosomal analysis (after achieving mitotic metaphase arrest). In RPL cases POC-KT yields negative results in 20%–40% of cases due to the absence of fetal tissue,culture failure or maternal cell contamination (MCC). Because of its low resolution, false-negative results are there in as high as one-third of cases.

Fluorescent In-situ Hybridisation (FISH) cytogenetic test is limited to testing of few chromosomes with larger limitations. With better available tests, its utility is limited.

Chromosomal microarray analysis (CMA)-This genetic test can detect submicroscopic genetic errors that are not picked up by KT. It is done by using array Comparative Genomic Hybridization(a-CGH) or Single Nucleotide Polymorphism (SNP) genotyping. It has better resolution with fewer inconclusive or false-negative results. Studies have reported a 13% rise in the detection rate of chromosomal errors by CMA compared to KT.ESHRE has therefore recommended a-CGH as the preferred genetic test for fetal tissue. Table 1 summarises the main difference among KT, CMA and FISH. It is better to do CMA in POC and KT in parents.

Table 1: Comparison among KT,CMA and FISH

Parameter	Karyotyping	Fish	CMA
Resolution	5 – 10 mb	13 18 21 X Y chromo-somes only	50 – 400 kb
Balanced Transloca-tions	Yes	No	no
Mosaicism	Yes	yes	yes
Triploidy	Yes	No	no
Uniparental Disomy	Yes	No	yes
Failure Rate	High 40%	10 – 30%	Low 5%
Method	Subjective evaluation	Objective	Bio-informatics evaluation
Turn Around Time	14 – 21 days	3 days	5 to 10 days

Recent additions to the genetic tests armamentarium are Next-Generation Sequencing(NGS) such as Whole genome Sequencing(WGS) and whole exome sequencing (WES) which can detect mutations. They have further enhanced resolution and are expensive. WGS analyses the entire genome including introns(non-coding regions) and exons(coding regions).Since introns sequencing has no clinical significance here in place of whole genome sequencing, WES (which examines the coding regions, exons) is done.

Practical Approach while Managing a Case

In women with RPL, POC should be subjected to genetic testing. Most often, clinicians can find an explanatory result where the recurrence risk is low (e.g., trisomy) and no further action is required. However, in subsequent pregnancy, appropriate prenatal diagnostic test should be offered.

In case of unbalanced translocations in fetal tissue, parental KT must be done to detect parental carrier status for balanced translocation(2%–5% of RPL cases). This condition has high recurrence risks. They can be offered options of Artificial Reproductive Technology (ART) with Preimplantation Genetic testing structural rearrangement (PGT - SR) to identify and transfer euploid embryos. In the pregnancy achieved after PGT testing,the parents are (again) counselled about chance and risks of a misdiagnosis and be informed about the need for prenatal testing.

In cases where cytogenetic testing of fetus/POC gives inconclusive results or cannot be done due to absence of tissue, parental KT may be done. Those with a genetic abnormality can then be subjected to Genetic Counselling. This counselling covers risks in further conceptions and options like PGT, prenatal testing (e.g., amniocentesis, chorionic villi sampling, and non-invasive testing) or gamete donation.

How to do POC/Fetal-Sample Collection and Transportation.

Small piece of tissue (POC),preferably chorionic villi can be sampled. If a woman is asked to carry POC from home, ask her to carry the specimen covered in a sterile gauze or the testing laboratory can be asked to provide her with the container with media in advance to collect the POC. The fetal tissue can be snipped using sterile scissors after clearing it off maternal blood using saline and then transferred in a sterile screw top container with tissue culture medium, Hanks balanced solution, or Earls medium preferably with an antibiotic(penicillin/streptomycin/gentamicin) added, in case sample needs long distance transportation. Never use formalin as a fixative or freeze the sample as these samples cannot be cultured.If needed, it can be refrigerated at 4 degrees but never frozen.

Other specimens that can be sampled are placental block 1x1 cm taken below cord insertion site and should include chorionic plate so as to minimise the risk of confined placental mosaicism, skin from the anterolateral aspect of thigh 1cm in size 3 mm deep so as to include dermis and underlying muscle or

one can take even internal organs.ACOG suggests costochondral junction or patella because of the longer viability of fibroblast in these tissues. Always mention or provide copy of tests like Noninvasive prenatal testing (NIPT) or maternal serum screening studies or describe the fetal phenotype when sending the sample to genetic lab.

Blighted Ovum/Anembryonic Pregnancy- POC testing not recommended, Parental KT can be done followed by Genetic counselling and further testing accordingly

Between 6-10 weeks – Identification of fetal tissue is important. In case of MCC, CMA result will fail. FISH/KT can be attempted.

Between 10-20 weeks-It is important to isolate fetal tissue for sampling. Always opt for CMA.

Beyond 20 weeks-If possible a fetal autopsy should be done. Pictures should be recorded for external abnormalities. If skeletal abnormalities are present X-Ray needs to be done. Based on these findings, further tests can be done. CMA, NGS-based tests, Clinical exome sequencing(CES) or targeted disease panel can be done.

Role of Male Partner

Recurrent pregnancy loss till now has been considered from female causes but recently several studies have compared sperm parameters of couples with RPL to fertile controls. Overall these studies found no significant differences in sperm volume or count. The percentage of motile sperm and those with normal morphology was reported to be lower in RPL men in some studies while others found no difference. Following the lack of consensus between studies on sperm parameters, recent studies mostly focussed on male genetic defects. These range from markers of Y chromosomal deletions, chromatin integrity and DNA damage. Some studies consistently reported higher sperm DNA fragmentation(SDF) in RPL men.

DNA fragmentation index(DFI) is a measure of the amount of DNA damage and may be described as a percentage of DNA fragmentation. Sperm DNA damage can occur at any level during spermatogenesis, spermiogenesis or epididymal transit. Sperms with damaged DNA can fertilize an egg and form a zygote but that embryo cannot grow beyond 4-8 cell stage. Sperm DNA integrity plays a vital role in embryogenesis and sperm DNA damage can result in pre or post-implantation pregnancy losses and congenital malformations. DNA fragmentation occurs due to improper packaging of sperm chromatin. DFI more than 30% is associated with infertility, poor embryo development and recurrent early pregnancy losses. Causes of DNA damage are not clear but oxidative stress plays an important role. Extrinsic factors such as smoking alcohol, radiotherapy and chemotherapy may be contributory.

Assays to detect DFI include (1) Sperm chromatin structure assay SCSA.(2) Terminal deoxynucleotidyl transferase-mediated nick end labelling TUNEL.(3) Comet (single cell electrophoresis) assay.(4) Sperm chromatin dispersion.(5) Acridine orange test. A DFI of 30% is recommended as a clinical threshold. SCSA and TUNEL assays are more commonly used to detect clinically significant damaged sperm, but they need fluorescent microscopy and flow cytometry and are expensive and time-consuming. Different tests have different cut-off values of DFI.

Recent systematic reviews and meta-analysis have shown a significant increase in RPL in men with high sperm DNA damage. Robinson et al, concluded that sperm DNA damage testing should be offered to couples following even a single miscarriage after fertility treatment. Recent studies have reported significantly increased sperm DNA fragmentation in RPL couples with natural conception. Esteves et al concluded that elevated SDF may contribute to RPL or lead to Intrauterine insemination (IUI) or IVF/ICSI failure. ESHRE also mentions the evidence supporting an association between RPL and SDF, and this association is independent of female factors. However, the guidelines highlighted that the effect of interventions to reduce SDF on RPL needs well-designed studies.

The main cause of DNA damage is oxidative stress and it is increased by smoking alcohol and excessive exercise. So male partners of RPL couples should be advised to have healthy lifestyles so as to prevent DNA damage. ESHRE recommends assessing lifestyle factors (smoking, alcohol, obesity and excessive exercise) in a male partner of RPL couples and considering assessment of SDF for explanatory purposes based on indirect evidence.

Case Scenarios: Practical Approach for Evaluation of Genetic Causes

Case 1: H/O RPL but no POC sample available, couple comes for preconception counselling

After evaluation and ruling out non-genetic causes, the first step is to rule out hereditary chromosomal abnormalities. Parental KT is to be done. If parental KT is normal,detailed genetic counselling should be done and accordingly further testing like couple carrier screening can be done. If no cause is identified, paternal evaluation by performing Sperm DNA fragmentation index can be done.

Case 2: H/O RPL, ongoing pregnancy, fetus is having one or more structural abnormalities on Ultrasonography.

Prenatal chromosomal Microarray (CMA) is recommended in a woman with USG suggestive of fetal structural abnormality. If fetal structural abnormality is any disease specific, Clinical Exome sequencing (CES)/ disease panel testing can be done. Depending on the result, further parental evaluation has to be done followed by appropriate genetic counselling.

Case 3: H/O RPL, POCs CMA suggestive of chromosomal Unbalanced translocation

This is an indication of one of the parents being a balanced translocation carrier, so parental KT followed by clinical and genetic risk evaluation should be done by genetic counselling. Management options include ART with PGT-SR followed by transfer of euploid embryo.It is recommended that in subsequent pregnancy prenatal diagnostic test has to be done to confirm PGT findings.

Case 4: H/O RPL/Non-consanguineous marriage/First two pregnancies terminated due to neural tube defect (NTD)/Third pregnancy terminated due to congenital heart defect(CHD).The current fetus has complex CHD on ultrasound and pregnancy termination planned.

POC to be subjected to CMA analysis

Important Note: It is not advisable to go for surgical termination of pregnancy, for the sake of getting the sample. The woman can very well undergo Medical Abortion and can do POC sampling. The sample is usually brought to the clinic by the woman. The tissue can be carefully cleaned with normal saline off the maternal blood to avoid MCC.

Case 5: H/o recurrent IUD in 2/3 trimester in previous pregnancies, again presents with IUD in second trimester.

This history is most commonly associated with a definitive phenotype which has a tendency to recur in two or more pregnancies.

Do a thorough USG evaluation of the phenotype. Fetal Autopsy/Images/X-ray to be done along with a thorough family history. Then it is subjected to an appropriate genetic testing like CMA/CES/disease panel testing.

Suggested Reading

1. Practice Committee of the American Society for Reproductive Medicine. Electronic address: asrm@asrm.org. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril. 2020 Mar;113(3):533-535. doi: 10.1016/j.fertnstert.2019.11.025. Epub 2020 Feb 27. PMID: 32115183.
2. ESHRE Guideline Group on RPL; Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp et al. ESHRE guideline: recurrent pregnancy loss: an update in 2022. Hum Reprod Open. 2023 Mar 2;2023(1):hoad002. doi: 10.1093/hropen/hoad002. PMID: 36873081; PMCID: PMC9982362.
3. Regan L, Rai R, Saravelos S, Li TC; Royal College of Obstetricians and Gynaecologists. Recurrent Miscarriage.Green-top Guideline No. 17. BJOG. 2023 Nov;130(12):e9-e39. doi: 10.1111/1471-0528.17515. Epub 2023 Jun 19. PMID: 37334488.
4. Melo P, Dhillon-Smith R, Islam MA, Devall A, Coomarasamy A. Genetic causes of sporadic and recurrent miscarriage. Fertil Steril. 2023 Nov;120(5):940-944. doi: 10.1016/j.fertnstert.2023.08.952. Epub 2023 Aug 28. PMID: 37648143.
5. ESHRE PGT Consortium Steering Committee; Carvalho F, Coonen E, Goossens V, Kokkali G, Rubio C, Meijer-Hoogeveen M et al. ESHRE PGT Consortium good practice recommendations for the organization of PGT. Hum Reprod Open. 2020 May 29;2020(3):hoaa021. doi: 10.1093/hropen/hoaa021. PMID: 32524036; PMCID: PMC7257038.
6. RCOG Green Top guidelines no 55 : late intrauterine death and still birth 2010.
7. Inversetti A, Bossi A, Cristodoro M, Larcher A, Busnelli A, Grande G et al. Recurrent pregnancy loss: a male crucial factor-A systematic review and meta-analysis. Andrology. 2023 Oct 25. doi: 10.1111/andr.13540. Epub ahead of print. PMID: 37881014.
8. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod. 2012 Oct;27(10):2908-17. doi: 10.1093/humrep/des261. Epub 2012 Jul 12. PMID: 22791753.
9. Esteves SC, Zini A, Coward RM, Evenson DP, Gosálvez J, Lewis SEM et al. Sperm DNA fragmentation testing: Summary evidence and clinical practice recommendations. Andrologia. 2021 Mar;53(2):e13874. doi: 10.1111/and.13874. Epub 2020 Oct 27. PMID: 33108829; PMCID: PMC7988559.

Genetics and Male Infertility: Solutions

Sangeeta Khatter
Consultant Medical Genetics
Jindal IVF Sant Memorial Hospital, Chandigarh, India

Introduction

WHO defines infertility as "a disease of the male or female reproductive system characterized by the failure to achieve a pregnancy after 12 or more months of regular unprotected sexual intercourse".

As per the 2022 global infertility prevalence estimates, prevalence of infertility is 1 in 6 people and it affects 15% of the couples in developed countries. In developing countries like India, the prevalence during 2019–20 was 18.7/1,000 amongst women who have been married at least for five years.The factors contributing to infertility are in 35% related to females,30% related to males, 15% in both and 15% cases remain unexplained.

Male factor infertility is multifactorial due to complex pathology of male reproductive system and involvement of approximately 3000 genes in spermatogenesis. Presentation of male infertility is in the form of abnormal sperm parameters like azoospermia (absence of spermatozoa in ejaculate) oligozoospermia (reduced sperm counts), asthenozoopsermia (impaired sperm motility) teratozoospermia (abnormal sperm morphology) or oligoasthenoteratozoospermia (reduced number with impaired motility and morphology). The etiology of deranged sperm parameters can be at the pretesticular, testicular, or post-testicular level and can be genetic or non-genetic. The genetic factors account for 20-25% of the cases. The etiology remains idiopathic in around 50% of the cases.

The advancement in new In vitro fertilisation (IVF) techniques such TESA (Testicular sperm aspiration) TESE (Testicular sperm extraction) ICSI (Intracytoplasmic sperm injection) have helped IVF specialists achieving successful pregnancies in male factor infertility.

In this article we will explore known genetic causes of male infertility such as chromosomal and single gene disorders associated with infertility and their fertility management.

The Genetic Causes of Male Infertility can be

Chromosomal abnormalities: These are seen in less than 1% of men with normal sperm concentration whereas the chromosomal abnormalities account for 5% cases of severe oligospermia (sperm concentration <5million/ml) and 10-15% cases of azoospermia.

These can be Categorised as

- Numerical chromosomal aberrations (Aneuploidies): There is change in number of chromosomes e.g. 47, XXY, 47, XYY, Mixed gonadal dysgenesis (45, X/46, XY), 46, XX males due to translocated SRY gene.
- Structural balanced rearrangements like reciprocal translocations, robertsonian translocations, and inversions .(Table 1)

  Y chromosomes microdeletion: The genes over Y chromosome play a vital role in spermatogenesis and their deletion can lead to impaired spermatogenesis. This deletion accounts for 5% of cases of non-obstructive severe oligospermia and 10% of non-obstructive azoospermia. The deleted region over the distal part of Y chromosome is assigned as AZF (Azoospermia factor region) and is further divided into three subregions i.e. AZFa, AZFb and AZFc according to the location of deletion (Table 1).

Table 1: Chromosomal anomalies in male infertility

Karyotypic anomaly	Phenotype	Diagnosis	Management
47, XXY (Klinefelter syndrome)	Most common, prevalence-1-2% in infertile males, 11% in azoospermia males C/F- azoospermia, tall stature, gynecomastia, sparse body hair, small and firm testis (<4cc)	Biochemical- raised FSH and LH levels (100%), decreased testosterone (50%) Biopsy-hyalinized seminiferous tubules, degenerated germ cells Genetic-Karyotype	Micro-TESE+ICSI±PGT
47, XYY	Rare- 0.1% Normospermia to severe oligospermia	Karyotype	Micro-TESE+ICSI±PGT

45, X/46, XY (Mixed gonadal dysgenesis)	Azoospermia, short stature, infertility, unilateral nonpalpable testis, contralateral streak gonad, persistent Mullerian structures	Karyotype	Micro-TESE+ICSI±PGT
46, XX male (De la Chapelle syndrome/testicular (DSD)	Similar to Klinefelter except for tall stature	USG-Persistent mullerian structures FISH-To detect SRY gene translocation to X chromosome	IUI or IVF with donor sperm
Balanced translocations-Reciprocal-balanced exchange of genetic material between 2 or more chromosomes,10 times more common in infertile males. Robertsonian- incidence is 0.9%in infertile males, involve acrocentric chr.13, 14, 15, 21 and 22 Inversion-Inversion of chromosomal segment by 180 degrees due to breakage and rearrangement.	Seen in 4% of Oligospermic males(<10miilion) With recurrent pregnancy loss	Karyotype	IVF self-sperm+PGT IUI or IVF with donor sperm
Y microdeletion	Non-obstructive azoospermia	Y-specific PCR AZFc deletion-80% AZFb-1-5%, AZFa- 0.5-4% AZFb+c -1-3%	AZFa and AZFb-No spermatogenesis, IUI or IVF with donor sperm AZFc-60% chances of success in micro-TESE+ICSI Genetic counseling

Abbreviations: C/F-tall stature, TESE-Testicular sperm extraction, ICSI-Intracytoplasmic sperm injection, PGT-Preimplantation genetic testing, DSD-disorder of sex development, FISH-Fluorescent in situ hybridization, IUI-Intrauterine insemination, IVF-In-vitro fertilization, AZF-Azoospermia factor, PCR-Polymerase chain reaction

Monogenic disorders in male infertility: A majority (60–70%) of infertile males remain without a clear diagnosis and are classified as unexplained.Studies have described association of more than 3000 genes with defective spermatogenesis. Nonetheless, less than 0.01% of these genes have been explored yet.

Few monogenic disorders implicated in male infertility are:

Kallman syndrome (KLS): This is an X-linked condition commonly due to KAL1 gene and autosomal FGFR1 genes variations and unexplained in 35 to 45% of cases. Incidence of KLS is 0.2% with male to female ratio of 5:1. The KAL-1 gene encodes the protein anosmin-1 which has role in the migration of both olfactory axons and GnRH neurons from the olfactory placode past the cribriform plate to the preoptic area of the hypothalamus and the defects result in anosmia and GnRH deficiency resulting in hypogonadotropic hypogonadism and anosmia. Other defects are cleft palate, an- or hyposmia, infertility, cryptorchidism, unilateral renal agenesis, and neurogenic deafness. Deficient GnRH hormone results in decreased levels of sex steroids leading to absence of sexual maturity and the secondary sexual characteristics.

Secondary testicular failure can occur in KS due to a lack of stimulation of the testicle to induce testosterone production and subsequent spermatogenesis.

Fertility Management for Kallman Syndrome

Testosterone replacement therapy to induce puberty. For fertility, Gonadotropin releasing hormone(GnRH), recombinant Follicle stimulating Hormone(rFSH) are used for spermatogenesis.The use of human Chorionic gonadotrophin(hCG) in adolescents with KLS for testicular growth and spermatogenesis is under research.

CBAVD (Congenital bilateral absence of the vas deferens): Cystic fibrosis (CF) is a lethal autosomal recessive disorder due to severe mutations in both copies of the CFTR gene and is characterized by chronic pulmonary obstruction, pancreatic insufficiency, high electrolyte concentration in sweat and male infertility. This gene maintains salt homeostasis through anion channels in epithelial cells and defect results in thickened secretions in the lungs, pancreas, and genital tract. CBAVD is the milder spectrum of CF due to single defected copy or two mildly defected copies of CFTR gene. Around 95% of men with cystic fibrosis have CBAVD. CBAVD is found in 1% to 2% in infertile males and in 25% with obstructive azoospermia.

Diagnostic criteria for CBAVD: Normal to small sized testicles, non-palpable or blind ending vas deferens, hypoplastic or absent seminal vesicles, epididymal or ejaculatory duct obstruction, normal plasma FSH, semen volume <1 mL, azoospermia, acidic pH and normal spermatogenesis.

Fertility management in CBAVD: Consultation with Geneticist for CFTR testing to understand the risk to children if wife is also carrier of CFTR gene variation. Reproductive options including Preimplantation genetic tests(PGT) can be discussed. Surgical sperm retrieval from the epididymal remnant through percutaneous epididymal sperm aspiration (PESA) or microsurgical epididymal sperm aspiration (MESA) can be done for IVF.

Genetic Syndromic Causes of Male Infertility

Prader-Willi syndrome (PWS): PWS is a neurodevelopmental disorder characterized by infantile hypotonia, intellectual disability, obesity, hypogonadism and infertility in both males and females. This is an imprinting disorder due to defect in parent of origin pattern of inheritance over chromosome 15q11-13 resulting in the absence of paternally expressed genes and hence the phenotype.

Primary ciliary dyskinesia (PCD): PCD is an autosomal recessive disorder of severely defected cilia leading to impaired mucociliary clearance of airways. Clinical features are chronic respiratory tract disease, bronchiectasis, rhinitis and sinusitis. Infertility is due to asthenozoospermia (poor motility) in males is due to defective sperm flagellar function. Kartagener syndrome is PCD and situs inversus (50%) Approximately 50%. Around 29 genes are known for PCD, DNAI1 and DNAH5 genes variations account for 30% of the cases.

Fertility management in PCD: Genetic consultation and testing is advisable to predict the risk in future offspring. IVF-ICSI with or without PGT is an acceptable option for these patients.

Noonan syndrome: This is an autosomal dominant disorder characterized by short stature, dysmorphic facies, and cardiac defect (Pulmonary valve stenosis or hypertrophic cardiomyopathy). About 50% of males with Noonan syndrome have azoospermia or oligozoospermia due to bilateral cryptorchidism. Among the many genes, PTPN11 gene accounts for 50% of the cases.

Myotonic dystrophy: Myotonic dystrophy is an autosomal dominant condition due to expansion of triplet (CTG) repeats over DMPK gene. The phenotypic features are grip myotonia, muscle weakness, cardiac conduction disturbances, cataract and progressive testicular atrophy in 60-80% of males.

Fertility management in syndromic male infertility: Each child of an individual with autosomal dominant disorder has a 50% chance of having the affected status. In autosomal recessive disorders, there is 25% chances of having an affected child if both partners are carrier of pathogenic gene variation.Referral to a Geneticist for genetic test, genetic counselling for recurrence risk, reproductive counselling and discussion of reproductive options including PGT is suggested in above mentioned genetic disorders.

Non-syndromic genes for male infertility.: In the modern era of genomics, next-generation sequencing (NGS) technologies, such as whole-exome and -genome sequencing, have enabled the research scientists to identify new genes for male infertility. Currently, >1000 genes have been reported to play a role in reproductive processes essential for male fertility. Most common gene is TEX11 on the X chromosome (Xq13.2). Other genes are involved in various processes like spermatogenesis (e.g. USP9Y, DBY, RBMY, TEX11, DAZ) (development of the male reproductive system (e.g. AR, FSHR, ADGR2) steroid hormone signalling (e.g. SHBG) etc. Research is going on and will possibly explain the 40% idiopathic male infertility in future.

Summary: Male factors contribute to infertility in approximately 50% of cases. The identifiable genetic abnormalities can explain 15-20% of the male infertility.Chromosomal analysis and testing for AZF deletions should be performed in cases of severe oligozoospermia and azoospermia. Karyotype analysis is the gold standard test recommended in diagnostic workup of all oligospermic men with less than 10 million spermatozoa/ml and in all nonobstructive azoospermia cases.

Male presenting with CBAVD should be advised CFTR testing along with partner. A referral to a Clinical Geneticist must be sought for decision of correct genetic testing, pre and post- test counselling for reproductive options including PGT. Expanding the use of NGS (Next generation sequencing) in research settings could lead to personalized diagnosis and improved management of infertile males.

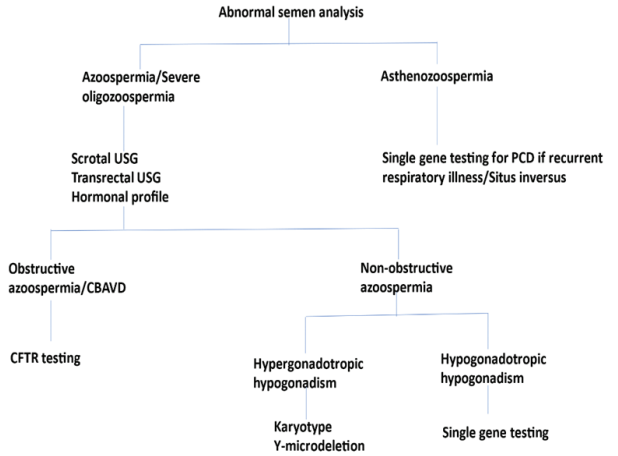


Figure 1: Flowchart for genetic evaluation in male infertility

Abbreviations: PCD-primary ciliary dyskinesia, CBAVD-Congenital bilateral absence of vas deferens, CFTR-Cystic fibrosis transmembrane conductance regulator

Suggested Reading

1. Infertility prevalence estimates, 1990–2021. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.
2. Agiwal V, Madhuri RS, Chaudhuri S. Infertility Burden Across Indian States: Insights from a Nationally Representative Survey Conducted During 2019-21. J Reprod Infertil. 2023 Oct-Dec;24(4):287-292. doi: 10.18502/jri.v24i4.14156.
3. Kuroda S, Usui K, Sanjo H, Takeshima T, Kawahara T, Uemura H et al.Genetic disorders and male infertility. Reprod Med Biol. 2020 Jun 27;19(4):314-322. doi: 10.1002/rmb2.12336.
4. Gardner, D.K., Weissman, A., Howles, C.M., & Shoham, Z. (Eds.). (2023). Textbook of Assisted Reproductive Techniques: Volume 1: Laboratory Perspectives (6th ed.). CRC Press. https://doi.org/10.1201/9781003268598
5. Krausz C, Casamonti E. Spermatogenic failure and the Y chromosome. Hum Genet. 2017 May;136(5):637-655. doi: 10.1007/s00439-017-1793-8. Epub 2017 Apr 29.
6. Rawal, L., Prabhash, S., Kumar, R. et al. 46,XX males with SRY gene translocation: cytogenetics and molecular characterization. J Rare Dis 3, 1 (2024). https://doi.org/10.1007/s44162-023-00025-8
7. Wayne W. Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics (Seventh Edition), Academic Press,2022. 121-147, ISBN 9780128152362, https://doi.org/10.1016/B978-0-12-815236-2.00010-2.
8. Los E, Leslie SW, Ford GA. Klinefelter Syndrome. [Updated 2023 Nov 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482314/
9. Zhang Y, Liu Z, Ou J, Zhang LX, Lu WY, Li Q, Ma Y. Successful surgical sperm retrieval from a patient with 45,X/46,XY mosaicism followed by in vitro fertilization pregnancy: A case report. Medicine (Baltimore). 2020 Oct 2;99(40):e22223..
10. Kouvidi Elisavet, Tsimela Hara, Lazaros Leandros, Manola et al. A 46,XX Karyotype in Men with Infertility Journal of Human Reproductive Sciences 15(3):p 307-317, Jul–Sep 2022.
11. Houston BJ, Riera-Escamilla A, Wyrwoll MJ, Salas-Huetos A, Xavier MJ, Nagirnaja L. et al. A systematic review of the validated monogenic causes of human male infertility: 2020 update and a discussion of emerging gene-disease relationships. Hum Reprod Update. 2021 Dec 21;28(1):15-29. doi: 10.1093/humupd/dmab030.
12. Sonne J, Lopez-Ojeda W. Kallmann Syndrome. [Updated 2023 May 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538210/
13. Kuroda S, Usui K, Sanjo H, Takeshima T, Kawahara T, Uemura H et al. Genetic disorders and male infertility. Reprod Med Biol. 2020 Jun 27;19(4):314-322. doi: 10.1002/rmb2.12336.
14. de Souza DAS, Faucz FR, Pereira-Ferrari L, Sotomaior VS, Raskin S. Congenital bilateral absence of the vas deferens as an atypical form of cystic fibrosis: reproductive implications and genetic counseling. Andrology. 2018 Jan;6(1):127-135. doi: 10.1111/andr.12450. Epub 2017 Dec 7.
15. Zariwala MA, Knowles MR, Leigh MW. Primary Ciliary Dyskinesia. 2007 Jan 24 [Updated 2019 Dec 5]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1122/

Unexplained Stillbirth: What the Obstetrician Must Know ?

Manisha Kumar*,Tuaiba Mufti**
Director Professor*, Resident **
Department of Obstetrics and Gynecology, Lady Hardinge Medical College, New Delhi

Introduction

The World Health Organization(WHO) defines that stillbirth (SB) is a baby born with absolutely no signs of life at or after 28 weeks of gestation, weight ≥ 1000 g, crown-heel length (CHL) ≥ 35 cm. Unexplained SB is actually unexplored stillbirth. If we are systematic in our approach and do full investigations, unexplained stillbirth can be reduced to a large extent. Investigations that are warranted in a stillbirth where the cause is not obvious are fetal autopsy, gross and histologic examination of placenta and umbilical cord along with genetic evaluation and fetal Deoxyribonucleic acid(DNA) storage. However, in a significant proportion of them the cause might not be known even after thorough investigations.

Triple Risk Model of Stillbirth

Warland et al in their study suggested a triple risk model and stated that the unexplained late stillbirths occur when a fetus who is somehow vulnerable dies as a result of encountering a stressor and/or maternal condition in a combination which is lethal for them. If the means of protecting the vulnerable fetus is found then this would essentially block the pathway to stillbirth.

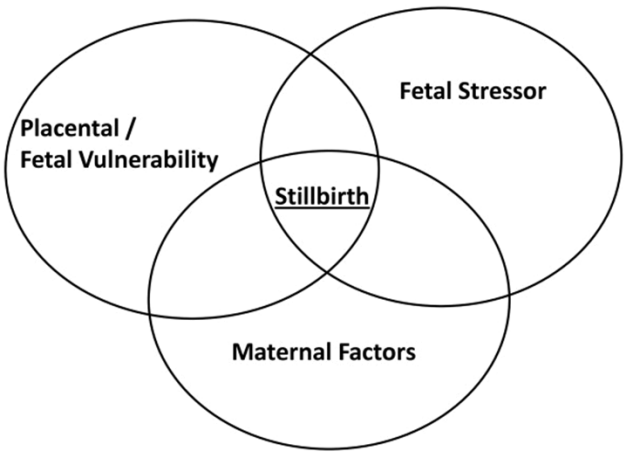


Fig 1: Triple risk model.

Warland J, Mitchell EA. A triple risk model for unexplained late stillbirth. BMC Pregnancy Childbirth.

What can be the practical approach towards the workup of any stillbirth?

- The following points are relevant with regards to workup of cases leading to SB

History	Send Maternal blood investigations if not done previously
• Time since loss of fetal movement • Leaking /bleeding PV, fever, itching • Antenatal visit – Place, timing of visits, timing of last visit • Maternal high risk conditions – HDP, DM, Anemia, hypothyroidism, twin pregnancy • Fetal high-risk conditions - FGR, Infection, birth defect • Review antenatal ultrasound records- Confirm POG • look for anomaly, liquor • fetal growth- weight & Abdominal circumference centile	• CBC, Blood group • TORCH serology – IgG and IgM • VDRL test • Maternal blood glucose Fasting, Postprandial and HbA1c • Maternal TSH • If there is Itching – LFT, Bile acids • if Fever, flu like symptoms, PROM, abnormal liquor - Blood culture, cervical and vaginal swab, urine culture sensitivity • If FGR or placental disease – Antiphospholipid antibody syndrome,Lupus anticoagulant. • Counsel parents before delivery regarding need for genetic tests • If Recurrent miscarriage – parental karyotype

HDP: Hypertensive disorders of pregnancy.DM: Diabetes Mellitus.FGR: Fetal growth restriction.POG: Period of Gestation.VDRL: Venereal disease research laboratory. LFT:Liver Function test. TSH: Thyroid stimulating Hormone.PROM: Preterm future of membranes.

After Delivery

In all cases

- Baby weight-
- Compare weight with centile chart given below
- Photograph*
- Examination of Placenta cord and membranes
- Take Placental weight
- Gross inspection of placenta, cord and membranes
- If Twin - chorionicity to be confirmed
- By ascertaining number of placenta
- whether fused
- intermembrane - two leaves/ four leaves
  - Placental tissue for HPE
  - In cases of FGR, unexplained, hydrops, suspected infection
  - Cord – length
  - Any other abnormality
  - Membranes - for culture in case of clinical features s/o infection
  - Delivery

In cases with birth defect, hydrops, unexplained

- Autopsy should be offered
- If consent not given - external examination from head to toe
- *Photograph – Anterior supine, lateral, back – should include hands and feet, genitalia
- Cord blood to be saved
  - Heparin (green cap)- for karyotype
  - EDTA (purple cap)- for genetic test, CMA
  - Red (Plain)- for CBC (in hydrops)
- In case of skeletal abnormality – Infantogram to be done.
- Placental tissue For genetic tests -
  - A 1 rupee coin shaped full thickness placental tissue at the region of cord insertion – to be kept in normal saline
  - A 2 cm wedge shaped tissue from inner thigh – kept in saline

HPE: Histopathological examination, CMA: Chromosomal Microarray, CBC: Complete blood count

When should we Offer Fetal Autopsy?

Although fetal autopsy is recommended in all cases of stillbirth, but it must be offered in cases where there is no accompanying high-risk in the mother or fetus. It should be also done in cases of birth defects and non-immune hydrops.

Can Fetal Autopsy be done without Internal Examination?

Fetal autopsy comprises history taking, pedigree drawing, photograph, infantogram, external examination and biometry, internal examination, histopathological examination of relevant tissue, along with examination of placenta and cord. It also includes sending membranes for culture, genetic testing from fetal tissue or placenta. Hence, internal examination plays a small part in autopsy. Though performing a complete autopsy is desirable, but in situations where consent is not given or if the facilities do not exist, limited autopsy can be done.

Newer alternatives to conventional autopsy

- Non-invasive autopsy involves doing post-natal Ultrasonography (USG) and Magnetic resonance Imaging(MRI) is being offered at some centres.
- Minimally invasive autopsy (MIA) consists of obtaining tissue samples for histopathology, using ultrasound guided needle biopsy.

When should we Examine Placenta?

Examination of placenta is considered to be the single most important investigation, in finding the cause of stillbirth. It should be done by a trained pathologist. Histological examination of placenta also provides clues if there is unexplained stillbirth, in cases of infection, FGR or fetal hydrops. Placental weight can also provide us information about uteroplacental insufficiency. Centile charts for placental weight for boy and girl has been published in literature.

Causes which are Frequently Missed if Extensive Workup is not done

- Infection: Is associated with 10-20% of the stillbirth, and is seen more when it is preterm. Sending membranes for culture, placental histopathological examination can give clue to the presence of infection as a cause of stillbirth. Early studies implicated inflammation associated with infectious agents. There are usually no overt signs of infection prior to fetal loss including: maternal fever or chills; abdominal discomfort; or fetal tachycardia. Ascending bacterial infection (before and after membrane rupture) was identified as the most common infectious cause of stillbirth. Infection can also occur from hematogenous spread. The most common organisms involved were Escherichia coli, group B Streptococcus pyogenes, and Ureaplasma urealyticum. The two most common viral infections associated with stillbirths were Parvovirus and Coxsackie virus. Cytomegalovirus (CMV) has been attributed to cause 15% of stillbirths.
- Umbilical Cord abnormalities accounts for 10 – 19 % of the stillbirth, examination of cord in terms of its length, presence of hyper coiling or hypo

coiling and abnormality of insertion in the placenta are important determinants regarding the cause responsible for the adverse event. In the study by Hammad et al for cord abnormalities in stillbirth 48% had compromised fetal microcirculation, 29% had cord entrapment, 27% knots, torsions, or stricture, and 5% had cord prolapse .

- Genetic evaluation should be guided by clinical history. Fetal autopsy is essential to look for dysmorphic features and minor abnormalities which can be missed on ultrasound. Abnormal karyotype can be found in approximately 6-13% of SB, it exceeds 20% if the anatomical abnormality is present or if there is associated FGR. Karyotypic abnormality underestimates the contribution of genetic abnormality, as in half of the cases, the culture does not grow. Prenatal extraction of fetal sample such as Amniotic fluid or placental biopsy will increase the yield of culture. However, doing microarray is a far better option as it not only detects aneuploidy but also the copy number variations less than 5MB. The CMA testing has been found to diagnose genetic cause in 41.9% of the cases. Whole exome sequencing(WES) may be indicated in syndromic causes and in those in which there is family history. Long QT syndrome has been reported to be a rare genetic cause of unexplained recurrent SB.
- Confined placental mosaicism –Chromosomal mosaicism is the presence of two or more cell lines with different chromosomal complements in an individual derived from a single zygote . It is a condition in which the placental cell line has aneuploidy but the fetus is euploid. The baby might have early onset FGR, large placenta, oligohydramnios due to placental abnormality. It may be the cause of unexplained SB, but currently it is not part of standard testing.

What is the Advantage of DNA Storage?

If the cause of the fetal demise is not determined in spite of best efforts, fetal DNA extraction and storage can be done. Fetal tissue from the thigh is generally taken, however any fetal tissue can yield DNA for storage, placental issue at the point of cord insertion can also be taken. Tissue is sent to the lab in normal saline for storage. If the blood sample is to be sent for DNA storage it is sent in EDTA vial. The extracted DNA is stored in -20^0 C. If the parents later want to opt for WES, it can be sent as Trio WES test which involves samples from mother, father and the proband(affected sibling/ progeny).

What is the Evidence Regarding cord Round the Neck as Cause of Stillbirth?

Single nuchal cords noted in between 20% and 35% of all deliveries at term. Multiple nuchal cords are considerably less frequent (3-5%). Single or multiple nuchal cord has not been found to be associated with SB in a large multicentric study by Carey et al published in the year 2000. They found that there was no association between the diagnosis of growth restriction and the presence of a cord encirclement. In a recent review article by Sherer et al, it was stated that while clearly single (and likely double) nuchal cords were almost uniformly associated with favorable neonatal outcomes, data suggested that cases of ≥3 loops of nuchal cords were more likely to be associated with an increased risk of adverse perinatal outcome (either stillbirth or compromised neonatal condition at delivery) .

For Patients with Unexplained Stillbirth in the Previous Pregnancy, how should the Clinical Management be done in next Pregnancy?

- Need for post-delivery and pre- pregnancy support and counselling

  It has been shown that the parents benefit from this knowledge in terms of a reduced risk of post-traumatic stress disorder. Knowing the cause is important, not only to understand the risk of recurrence but also relieving the couple of the guilt and gives them hope for the future.
  - o Identify high risk contributory factors
  - o SB in twins is 2.5 times that of singletons.
  - o The risk of recurrence in next pregnancy, with one stillbirth has the OR of 4.83
  - o The risk of unexplained stillbirth is high if there is adverse outcome such as FGR in previous pregnancy
  - o There is 2 - 5 fold increase in the incidence of stillbirth in pregestational and gestational diabetes
  - o Strict blood pressure control reduces the risk of stillbirth.
    - · Antepartum surveillance
  - o Increase in quality and quantity of antepartum care
  - o Daily fetal movement count, especially the change in its pattern needs to be monitored.

Suggested Reading

- Stillbirth (who.int) https://www.who.int/health-topics/stillbirth#tab=tab_1. Last accessed 10/08/2024.

- Silver RM, Varner MW, Reddy U, Goldenberg R, Pinar H, Conway D et al. Work-up of stillbirth: a review of the evidence. Am J Obstet Gynecol. 2007 May.
- Pasztor N, Kereszturi A, Kozinzky Z, Pal A. Identification of causes of stillbirth through autopsy and placental examination reports. Fetal & Paediatric Pathology. 2014;33(1):49–54.
- Addison S, Arthurs OJ, Thayyil S. Post-mortem MRI as an alternative to non-forensic autopsy in foetuses and children: from research into clinical practice. Br J Radiol. 2014 Apr;87(1036):20130621. doi: 10.1259/bjr.20130621.
- Christopher Flatley, Pol Sole-Navais, Marc Vaudel, Øyvind Helgeland, Dominika Modzelewska, Stefan Johansson et al.Placental weight centiles adjusted for age, parity and fetal sex,Placenta,Volume 117, 2022, Pages 87-94,
- Gibbs RS. The origins of stillbirth: infectious diseases. Semin Perinatol (2002) 26(1):75–8. doi:10.1053/sper.2002.29839
- McClure EM, Goldenberg RL. Infection and stillbirth. Semin Fetal Neonatal Med (2009) 14(4):182–9. doi:10.1016/j.siny.2009.02.003
- Hammad IA, Blue NR, Allshouse AA, Silver RM, Gibbins KJ, Page JM et al. NICHD Stillbirth Collaborative Research Network Group. Umbilical Cord Abnormalities and Stillbirth. Obstet Gynecol. 2020 Mar;135(3):644-652.
- Stillbirth Collaborative Research Network Writing Group. Causes of death among stillbirths. JAMA. 2011 Dec 14;306(22):2459-68.
- Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. BMJ. 2005;331(7525):1113–7
- Martinez-Portilla RJ, Pauta M, Hawkins-Villarreal A, Rial-Crestelo M, Paz Y Miño F, Madrigal I et al. Added value of chromosomal microarray analysis over conventional karyotyping in stillbirth work-up: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2019 May;53(5):590-597.
- Warland J, Mitchell EA. A triple risk model for unexplained late stillbirth. BMC Pregnancy Childbirth. 2014 Apr 14;14:142.
- Kalousek DK. Current topic: confined placental mosaicism and intrauterine fetal development. Placenta. 1994 Apr;15(3):219-30.
- Carey JC, Rayburn WF. Nuchal cord encirclements and risk of stillbirth. Int J Gynaecol Obstet. 2000 May;69(2):173-4
- Sherer DM, Roach C, Soyemi S, Dalloul M. Current Perspectives of Prenatal Sonographic Diagnosis and Clinical Management Challenges of Complex Umbilical Cord Entanglement. Int J Womens Health. 2021 Feb 24;13:247-256.
- ACOG Practice Bulletin No. 10: Management of Stillbirth. Obstetrics & Gynecology . Vol 135, No 3, March 2020, e110

Turner Syndrome: Optimising Quality of Life

Kavita Mandrelle Bhatti
Professor & Head, Dept of OBGYN, Christian Medical College & Hospital, Ludhiana, Punjab

Turner syndrome (TS) is a genetic disorder with presence of one X chromosome and partial or total absence of the second X chromosome in a genotypic female, its incidence being 1in 2000 girls.The former condition is known as Turner Mosaic where some cells have both X chromosomes while some cells do not in contrast to latter where all cells have only one X chromosome. Approximately every 3 out of 10 girls with TS are Turner mosaic. The main characteristic features of TS is short height and ovarian failure. They may have other severe medical issues and a high mortality risk. They also appear to have more social, emotional, and cognitive behavioural issues.

Girls with TS do not experience pubertal development without estrogen replacement therapy, and a majority of them are infertile. Other ailments of TS include eye problems and hearing difficulties, heart and kidney abnormalities, diabetes, thyroid dysfunction, liver dysfunction, hypertension, and varying degrees of dysmorphic traits. Clinical indicators of TS, such as ovarian insufficiency, cardiovascular anomalies with aortic dissection risk, osteoporosis, hypothyroidism, hearing loss, neurodevelopmental issues, and social anxiety, demand continuous monitoring throughout adulthood. Girls with Turner mosaic experience milder degree of problems and lead relatively healthier lives.

The quality of life of TS is affected by the presence of severe medical problems. Patients with Turner Syndrome may have a lower quality of life due to mental and emotional concerns. They have lower self-esteem and a higher prevalence of depression and anxiety.

Therefore, it is vital to analyze the impact of symptoms of TS and subsequent treatments on health-related quality of life.

Objectives of Care in TS should Include

Screening for and controlling potentially linked co morbid illnesses and to reduce mortality.

Optimising Puberty Growth

Providing continuous care and promoting easy transition from pediatric to adult healthcare.

Providing for Adult Follow-up

Improving the quality of life for patients of TS during childhood and adult life

Co- Morbid Conditions in TS

Congenital Heart Disease

Abnormalities of the heart are identified in up to half of the patients. Coarctation of the aorta can be diagnosed in early infancy, necessitating immediate surgery. Severe aortic stenosis is seen in some patients of TS and requires surgery. Routine echocardiograms may show bicuspid (rather than tricuspid) aortic valves. This finding necessitates ongoing surveillance and endocarditis prevention.

Referral to a cardiologist is recommended at the time of diagnosis. Re-evaluation by echocardiography at 10 years of age and also in adulthood is mandatory.

1. **Hypertension**

   Adolescents of TS are more likely to develop hypertension. It is recommended to measure blood pressure annually beginning at school age and charting the results on an age-specific chart. If chronic hypertension is confirmed, antihypertensive therapy is required.

❖ Short stature

Girls with Turner syndrome are typically short in relation to the height of their parents. On average, adult women with untreated Turner syndrome are 20 cm shorter than adult women without the syndrome. Growth hormone (GH) medication should begin from 8 years of age if induction of puberty is planned for 12 years. GH is given as a subcutaneous injection at night time. Monitoring every 4-6 monthly is recommended to evaluate height velocity, adjust dose of GH and to check for compliance. The child should be under the care of Paediatric endocrinologist.Treatment with additional high-dose growth hormone reduces this difference by about 5cm (about 2 inches) on average. Limb lengthening surgeries are available and need to be appropriately counselled for vis a vis associated complications by orthopaedic surgeons.

Primary ovarian failure

Most girls with Turner syndrome do not produce enough of the sex hormones, which means

- they may not begin sexual development or fully develop breasts without female hormone replacement therapy (HRT)

- they may begin sexual development but not complete it
- they will have primary or secondary amenorrhoea.

Most of the girls with TS require pubertal induction with estrogen therapy and also need hormone replacement during adulthood.

Fertility

It is important to address fertility issues during childhood itself. Majority of women will need assisted conception in the form of egg donation which should be discussed with parents. Girls with some ovarian function may conceive spontaneously. Genetic counselling is required to explain the risk of congenital defects and miscarriage. A hypoplastic uterus increases the risk of miscarriage. The need of adhering to long-term estrogen replacement therapy is essential.

Renal abnormalities

Structural renal malformations, such as horseshoe kidneys, and duplex systems are common in TS. It is essential to perform a renal ultrasonogram assessment at diagnosis.

❖ Liver function

Adults with TS have been found to have abnormal liver function, with a five-fold increased risk of cirrhosis. Liver function tests should be performed before pubertal induction and again before adulthood.

❖ Thyroid Abnormalities

Autoimmune thyroiditis is prevalent in TS. Thyroid function should be evaluated yearly. Preventing hypothyroidism is crucial for girls undergoing growth hormone (GH) medication.

❖ Glucose intolerance, insulin resistance, and diabetes mellitus

Glucose intolerance and insulin resistance may occur in those getting GH injections. These return to normal once the treatment is discontinued. In adults insulin resistance may cause cardiovascular disease.

❖ Ear problems

Middle ear illnesses such as suppurative otitis media, conductive deafness, suppurative middle ear disease with perforation are all frequently encountered. Specific treatment and monitoring should be done in the pediatric clinic. Regular audiological examinations with timely referral to ENT specialists is indicated.

❖ Eye problems

Ocular abnormalities such as strabismus, ptosis, and amblyopia are common. Eye examination and ophthalmological treatment may be required.

❖ Foot problems

Girls with TS also have foot issues which are structural, such as short and broad feet. Lymphoedema can cause risk of infection and cellulitis.

❖ Obesity

Obesity is common. Parents and health care providers should promote healthy nutrition and physical activity.

❖ Neck webbing

Intrauterine lymphoedema, usually resolves before birth leaving folds at neck. Significant webbing of neck is uncommon. Surgery can be undertaken in extreme cases but the cosmetic outcome may be disappointing due to keloid growth in the scars.

❖ Feeding difficulties

Feeding difficulties due to high arched palate maybe present which requires involvement of a nutritionist and a speech therapist.

❖ Behaviour patterns

High activity levels, a short attention span, and poor sleeping habits are some of the difficulties encountered in childhood. Referral to a clinical psychologist is recommended.

❖ Schooling difficulties

Most girls with TS have normal intelligence, which is comparable to the general population. A limited attention span and hearing loss are problems faced during school life. Some girls may require psychometric testing and psychological counselling.

❖ Psychosocial aspects

Girls with TS have less social involvement which needs to be addressed by the health care provider in consultation and guidance of a psychosocial expert. Social involvement can be less due to multiple co mobilities such as lack of hearing, short stature, physical appearance etc.

Surveillance - Women with TS require surveillance to promote health and hormone replacement, and to address comorbid conditions. Early diagnosis and treatment of conditions like hypothyroidism, hearing loss and eye problems. Estrogen replacement and cardiovascular health require more vigilance in these patients.

Estrogen replacement- Estrogen replacement maintains heart and bone health. Standard estrogen therapy is given orally. Transdermal estrogen is preferred in those with cardiovascular risk factors. Since these women are also at a higher risk of osteoporosis and fractures, estrogen replacement is essential. Progesterone can be started after estrogen therapy and usually leads to onset of menstruation.

1. Cardiovascular health – Hypertension, ischemic heart disease, aortic root dilatation and death due to aortic dissection/rupture cause increased morbidity and mortality. Measurement of aortic root diameter and cardiac evaluation by echocardiography and Magnetic resonance imaging (MRI)scanning should be done every few years in adulthood.

Genetic Counselling

Counsel on the need for postnatal karyotyping and screening for Y material as there is an associated risk of gonadoblastoma. Screening for heart and renal abnormalities should be highlighted. Provide information to the parents regarding short stature and premature ovarian failure and the treatment options available.Counselling should be provided regarding assisted reproduction and egg donation.Spontaneous pregnancies are uncommon. Fertility preservation can be offered when puberty occurs spontaneously and the girl has natural menstruation.

Information regarding autoimmune illnesses, cardiovascular diseases, metabolic disorders and hepatic diseases should be given. Recommend and counsel for a full psychological examination and management.

Parental Counselling

Parental counselling is an important element for optimization of quality of life along with a clinical geneticist and a pediatric endocrinologist. Counselling should include risk of short stature, ovarian insufficiency, its consequences in terms of infertility. It is important to address social skill issues and learning challenges for which psychological therapy is suggested such as cognitive behavioural therapy.

Care During Childhood

Clinical examination should include height, weight, BMI and pubertal growth. Screening for heart murmur, blood pressure, morphological features, skin signs, examination of the hips in infants and evaluation of possible scoliosis and kyphosis should be included. Analyse growth curve.

Check for related comorbidities such a hearing loss. Hormones FSH, LH, AMH, and oestradiol levels are measured. Antibodies against thyroid peroxidase (anti-TPO Ab), TSH, and FT4 should be included in the panel of investigations. Oral glucose tolerance tests and HbA1c ± glucose measurements should be done.

Tests for dyslipidemia should include triglyceride, total cholesterol, HDL and LDL values. Complete blood count and 25(OH)D [25-hydroxyvitamin D] should be sent for. In case of renal abnormalities or hypertension, renal function tests are required.

Y chromosomal material to be screened for and in case it is present, a pelvic ultrasound scan or MRI should be performed to rule out gonadoblastoma.

X-rays of the wrist and hand to assess bone age prior to growth hormone treatment.

Electrocardiogram(ECG) and Cardiac imaging in consultation with a cardiologist. Renal ultrasound scan to check for any abnormalities. ENT consultation along with hearing tests.

Ophthalmology consultation between the ages of 12 and 15 months to check for related abnormalities. Biannual appointments with a dentist should begin in the early childhood period. Psychometric tests for learning difficulties and psychological consultation is required.

Although cognitive performance is generally good, certain types of learning may provide difficulties. Evaluate academic performance and response to schooling.

Care During Adulthood

Height, weight, BMI and waist-to-height ratio, screening for a heart murmur, blood pressure measurement is recommended. Provide information that growth hormone treatment is not available after the developing cartilage has fused.

Suggest fertility preservation and egg donation. The importance of long-term interdisciplinary monitoring should be emphasized.

Cardiac ultrasonography and/or MRI along with an ECG. Renal ultrasound and ENT consultation followed by a hearing evaluation using audiogram. Bone density measurement and evaluation of calcium and vitamin D levels.

Ultrasound for uterine size assessment, endometrial thickness, ovarian size and antral follicle count. Blood tests, should include FSH, LH, estradiol, AMH, anti-TPO, TSH, FT4, HbA1c, cholesterol (total, HDL, LDL), triglycerides, liver function tests, antitransglutaminase

(IgA) Ab, blood creatinine, and microalbumin screening.

Psychological therapy is needed. When faced with socio-professional issues, personalized support should be suggested.

Multidisciplinary Team for Improving Health Related Quality of Life

Multidisciplinary patient care includes pediatric endocrinologists, adult endocrinologists, and gynecologists. Specialists such as cardiologist, clinical geneticist, nephrologist, medicine specialist, ophthalmologist, ENT specialist, psychiatrist and reproductive medicine specialist need to be involved in the care of the patient with TS. Health care professionals such as psychologist, educational therapy nurse, speech therapist, social worker, and dietician should also be included. Follow-up depends on the severity of the disease.

Therapeutic Education

Therapeutic education should focus, in particular, on the following points:

Assessments and knowledge dissemination regarding Turner syndrome and its therapies should be included in therapeutic education to ensure that patients and relatives completely understand and follow treatment plan.

It necessitates collaboration among many health experts working in hospitals or locally, including doctors, nurses, dieticians, psychologists, speech therapists, psychomotor therapists, physiotherapists, occupational therapists, and so on.

Associations

Families must be aware of patient associations, centres specializing in uncommon endocrine illnesses. By collaborating with patients, families, and carers, patient associations contribute to better overall Turner syndrome management. These groups can build on established care networks and educate families about the significance of regular follow-up. This promotes a relationship between patients and families and provides constant family supervision.

Suggested Reading

1. Ertl DA, Gleiss A, Schubert K, Culen C, Hauck P, Ott J et al. Health status, quality of life and medical care in adult women with Turner syndrome.Endocr Connect. 2018 Apr;7(4):534-543. doi: 10.1530/EC-18-0053. Epub 2018 Mar 7. PMID: 29514898; PMCID: PMC5881433.
2. Reis CT, de Assumpção MS, Guerra-Junior G, de Lemos-Marini SHV. Systematic review of quality of life in Turner syndrome. Qual life Research: Int J Quallife Aspects Treat care Rehabilitation. 2018;27(8):1985–2006.
3. Shankar RK, Backeljauw PF. Current best practice in the management of Turner syndrome. Ther Adv Endocrinol Metab. 2018 Jan;9(1):33-40. doi: 10.1177/2042018817746291. Epub 2017 Dec 18. PMID: 29344338; PMCID: PMC5761955.
4. Fiot E, Alauze B, Donadille B, Samara-Boustani D, Houang M, De Filippo G et al. Turner Syndrome: French National Diagnosis and Care Protocol (NDCP; National Diagnosis and Care Protocol) Orphanet J. Rare Dis. 2022;17:261. doi: 10.1186/s13023-022-02423-5.

Non - Immune Hydrops Fetalis

Dipika Deka
Senior Consultant Fetal Medicine
Global Ultrasound Fetal Medicine, Dwarka
Ex Prof & HOU, Chief Maternal Fetal Medicine, OBGYN, AIIMS, Delhi.

Hydrops fetalis (HF) is described when there is abnormal fluid collection in at least two body cavities of a fetus including pleural cavity, pericardial or peritoneal cavity or generalized skin edema (> 5 mm), placentomegaly or polydramnios. Immune hydrops develops as a result of Rh isoimmunization whereas non-immune hydrops refers to the other causes of HF. As the incidence of isoimmunization are decreasing due to routine Rh Anti-D use, fetal hydrops due to other causes are increasing. It is a grave condition with an overall poor prognosis, requires thorough evaluation to find the cause. Fetal and neonatal mortality is high and often the pregnancy is terminated. Intrauterine therapy is possible if the cause found is amenable to treatment with a good prognosis.

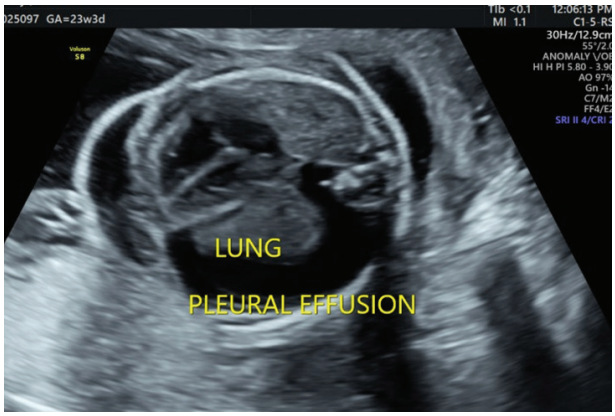


Fig. 1. Bilateral Pleural Effusion in a 23-week fetus (Deka et al).

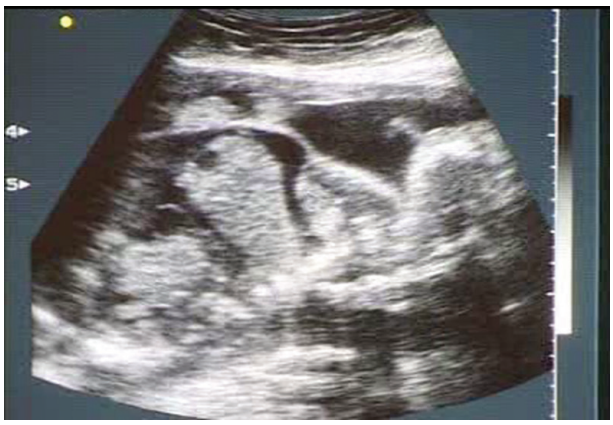


Fig. 2. Fetus with NIHF, liver floating in ascites, at 24 weeks pregnancy (Deka et al).

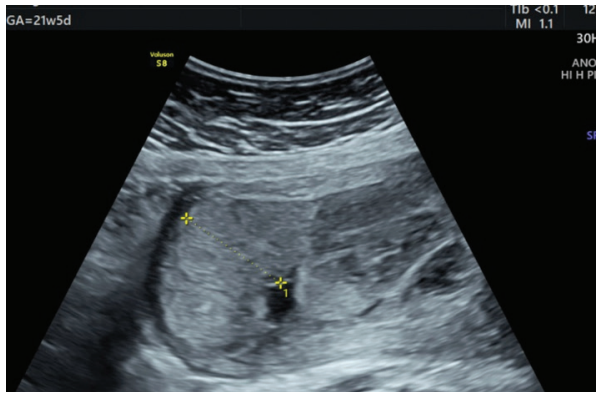


Fig. 3. Massive placentomegaly in a 21-week fetus with NIH (Deka et al).

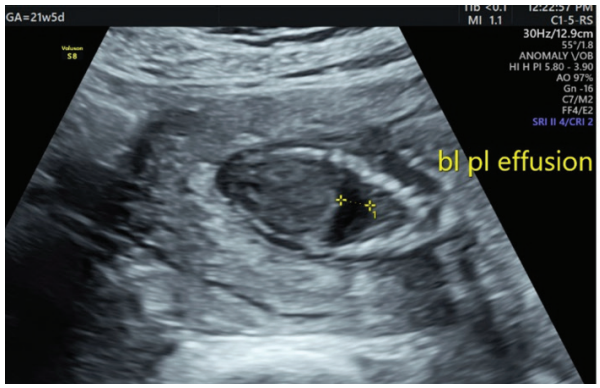


Fig. 4. Bilateral Pleural Effusion and severe oligo hydramnios in a 21-week fetus (Deka et al).

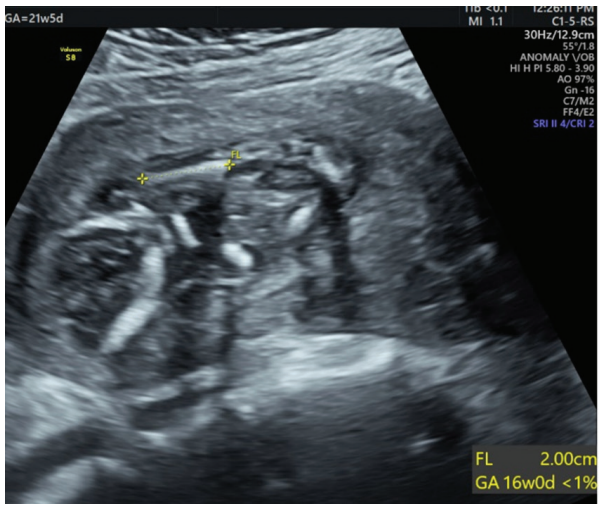


Fig.5. Short long bones with pleural effusion and severe oligohydramnios in a 21- week fetus (Deka et al).

Ultrasound Features

NIHF is identified very easily on ultrasound, with skin edema in parts of the body such as the scalp or nape, all over body, pleural or pericardial effusion, ascites and placentomegaly.

Causes

The basic mechanism of abnormal fluid accumulation is transudation and lymphatic obstruction. This can result from abnormal development of the fetal circulatory and drainage system and several structural abnormalities.

NIHF

Basic mechanism:

i. high plasma oncotic pressure,

ii. increased central venous pressure

iii. reduced lymphatic flow.

Hydrops is therefore a sign, not a diagnosis and the possible cause needs to be found by systematic thorough examination and investigation. The various causes could be grouped as maternal, maternal-fetal or fetal-placental.

1. Fetal causes:

A. Congenital Malformations:

Cardiac anomalies: may result in increased central venous pressure - paroxysmal supraventricular tachycardia, atrial flutter, or heart block (decreased ventricular filling), endocardial cushion defects, congenital pulmonary airway malformation, hypoplastic left or right heart, atrio-ventricular septal defects, aortic stenosis or atresia, pulmonary stenosis or atresia, tetralogy of Fallot, premature closure of the ductus. Myocarditis as a result of Coxackie, CMV and parvovirus B-19infections, can cause the hydrops.

Extra cardiac (lungs, abdominal, renal) anomalies: obstructed lymphatic drainage in thoracic, abdominal cavities - bowel obstruction/infarction, intestinal or esophageal atresias, volvulus, diaphragmatic hernia, meconium peritonitis, bronchopulmonary sequestration, Congenital pulmonary airway malformation (CPAM), Polycystic kidneys, protein loss and decreased colloid osmotic pressure, urinary ascites, nephrotic syndrome, Congenital nephrosis and prune belly syndrome. Hepatic disorders such as cirrhosis and necrosis.

Tumours: shunting of blood causing high output cardiac failure – neuroblastoma, saccro-coggygeal teratoma,

B. Infections:

TORCHE group, Parvovirus B19, can cause severe anemia and cardiac failure.

C. **Genetic:**

i. **Chromosomal aberrations (7-16% of NIHF):** may cause various cardiac and non-cardiac anomalies - Monosomy X - Turner's syndrome in 42-67% of aneuploid cases, Trisomy 21 in 20-30%, Trisomy 13 /18 /12 -10%, triploidy / tetraploidy, mosaicisms, unbalanced translocation.

It is now realised that when the karyotype is normal in the setting of fetal malformations or syndromes, a genetic basis is often present and results from pathogenic copy number variants (CNVs) in a gene, and other Rasopathies.

ii. **Monogenic Syndromes due to gene mutations (5-10% of NIHF):** can cause decreased erythropoiesis, anaemia, hypoproteinaemia: Noonan syndrome, Miller–Dieker syndrome, Neu–Laxova syndrome, Mucopolysaccharidosis, Cornelia de Lange, tuberous sclerosis, myotonic dystrophy are a few.

iii. Inborn errors of metabolism (IEM) (5-10% of NIHF) - Niemann-Pick disease type-C (NPC), Gaucher disease type 2, and beta-glucuronidase enzyme deficiency, skeletal dysplasias.

- Lysosomal storage disease (LSD) (25% of all NIHF cases, especially in recurrent NIHF and positive family history):

Mucopolysaccharidosis type VII (MPSVII, caused by a mutation in the gene encoding beta-glucuronidase)

Gaucher disease (GD, caused by a mutation in the gene encoding acid beta-glucosidase)

GM1-gangliosidosis (caused by a mutation in the gene encoding beta-galactosidase-1)

2. Maternal causes: Severe hypertensive disorders of pregnancy (HDP), uncontrolled thyroid disorders or diabetes can lead to fetal growth disorders – growth restriction (IUGR) and hypoxia. This leads to fetal high output cardiac failure state, increased catecholamine release, redistribution of blood to the vital organs (brain, heart, adrenals, and ductus venosus) and decreased hepatic, renal blood flow.

3. **Maternal/ Paternal-Fetal causes:** Alpha thalassemia, other blood group iso- immunization (Minor antigens like Kell, Duffy, Kidd, others), chromosomal (parental balanced translocation) and various genetic, metabolic diseases, Feto-maternal haemorrhage, Maternal TORCH infections leading to fetal infection.

4. **Placental:** chorioangioma, thrombosis or torsion of umbilical vein/cord, hypertrophy, feto-maternal haemorrhage.

Hydrops has a distinctive correlation to period of gestation as in early pregnancy the cause is mainly genetic, whereas after 22-24 weeks, hydrops is the result of malformations or infections. Most studies find the following order of prevalence - cardiovascular (21.7%), idiopathic (17.8%), genetic (13.4%), haematological (10.4%), infectious (6.7%), and metabolic disorders (1.1%), chest tumours (6.7%), urogenital conditions (2.3%), monochorionic twin pregnancy complications (TTTS, TRAP)) (5.6%) and gastrointestinal problems (0.5%).

About 40 types of LSDs account for up to 25% of NIHF cases, and one study described the overall incidence as 5.2% in all NIHF cases tested for any LSD, 17.4% in idiopathic NIHF cases, and 24.6% in idiopathic NIHF cases for which a comprehensive LSD workup was done.

CLINICAL EVALUATION: The Obstetrician's challenge is in the systematic, extensive search for cause of the hydrops. Idiopathic cause being the second most common, counselling of the couple is of utmost importance before ordering serological and genetic tests which are time consuming and costly too.

HISTORY:

Obstetric History: Previous fetal congenital malformation / Down syndrome or IEM, intrauterine deaths, miscarriages, developmental delay.

Family history: congenital malformation / Down syndrome or IEM, recurrent NIHF, genetic condition, developmental delay. Consanguinity is a very important cause of IEMs and many genetic syndromes, especially if recurrent or there is a family history.

Medical history: Fever in first trimester, exposure to infection, intake of teratogenic drugs, hypertension, medical disorders.

Examination and Investigations:

General physical examination:

Presence of high Blood Pressure, anemia, pedal or generalized edema, dry skin, abnormal facial features or structural defects suggesting syndromic appearance.

Obstetric examination:

Smaller fundal height suggestive of fetal growth restriction or distended abdomen indicating polyhydramnios.

Presence of bradycardia or abnormal fetal heart rate/ rhythm.

Ballantyne or Mirror syndrome – severe hypertension, edema, anemia, distended abdomen due to acute polyhydramnios.

Blood: Haemogram, Indirect Coomb's test (ICT), Rh and extended blood grouping for minor antigen. Screening for hemoglobinopathies. TORCH and Parvovirus IgG IgM and Avidity)

Urine: Protein and Casts.

Fetal Ultrasound:

Should be detailed, to look for any relevant cause of NIHF. Fetal growth and doppler, Middle cerebral artery (MCA) peak systolic velocity to detect fetal anaemia, hypoxia. Congenital malformations of all organ systems, in pleural effusions and lung anomalies the deviation of the heart to the right is an important prognostic factor. There could be contractures, skeletal abnormalities. Fetal echocardiography, neuro-sonography where necessary. Placental, amniotic fluid and cord anomalies should be looked for.

IEMs have some subtle pointers: the skin edema is a very prominent feature, especially nuchal fold, massive ascites hepatosplenomegaly. Usually, IEMs especially LSDs do not have major congenital abnormalities.

Genetic Testing:

Invasive testing (Amniocentesis or cordocentesis) to be offered to the couple after proper counselling regarding indication, yield, turnaround time and cost for the specific genetic tests[8,12,13]. Pleural, pericardial or ascitic tap sampling can be done if indicated and fluid can be sent for biochemical evaluation. Non-invasive prenatal screening (NIPs) does not have a role in this condition.

1. Karyotype
2. Chromosomal microarray (CMA)
3. Enzyme studies 'panel for NIHF' for specific IEM metabolites
4. Whole Exome sequencing (WES) or even whole genome sequencing (WGS)
5. TORCH-PCR, Parvovirus -PCR
6. Fetal blood grouping, Hemogram and hemoglobinopathy screening

Karyotype or FISH may not detect microdeletions or duplications; hence CMA is now preferred as the investigation of choice.

Gene sequencing is required to test for monogenic disorders by performing the 'gene panel' for NIHF. Whole exome sequencing testing (WES) may pick up novel mutations too. However, expert genetic

counselling is required to inform couples about the results of variant of unknown significance (VOUS).

In IEMs it is necessary to test index case (if present) for Prenatal diagnosis.

In some cases, Trio testing (couple and fetal genetic testing) for "Whole Exome or Whole Genome Panel." The autosomal dominant 'de novo' genetic mutations (no parental abnormality or affection) have poor prognosis, as in RASopathies, such as Noonan syndrome. However, it has a lower recurrence rate. A heterozygous parent could be a carrier but have lesser penetration and not show phenotypic abnormalities.

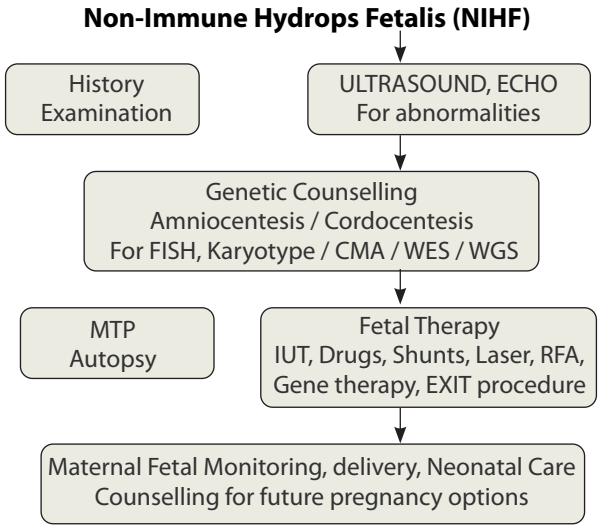


Fig. 5. Flow chart on Diagnosis and Management of NIHF.

Management

In 60-70% cases a cause can be detected and further cause specific management with options of available fetal therapy can be offered. These are as follows

A. Medical Termination of Pregnancy (MTP) as an option in potentially lethal gross congenital malformation or genetic disorders to be discussed and offered to the couple. If prenatal testing cannot be performed in such cases, fetal DNA sample can be obtained after the MTP for genetic molecular testing. Generally, culture for karyotype does not grow well from stillborn fetuses. Option and benefit of fetal autopsy should be reiterated while counselling for MTP.

B. Fetal Therapy can be attempted quite successfully in some indications, when the fetus is not detected to have a genetic or chromosomal aberration.

1. Intrauterine fetal blood transfusion (IUT) has a good prognosis in cases of fetal anemia due to Parvovirus infection, Feto-maternal haemorrhage. Alpha thalassemia can also be treated by IUT if couples wish to give birth the baby.
2. Maternal antibiotics or antivirals in CMV, herpes simplex, Toxoplasma gondii, treponema pallidum fetal infections
3. Cardiac arrythmias can be treated by maternal or direct fetal antiarrhythmic drugs like digoxin
4. Pleural tap or pleuro-amniotic shunt if necessary
5. Ascitic tap or drainage
6. Vescicocentesis, vescico-amniotic shunt or fetal cystoscopic laser ablation of posterior urethral valve by cystoscopy
7. Fetal aortic valvuloplasty in critical aortic stenosis HLHS
8. In utero Fetal pulmonary valvuloplasty for Pulmonary atresia.
9. In-utero resection, radiofrequency ablation (RFA), targeted laser in chorioangioma, teratoma.
10. In-utero enzyme replacement therapy, stem cell therapy, gene therapy for thalassaemia, lysosomal storage disorders
11. In complicated Monochorionic twin pregnancy: Fetoscopic laser ablation, or selective termination by bipolar cord coagulation, laser ablation or Radiofrequency ablation for complicate monochorionic twin such a TTTS, TRAP.
12. Ex Utero Intrapartum Treatment (EXIT) procedure for delivery in large neck tumours, intubation of baby with placental circulation in situ.

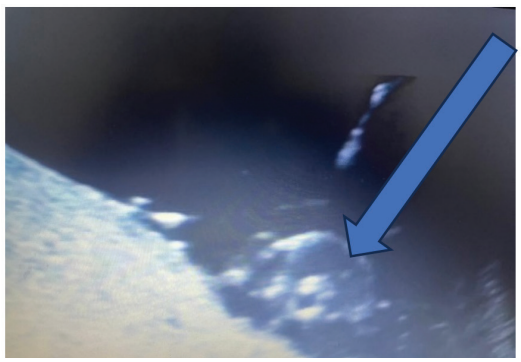


Fig. 6. IUT procedure, needle inside the Umbilical Vein at its origin from posterior Placenta (Deka et al).

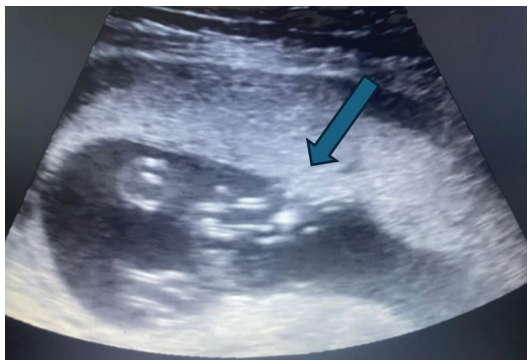


Fig. 7. IUT procedure in an 18 weeks fetus, needle inside the Umbilical Vein at its origin from anterior Placenta (Deka et al).

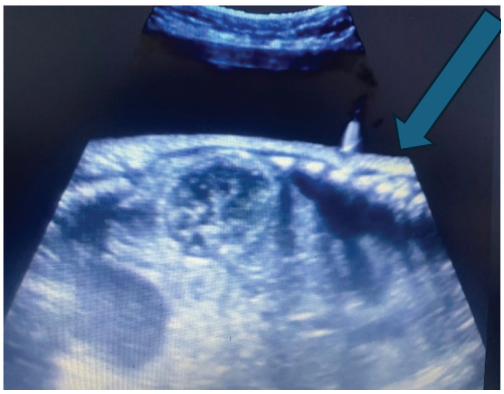


Fig.8. Pleuro-amniotic shunt, needle inside the chest wall (Deka et al).

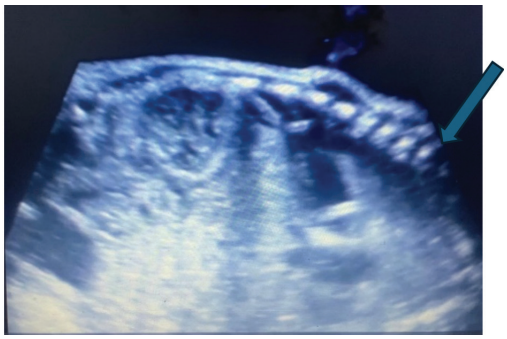


Fig.9. Double pigtail Pleuro-amniotic shunt inside the pleural cavity (Deka et al).

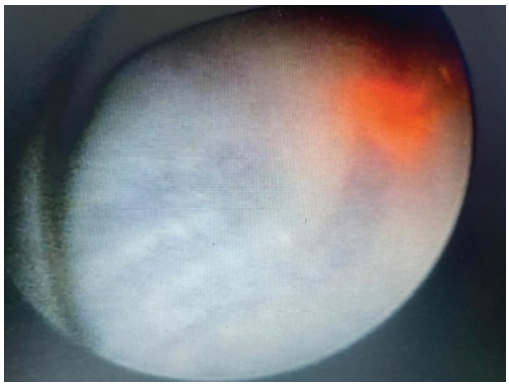


Fig.9. Endoscopic Laser coagulation of placental vessels in TTTS (Dipika Deka et al).

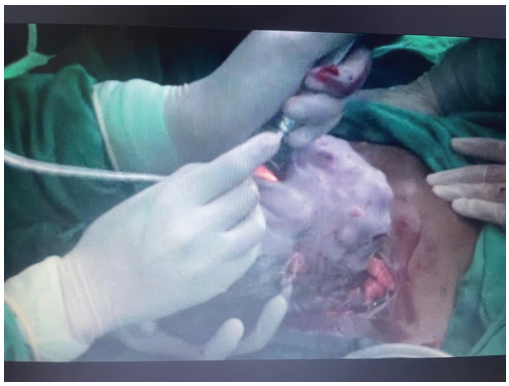


Fig.10. EXIT procedure delivery in large neck tumours (Dipika Deka, Vatsla Dadhwal et al)

Conclusion

NIHF is a challenging fetal condition with diverse etiology. Idiopathic causes being the second most common cause and with a potentially poor prognosis this condition is very tricky to manage. It requires thorough investigation and wherever possible intensive monitoring and Fetal therapy can achieve a good fetal outcome. Genetic testing plays a pivotal role for predicting the risk of recurrence. Pre-conception care would be tailored accordingly by Fetal Medicine specialist and geneticist before future pregnancy and recurrent causes should be managed from very early presentation.

Suggested Reading

1. Swearingen C,Colvin, Z.A.Leuthner, S.R. Nonimmune Hydrops Fetalis. Clin Perinatol. 2020, 47, 105–121
2. Hamza Bajwa, Hajira Basit. Thalassemia. In: StatPearls [Internet]. StatPearls; 2024
3. Przemyslaw Kosinski, Pawel Krajewski, Miroslaw Wielgos, Aleksandra Jezela-Stanek. Nonimmune Hydrops Fetalis—Prenatal Diagnosis, Genetic Investigation, Outcomes and Literature Review. J Clin Med, 2020
4. Norton, M.E.; Chauhan, S.P.; Dashe, J.S. Society for maternal-fetal medicine (SMFM) clinical guideline #7: Nonimmune hydrops fetalis. Am. J. Obstet. Gynecol. 2015, 212, 127–139
5. Thammavong K, Luewan S, Wanapirak C, Tongsong T. Ultrasound Features of Fetal Anemia Lessons from Hemoglobin Bart Disease. J Ultrasound Med. 2021 Apr;40(4):659-674
6. Leung KKY, Hon KL, Yeung A, Leung AKC, Man E. Congenital infections in Hong Kong: an overview of TORCH. Hong Kong Med J. 2020 Apr;26(2):127-138.
7. Meng D, Li Q, Hu X, Wang L, Tan S, Su J et al. Etiology and Outcome of non-immune Hydrops Fetalis in Southern China: report of 1004 cases. Sci Rep. 2019 Jul 24;9(1):10726
8. W. Kurdi. Non-immune fetal hydrops: Are we doing the appropriate tests each time? J Prenat Med, 2007 Jan-Mar; 1(1): 26–28.
9. Sileo FG, Kulkarni A, Branescu, I, Homfray T, Dempsey, E Mansour S et al. Non-immune fetal hydrops: Etiology and outcomes according to gestational age at diagnosis. Ultrasound Obstet. Gynecol. 2020.
10. Al-Kouatly, H.B Felder, L. Makhamreh, M.M. Bs, S.L.K, Vora N.L et al. Lysosomal storage disease spectrum in nonimmune hydrops fetalis: A retrospective case control study. Prenat. Diagn. 2020, 40, 738–745.

11. Mathias CR, Rizvi C. The diagnostic conundrum of maternal mirror syndrome progressing to pre-eclampsia – A case report. Case Rep Womens Health 2019;23:e00122.

12. Mone F, Eberhardt RY, Hurles ME, McMullan DJ, Maher ER, et al. Fetal hydrops and the Incremental yield of Next-generation sequencing over standard prenatal Diagnostic testing (FIND) study: prospective cohort study and meta-analysis. Ultrasound Obstet Gynecol 2021;58:509–18.

13. Williams D, Chitty LS, Maher ER, Kilby MD. Evidence to Support the Clinical Utility of Prenatal Exome Sequencing in Evaluation of the Fetus with Congenital Anomalies: Scientific Impact Paper No. 64 [February] 2021. BJOG 2021;128:e39–e50

14. Nassr AA, Ness A, Hosseinzadeh P, Salmanian B, Espinoza J, Berger V et al. Outcome and Treatment of Antenatally Diagnosed Nonimmune Hydrops Fetalis. Fetal Diagn Ther. 2018;43(2):123-128

Prediction of Preeclampsia

Aruna Nigam, Neha Bharadwaj*
Prof & HOD, Department of Obstetrics & Gynaecology, Hamdard Institute of Medical Science & Research, Jamia Hamdard, New Delhi, *Senior Resident, BLK Max Superspeciality Hospital, Delhi

Introduction

Worldwide, Pre-eclampsia, a pregnancy-specific disease is a leading cause of maternal and perinatal morbidity and mortality. This disorder affects 2% to 8%[1] of pregnant women. It is one of the leading cause of maternal morbidity and mortality. Globally, 76,000 women and 500,000 babies die annually from this disorder. With delivery being the only current cure, preeclampsia contributes significantly to prematurity, neonatal morbidity and perinatal mortality. Therefore, it is necessary to screen patients who are at higher risk of developing pre-eclampsia.

Various PE predictors have been identified and are currently undergoing research for their application in clinical settings in terms of cost, availability and accessibility.

ISSHP 2022 guidelines define pre-eclampsia as gestational hypertension at ≥20 weeks gestation accompanied by one or more new onset of proteinuria or maternal end-organ dysfunction which includes neurological complications, pulmonary oedema, hematological complications, Liver or kidney involvement and Uteroplacental dysfunction.[2]

In 2016, WHO increased the Antenatal visits from 4 visits to 8 visits with the idea to increase opportunities to detect and manage potential complications of pregnancy. However, recent studies shows that the majority of pregnancy complications arising later in pregnancy can be predicted in the first trimester with a multitude of tests. Thus, inverting the pyramid of antenatal care i.e. shifting the focus towards prediction rather than managing complications.[3]

As evidenced by a randomized controlled trial [PHOENIX: Planned early delivery or expectant management for late preterm pre-eclampsia; 34+ 0and 36+ 6weeks, identifying patients at higher risk of pre-eclampsia enables increased surveillance, well-timed delivery and reduction in maternal morbidity, severe hypertension compared with expectant management.[4]

Pathogenesis

Pathogenesis of PE has been well explained by The two-stage theory.

During early placentation, trophoblast cells invade the wall of the maternal uterine vasculature. Environmental factors, immunological factors and genetic factors result in defective cytotrophoblast invasion into maternal uterine spiral arteries. This is considered stage 1 in two-stage theory. This results in hypoperfused placenta which releases anti-angiogenic factors like soluble fms-like tyrosine kinase 1 (sFlt1) in maternal circulation causing endothelial dysfunction and systemic vascular dysfunction (stage 2).

Various studies have suggested that sFlt1 and endoglin (sEng)-TGF-β coreceptor, correlates well with preeclampsia severity before the onset of PE symptoms.[6] sFlt1 is a spliced version of VEGFR1 (Flt1). In normal pregnancy, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) bind to VEGFR1 to induce angiogenesis. In preeclampsia, the hypoperfused placenta produces excessive soluble form of VEGFR1 (sFlt1) that antagonizes VEGF and PlGF in maternal serum and impairs angiogenesis as well as VEGF- and PlGF-induced microvascular relaxation.[5] (Figure1)

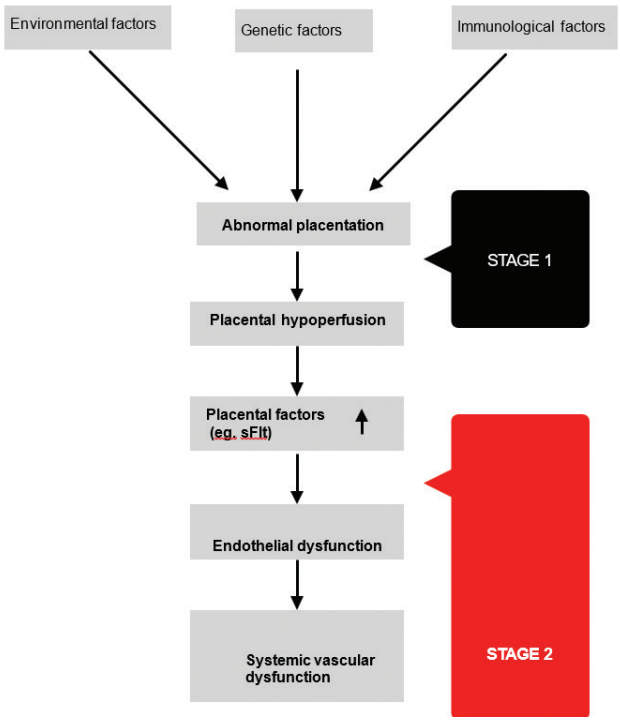


Figure1: Two Stage theory of Pathogenesis of Preeclampsia

Predictors of pre-eclampsia

Screening is necessary to contextualize risk and plan accordingly. Recently, various predictive biomarkers for

preeclampsia have been identified for risk stratification, targeted surveillance in early pregnancy (less than 16 weeks) and timely delivery.[6] Added to this it may also identify lower risk patients, hence, safely reducing their antenatal visits.

1. Maternal Risk Factors

Several professional societies such as the National Institute for Health and Care Excellence (NICE) and the American College of Obstetricians and Gynecologists (ACOG) have proposed screening for preeclampsia based on maternal risk factors. Recent evidence has shown that maternal risk factor stratification based on the NICE and ACOG approach has suboptimal performance. NICE recommendation only achieves detection rates of 41% and 34% with a 10% false-positive rate (FPR) for preterm and term pre-eclampsia respectively.[7]

The expanded list of clinical risk factors included in The U.S. Preventive Services Task Force (USPSTF) recommendation has led to a considerable improvement in the detection rates to 90% and 89% for preterm and term pre- eclampsia respectively. Though, FPR has increased to 64%. An updated version of USPSTF guideline has now been endorsed by ACOG and Society for maternal and fetal medicine (SMFM).[8]

USPSTF recommends 3 categories of maternal risk factors: High, Moderate, and Low Risk (Table1)

Table 1: Three categories of maternal risk factors as recommended by USPSTF

High Risk factors	Moderate risk factors	Low Risk
History of preeclampsia, especially when accompanied by an adverse outcome	Nulliparity	Previously uncomplicated pregnancy and delivery
Multifetal gestation	Obesity (body mass index >30 Kg/m^2)	
Chronic hypertension	Family history of preeclampsia (mother or sister)	
Pregestational type 1 or 2 diabetes	Black persons (due to social, rather than biological, factors)	
Kidney disease	Lower income	
Autoimmune disease (i.e. systemic lupus erythematous, antiphospholipid syndrome)	Age 35 years or older	
Combinations of multiple moderate-risk factors	Personal history factors (low birth weight or small for gestational age, previous adverse pregnancy outcome, >10-year pregnancy interval)	
-	In vitro conception	

Role of Aspirin

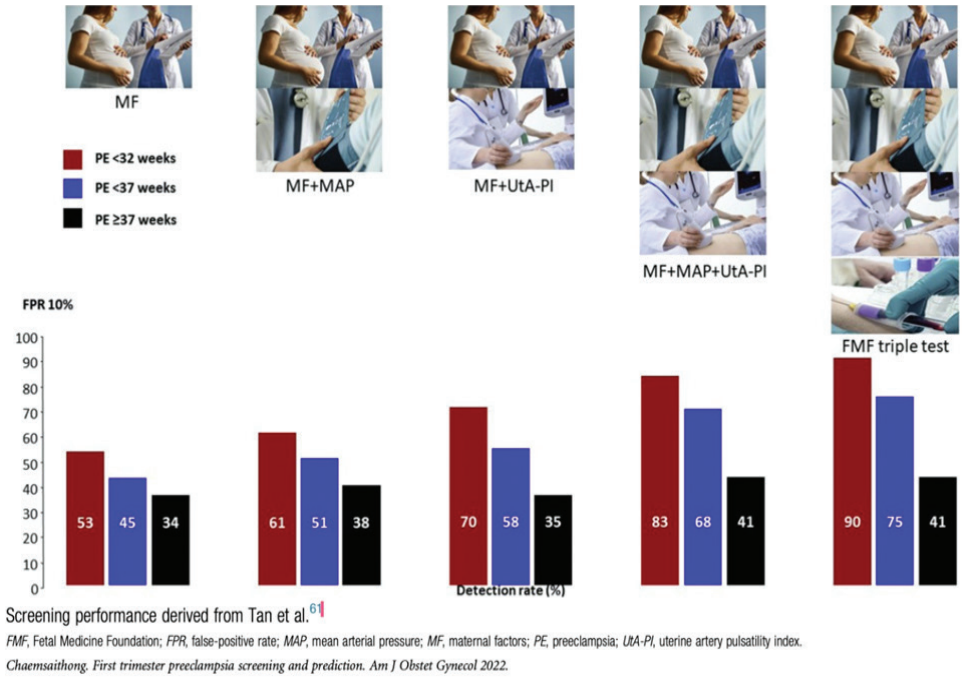
USPSTF confirms recommendations on the usage of aspirin for the prevention of preeclampsia. USPSTF reaffirms the 2014 recommendations for usage of low-dose aspirin (typically 81mg) starting between 10-16 weeks of pregnancy to prevent preeclampsia. Aspirin is recommended if patient has ≥1 of high-risk factors, ≥2 moderate-risk factors. Aspirin is not recommended for patients at low risk.[9] ASPRE Trial in 2017 (Combined Multimarker Screening and Randomized Patient Treatment with Aspirin for Evidence-Based Preeclampsia Prevention) involved 26,941 singleton pregnancies from 13 maternity hospitals in 6 countries (the United Kingdom, Spain, Italy, Belgium, Greece, and Israel). It was found that the detection rates of preterm was 77% and term pre-eclampsia was 43% with FPR of 9.2%.

2. Biomarkers -

Maternal risk factors alone detects 44.8% and together with MAP it detects 50.5% preterm pre- eclampsia**.** False positive rate of both is 10%.[10]

FIGO recommends baseline test of combination of maternal factors with MAP in low resource countries.[11]

FMF recommends triple test as the best predictive test for pre-eclampsia.[10] Triple test is a combination of maternal factors, MAP, Uterine artery pulsatility index(UtA-PI), Serum PlGF with detection rates of 90%, 75% and 41% for very early (<32 weeks), preterm and term pre- eclampsia.



Screening performance derived from Tan et al.[61]

FMF, Fetal Medicine Foundation; *FPR*, false-positive rate; *MAP*, mean arterial pressure; *MF*, maternal factors; *PE*, preeclampsia; *UtA-PI*, uterine artery pulsatility index.

Chaemsaithong. First trimester preeclampsia screening and prediction. Am J Obstet Gynecol 2022.

Figure 2: Screening performance of the first trimester FMF prediction model for PE according to the different combinations at FPR of 10%.[10]

Table 2 summarizes various predictors of PE.

	Predictors	Specifications	Advantage	Disadvantage
1	Clinical risk factors (USPSTF)	High or moderate risk factors	• Readily accessible • Applies to all women at the first visit • Aspirin prophylaxis • Cost effective	44.8% detection rate
2	First trimester combined algorithm for preeclampsia (FMF recommended)	Maternal factors, MAP, UtA-PI, Serum PlGF to stratify risk	• Detects women at risk of early pre- eclampsia • Aspirin prophylaxis	• Does not predict term pre-eclampsia • Expensive
3	sFlt1:PlGF ratio	>38 represents screen positive	• If sFlt1:PlGF is 38 or less there is 99.3% negative predictive value for pre- eclampsia within a week a strong "rule out" test	• Limited to suspected preeclampsia at <37 weeks not applicable to the general pregnant population
			• Reduces admissions for women with suspected preeclampsia	• Unable to accurately predict ("rule in") who will develop pre- eclampsia, with low sensitivity and positive predictive values
4	PlGF alone	<100pg /ml represents screen positive	• Screen positive in women with suspected preeclampsia at <35 weeks achieves 96% sensitivity and 98% negative predictive value for preeclampsia developing within 2 weeks • Its use has been shown to reduce the time to diagnosis, adverse maternal outcomes, outpatient attendances and costs to healthcare service	Limited to suspected preeclampsia at <35 weeks not applicable to the general pregnant population (ii) Unable to accurately predict ("rule in") who will develop pre- eclampsia at term.
	Newer Biomarkers			

5	Placenta markers			
5a	Placental RNA	EVT signature Adm	EVT signature increases during 11-17weeks	Modest predictive efficacy
			gestation and at	
			32-34weeks.	
			Adm- reduced in Term	
			PE	
5b	Placental proteins	PlGF PPI3 PAPP-A AFP GDF-15	Associated with PE	Other biomarkers need to be added to enhance the predictive performance of sFlt-1, PlGF and PP13
6	Endothelial markers			
6a	Endothelial RNA's	Gata2 miR126	Reduced in early onset PE	Modest predictive efficacy
6b	Endothelial proteins	ADMA ET1	Deranged in early onset PE	Modest predictive efficacy
		CT- pro-ET1		
		VCAM1		
		Endocan		

USPSTF (The U.S. Preventive Services Task Force), FMF (Fetal Medicine Foundation), MAP (Mean arterial pressure), UtA-PI (Uterine artery pulsatility index), PlGF (Placental growth factor), sFlt1 (Soluble fms-like tyrosine kinase 1), RNA (Ribonucleic acid), EVT (Extravillous cytotrophoblast), Adm (Adrenomedullin), PPI3 (Protein 13), PAPP-A (Pregnancy-associated plasma protein A), AFP (alpha-fetoprotein), GDF-15 (Differentiation Factor 15), PE (Pre-eclampsia), ADMA (Asymmetric dimethylarginine), ET1 (Endothelin-1), CT- pro-ET1 (C-terminal proendothelin-1), VCAM1 (Vascular cell adhesion molecule 1)Table 2: Various predictors of Preeclampsia sFlt-1 is antiangiogenic factor whereas PlGF is a pro-angiogenic factor. PROGNOSIS study showed that a sFlt-1:PlGF ratio of 38 or lower can accurately rule out the likelihood of developing preeclampsia among women at less than 37 weeks with negative predictive value of 99.3%.[12] sFlt-1:PlGF ratio >38 is suggestive of women at risk of developing PE within four weeks with positive predictive value of 36.7% and sensitivity of 66.2%.

PlGF alone has also been evaluated to triage care in women with suspected preterm preeclampsia. With high negative predictive value it has also been shown to predict preeclampsia requiring delivery within two weeks with modest predictive accuracy.

Placental Markers- Placental RNA

Tarca et al studied 20 circulating mRNAs deemed as "extravillous cytotrophoblast" (EVT) specific by single-cell RNA sequencing across gestation. EVT signature is increased as early as 11-17 weeks gestation and it further increases at 32-34 weeks once diagnosis is established.[13]

Adrenomedullin (Adm), a placenta-enriched RNAs was assessed and found to be significantly reduced at both 28 and 36 weeks in those destined to develop term PE. Other circulating biomarkers like mRNAs NR4A2, EMP1, PGM5, SKIL, and UGT2B1 have been identified as possible predictive markers and are still under research.[14]

Placental Proteins

PlGF and placental protein 13 (PP13) as considered the best predictive biomarkers. PlGF has 65% sensitivity at 89% specificity and PP13 has 27% sensitivity with a specificity of 88%. Differentiation Factor 15 (GDF-15), a member of the transforming growth factor b superfamily is found to be increased in the circulation of patients with preterm preeclampsia and is under research.[15]

Endothelial Markers- Endothelial RNA's

Endothelial cells release miRNAs that plays an important role in regulating endothelial and possibly cardiovascular function. Markers that have been investigated include miR149 and miR363 among others with 45% sensitivity at 90% specificity respectively. Gata2 and miR126 were both reduced in the circulation of patients with early onset preeclampsia (<34wks).[16]

Endothelial Proteins

NO is synthesized by Nitric oxide synthase (NOS) using L-arginine as a precursor. NO maintains endothelial integrity by regulating vasodilation, adhesion of leukocytes and platelet aggregation. A methylated

product of L-arginine-Asymmetric dimethylarginine (ADMA) has been found to have bio-marker potential for preeclampsia. ADMA endogenously inhibits NOS to reduce NO production.[17] Hence, causing endothelial dysfunction and further systemic vascular dysfunction as described earlier in two-stage theory of PE.

Other endothelial biomarkers still under research- Endothelin-1 (ET-1) or its precursor protein - C- terminal proendothelin-1 (CT- pro-ET1), VCAM and Endocan.[18] A combination of CT-pro-ET1, sFlt-1 and systolic blood pressure demonstrated sensitivity of 80% at 90% specificity for development of severe preeclampsia within 1 week among women at high risk.[19]

Conclusion

Early screening should be done to identify women at higher risk of developing PE. In low resource settings basic testing with maternal risk factors and mean arterial pressure should be done. Predictors in LMIC should be cheap, easy-to-use and that requires minimal training. FMF- Triple testing is recommended and future research is required to identify the predictor which are cost effective. Potential predictive tests are undergoing research currently to ascertain their use as a predictive too.

References

1. Guidelines [Internet]. Isshp.org. Available from: https://isshp.org/guidelines/ [cited 2024 Jun 2].
2. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009 Jun;33(3):130-7.
3. Nicolaides KH. A model for a new pyramid of prenatal care based on the 11 to 13 weeks' assessment. Prenat Diagn. 2011 Jan;31(1):3-6.
4. Chappell LC, Brocklehurst P, Green ME, Shennan A; PHOENIX Study Group. Planned early delivery or expectant management for late preterm pre-eclampsia (PHOENIX): a randomised controlled trial. Lancet. 2019;394(10204):1181-1190.
5. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. Placenta. 2009 Mar;30 Suppl A(Suppl A):S32-7.
6. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004; 350: 672–683.
7. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, et al. Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. Ultrasound Obstet Gynecol. 2017 Jun;49(6):756-760.
8. Preeclampsia - US preventative services task force affirms recommendations on aspirin usage for preeclampsia prevention [Internet]. Preeclampsia Foundation - Saving mothers and babies from preeclampsia. Available from: https://www.preeclampsia.org/the-news/research-news/us-preventative-services-task-force-affirms-recommendations-on-aspirin-usage-for-preeclampsia-prevention [cited 2024 Jun 2].
9. Rolnik DL, Wright D, O'Gorman N, Nicolaides KH. ASPRE trial: performance of screening for preterm pre-eclampsia. Ultrasound Obstet Gynecol. 2017 Oct;50(4):492-495.
10. Tan MY, Syngelaki A, Poon LC, Rolnik DL, O'Gorman N, Delgado JL, Akolekar R et al. Screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. Ultrasound Obstet Gynecol. 2018 Aug;52(2):186-195.
11. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, McAuliffe F et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. Int J Gynaecol Obstet. 2019 May;145 Suppl 1(Suppl 1):1-33
12. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. N Engl J Med. 2016 Jan 7;374(1):13-22.
13. Tarca AL, Romero R, Erez O, Gudicha DW, Than NG, Benshalom-Tirosh N et al. Maternal whole blood mRNA signatures identify women at risk of early preeclampsia: a longitudinal study. J Matern Fetal Neonatal Med. 2021 Nov;34(21):3463-3474.
14. Hannan NJ, Stock O, Spencer R, Whitehead C, David AL, Groom K, et al. Circulating mRNAs are differentially expressed in pregnancies with severe placental insufficiency and at high risk of stillbirth. BMC Med. 2020 May 22;18(1):145.
15. Townsend R, Khalil A, Premakumar Y, Allotey J, Snell KIE, Chan C, Chappell LC et al ; IPPIC Network. Prediction of pre-eclampsia: review of reviews. Ultrasound Obstet Gynecol. 2019 Jul;54(1):16-27.
16. Whigham CA, MacDonald TM, Walker SP, et al. Circulating GATA2 mRNA is decreased among women destined to develop pre- eclampsia and may be of endothelial origin. Sci Rep 2019;9(1):235.
17. Németh B, Murányi E, Hegyi P, Mátrai P, Szakács Z, Varjú P, et al Asymmetric dimethylarginine levels in preeclampsia - Systematic review and meta-analysis. Placenta. 2018 Sep;69:57-63.
18. Valencia-Ortega J, Zarate A, Saucedo R. Placental Proinflammatory State and Maternal Endothelial Dysfunction in Preeclampsia. Gynecol Obstet Invest 2019;84(1):12–9.
19. Saleh L, Verdonk K, Visser W. The emerging role of endothelin-1 in the pathogenesis of pre- eclampsia. Ther Adv Cardiovasc Dis 2016;10(5):282–93.

Rh Alloimmunization: Combating the Spectre

Charu Sharma[1], Pratibha Singh[2], Divya Chennaboina [3], Kratika Badi[3]
[1]Additional Professor, [2]Professor, [3,4]Junior Residents.
Department of Obstetrics & Gynecology, All India Institute of Medical Sciences, Jodhpur, Rajasthan

Introduction

Rhesus (Rh) alloimmunization poses a significant obstetric challenge and can result in serious complications for the fetus and newborn, such as hemolytic disease of the newborn, hydrops fetalis, and fetal demise. This condition usually occurs when an Rh-negative woman is exposed to Rh-positive red blood cells, often during a previous pregnancy or delivery, leading to the production of Rh antibodies that can cross the placenta and attack the fetal red blood cells.

The incidence of Rh-negative pregnancies in Indian women has been reported to be low, varying from 3.0 to 5.7% as compared to the Africans.[1] The alloimmunization rate varies from 0.4% to 2.7% among pregnant women worldwide. There are only a few studies from India that have studied alloimmunization in pregnancy. The alloantibody prevalence in India was found to be 1.25% in a study by Pahuja et al who also reported the prevalence of alloimmunization to be higher (5.5%) in pregnant women with bad obstetric history (BOH) than in normal pregnancies (0.5%).[2]

Additionally, the data on the utilization of prophylactic treatment among Rh-negative pregnant women, pregnancy complications and Rh incompatibility-associated adverse events (AEs) are also lacking. The lack of such information might result in an inability to formulate strategies to meet the real-world situations in eradicating Rh-negative pregnancy-related morbidities and mortalities in India.

Pathophysiology for Rh allo-immunisation

The formation of maternal antibodies to fetal red blood cell (RBC) antigens is called **RBC alloimmunization previously called Rh isoimmunisation.** The antigens which are present on the human RBCs are mainly ABO antigens (A, B, AB), rhesus D antigen (Rh-D) and infrequently rhesus (Rh) non D antigens like C, c, E, e, other atypical antigens like Kell (K), Duffy (Fya), Kidd (Jka, JKb), M and S.[3]

In most cases, ABO incompatibility does not lead to significant fetal hemolysis and anemia as the antibodies destroy the fetal red blood cells carrying the D antigen before any immune response occurs. However, it's important to note that **Rh(D) and Kell** are the most common antigens causing alloimmunization.

Upon initial exposure to these antigens, the body produces IgM antibodies, which are too large to cross the placenta and thus do not harm the fetus. However, during subsequent pregnancies, a secondary immune response triggers the production of IgG antibodies. These IgG alloantibodies can pass through the placenta and potentially lead to hemolysis, resulting in hemolytic disease of the fetus and newborn (HDFN). **(Figure 1)**

Kell alloimmunization is primarily caused by previous blood transfusions but can also occur due to feto-maternal haemorrhage (FMH) during pregnancy.

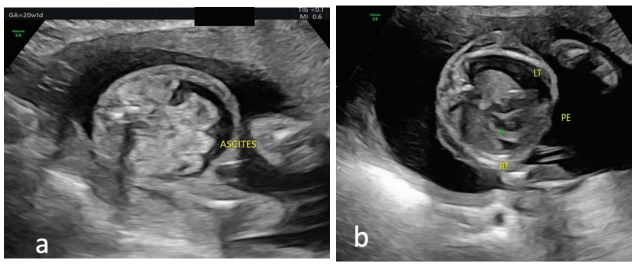


Figure 1: Ultrasound depicting a transverse view of the abdomen (a) depicting fetal ascites and (b) pleural effusion suggesting fetal hydrops

It has been found that the amount of FMH is very low, around 0.03 mL, during the first and second trimesters, with an incidence of less than 1%. However, it can be as high as 25 mL with an incidence of 46% during the third trimester. The **Kleihauer-Betke** test (Box 1) can detect fetal blood cells in maternal circulation through an acid elution test.[4] Another method of calculating FMH is **flowcytometry.**

BOX 1

Kleihauer-Betke test

- To perform the Kleihauer-Betke test, a maternal blood smear is exposed to an acid solution. HbF is resistant to acid, while HbA is removed. The smear is then stained using Shepard's method, which causes fetal cells to appear "pink-rose" and maternal cells to have a "ghost-like" appearance. The percentage of fetal to maternal red blood cells is calculated.
- The fetomaternal hemorrhage volume is calculated by multiplying the percentage of fetal red blood cells by 50, as 50 represents the average maternal blood volume (5 liters).
- The number of vials needed is determined by dividing the fetomaternal bleeding volume by 30 (one vial or 300 µg can prevent alloimmunization from exposure to up to 30 ml of fetal blood)

1. Prevention of Rh Alloimmunisation

2.1 Approach for a Rh Negative Woman

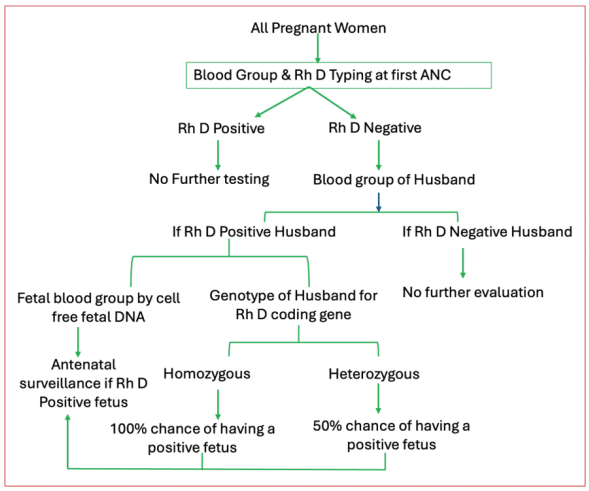


Figure 2: Approach for a Rh Negative pregnant woman

Most of the Western guidelines recommend finding the fetal blood group from circulating cell-free fetal DNA in maternal blood. However, in India, the facilities for testing zygosity for the Rh-D gene and fetal blood group from circulating cell-free fetal DNA in maternal circulation are available only in a few centres. Therefore, a pregnancy, when the mother is Rh-D negative and the father is RhD positive, is considered potentially at risk of allo-immunization.[5, 6]

1.1 Screening for Rh Alloimmunization

Small amount of FMH (total of less than 15 mL) is inevitable during the course of pregnancy. To detect the sensitization of the mother, presence of anti-D antibodies in maternal circulation (antibody screening) is usually detected by indirect coombs test (ICT). Antibody screening identifies pregnancies at risk of Haemolytic disease of fetus and new born(HDFN) and is used to stratify the risk and to guide management.

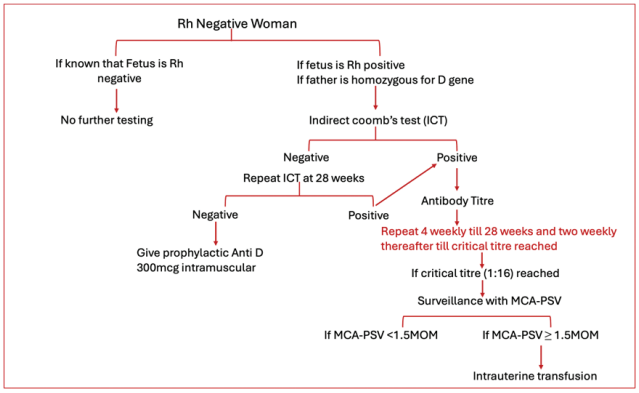


Figure 3: Screening for Rh alloimmunisation

2.3 What is Indirect Coombs test (ICT)

ICT is done on maternal serum by **tube agglutination test**. Maternal blood is treated with Rh positive RBCs. If antibodies are already present, they will bind to the D antigens of Rh positive RBCs. These are then washed and suspended in coombs serum containing antihuman immunoglobulin. This causes the agglutination of antibody-bound RBCs and is a positive test. The titre is estimated by diluting the maternal serum serially and incubating every time by positive RBCs to look for agglutination on adding coombs serum. The positive titres are represented as 1:4, 1:8, 1:16, 1:32 and so on. Critical titre lies between 1:16 and 1:32 depending on the method used whether tube or gel microcolumn assay and is generally considered as 1:16.[7]

1.2 How to prevent sensitisation?

For women who are not yet allo-immunised, the aim is to prevent sensitization. It can be achieved by giving a prophylactic dose of anti-D immunoglobulins to cover for the spontaneous FMH and also any antepartum event which has the potential to cause additional FMH. If no prophylaxis is given, it is estimated that 1 % of Rh-D-negative women will develop antibodies by the end of the first Rh-D-positive pregnancy. Around 7–9 % of additional women would be sensitized at the time of delivery. Another 7–9 % would develop antibodies during 6 months following delivery. Therefore, around 17 % of women would become sensitized by the second pregnancy.

1.3 When to administer Anti D

Anti-D immune globulin prophylaxis reduces the rate of Anti-D alloimmunization to less than 0.2% if given both antepartum at around 28 weeks and postpartum within 72 hours (ACOG recommendations).[5] Anti D should also be given after any potential sensitising event during pregnancy. (Box 2)

BOX 2

Potential Sensitizing Events in Rh D-Negative Women in Pregnancy

- Chorionic villus sampling, amniocentesis, cordocentesis
- Threatened miscarriage or miscarriage
- Ectopic pregnancy
- Evacuation of molar pregnancy
- Therapeutic termination of pregnancy
- Antepartum hemorrhage
- Abdominal trauma
- Intrauterine fetal death
- External cephalic version
- Delivery

1.4 What is the Dosage of Anti D

A standard dose of 300 µg of anti-D immunoglobulin can prevent Rh (D) alloimmunization following exposure to up to 30 mL of Rh (D) positive fetal whole blood or 15 mL of Rh (D) positive fetal RBCs. Despite giving Anti-D, certain women still develop Rh alloimmunisation. The reasons are depicted in Box 3.

BOX 3
Reasons for alloimmunisation despite Giving Anti D
Inadequate Dosage due to large inoculum (FMH)
Antenatal Dose is missed and the sensitisation has already occurred in third trimester
Poor maintenance of cold chain.
Improper site of administration (correct site being deltoid and not gluteal region).
Presence of extended antibodies.

How to manage an Rh allo-immunised pregnancy

Once ICT is positive, woman is said to be allo-immunised. It has to be taken into account that a low maternal anti Rh (D) titer (typically <4) (ICT +ve) can be detected for several weeks after the administration of Rh immunoglobulin.

The management of Rh allo-immunization involves a multidisciplinary approach, including

a. Close antenatal surveillance,

b. In severe cases, intrauterine fetal transfusions or even early delivery.

c. Maternal immunosuppressive medications may also be considered in certain cases to mitigate the antibody response.

Antenatal surveillance

3.1 Antibody Titre by ICT

In allo-immunised pregnancy, the antibody titres should be repeated at 4 weekly intervals until 28 weeks and thereafter at two weekly intervals. Once a critical titre (1:16) is reached, surveillance by ultrasound colour doppler by measuring the middle cerebral artery peak systolic velocity (MCA-PSV) is recommended for early detection of fetal anemia.

3.2 How to do Middle cerebral artery Peak systolic velocity(MCA-PSV) surveillance

Ultrasound is the preferred method for screening fetal anemia which is characterized by reduced blood viscosity, leading to increased venous return, elevated cardiac output, and greater blood flow velocity in all vessels.

While various vessels and aspects of the MCA Doppler wave form have been studied to predict fetal anemia, it has been consistently found that the peak systolic velocity (PSV) offers the best test performance. The MCA-PSV value for diagnosing fetal anemia was first proposed in 1990. Since then, it has been used as one of the best non invasive method to detect fetal anemia.

The MCA-PSV should be measured in the axial section of fetal head in trans thalamic plane at its proximal point, following its origin from the internal carotid artery, at a 0° angle. Color flow mapping should be used to identify the circle of Willis and the proximal MCA, just caudal to the transthalamic plane.The pulsed-wave Doppler gate should then be placed at the the proximal third of the MCA, close to its origin in the internal carotid artery (the systolic velocity decreases with increasing distance from the point of origin of this vessel) (8) **(Figure 4a, 4b)**

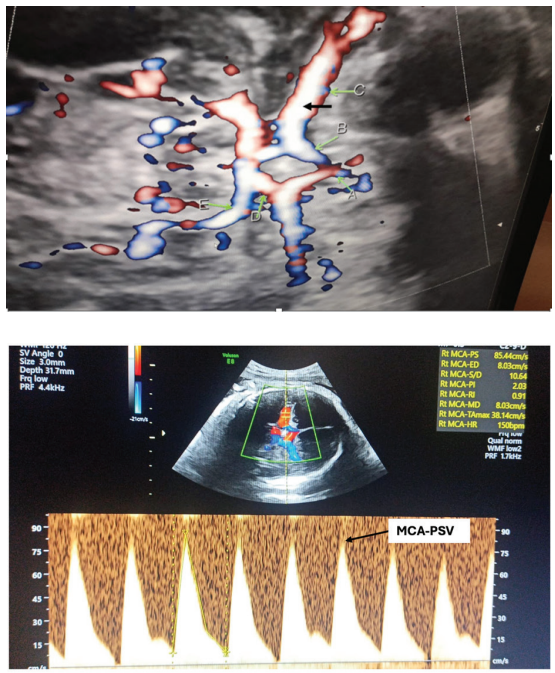


Figure 4a: Circle of Willis showing A- Anterior Cerebral Artery, B-Anterior Communicating Artery, C-Middle Cerebral Artery, D- Posterior Communicating Artery, E- Posterior Cerebral Artery; Black arrow shows the proximal part of MCA where peak systolic velocity is measured. Figure 4b: Colour Doppler showing MCA waveforms and peak systolic velocity.

The MCA-PSV had a sensitivity of close to 100% (95% CI 0.86–1.0) for detection of moderate to severe fetal anemia with a false-positive rate of 12% at 1.50 MoM. A meta-analysis estimated that MCA-PSV ≥1.5 MoM had a sensitivity of 86% and a specificity of 71% for the

diagnosis of severe anaemia in un transfused fetuses.[9]

If MCA PSV is ≥1.5MoM, Intrauterine Transfusion (IUT) should be Planned

3.3 Intrauterine transfusion (IUT)

There have been no trials evaluating the best management for pregnancies with red blood cell alloimmunization. However, fetal transfusion is likely the most effective treatment available in cases of fetal anemia. This technique involves ultrasound guided needle placement followed by transfusion of a specially prepared blood component. The Procedure is usually done after 18 weeks of gestation if indicated. The steps involved in IUT are summarised as below.

i. Choosing the Right Patient & taking Informed Consent
ii. Information regarding failure to gain access, fetal bradycardia (5-10%), cord accident, intra-uterine fetal demise (1-2%), need for emergency caesarean section and preterm labour (10%) should be provided.
iii. Blood Preparation
   a. O negative blood if D antibody
   b. Type specific blood if Non-D
   c. Infection Screening (Specifically CMV along with other infections)
   d. Fresh blood < 7days old
   e. Gamma irradiated (25Gy)
   f. Leuko-depleted and cross-matched packed red blood cells having haematocrit of 75%-85% to be transfused
iv. Calculation of blood for Intravascular Transfusion- Volume depends on
   - Initial Fetal hematocrit (HCT)/ Expected fetal Haematocrit
   - Fetoplacental Volume
   - Donor HCT
   - Target HCT- generally taken as 45%-50%. (Split up before 24 weeks)
   - Volume to be transfused= Fetoplacental Volume X (desired HCT- Expected Fetal HCT)/ Donor HCT
     [Fetoplacental Volume= 1.046+[USG estimated weight of fetus X 0.14]

   Note: Can also use the Online Calculator on Perinatology.com
v. Maternal Preparation: No anaesthesia is generally required. However, local anaesthesia can be considered.
vi. OT setting
   a. Pancuronium or vecuronium (0.1mg/kg of effective fetal weight) and/or atracurium-(0.4mg/Kg) should be available.
   b. Adrenaline prefilled syringe must be kept ready
   c. To maintain sterility, the ultrasound probe should be covered by a sterile glove, and a camera cover can be put over the cord.
   d. Anaesthetist and Neonatologist should be pre-informed, unless there is a need for emergency caesarean.
   e. The materials required on OT trolley should be ready and checked. (Box 4), Figure 5

BOX 4

Materials required on OT trolley for Intrauterine transfusion

a. 6-7 heparinised 10 ml syringes to draw blood from blood bag.
b. 2 heparinised 5 ml syringes
c. 1 heparinised 2ml syringe
d. 1 non heparinised 1ml and 10 ml syringe to prepare vecuronium (labelled to avoid confusion)
e. EDTA Vial
f. Plain vials
g. 22-gauge spinal needle for intra-muscular fetal paralysis
h. 20-gauge spinal needle (with yellow hub)
i. long connector with 3-way stopcock
j. Blood transfusion set
k. Adrenaline loaded syringe
l. A sterile cover to drape the USG wire and glove to cover the USG probe.
m. 20 ml syringe loaded with saline

Fi**gure 5:** Materials required for Intra-uterine Transfusion

vii. Procedure:
   - Under ultrasound guidance, identify a fixed segment of the cord.
   - Try to localise the umbilical cord at its placental insertion site i.e. where the cord inserts into placenta, zoom it and confirm the vein by colour flow.
   - Avoid free cord loops as they are more inclined to move and tear. However, if free loop has to be used, it can be stabilised by uterine sidewall, but it is less desirable.
   - The procedure is easiest when the placenta is anterior and site is approached transplacentally, however penetration of placenta increases the risk of feto-maternal haemorrhage and fetal loss 3.6% versus 1.3%.
   - Fetal Paralysis: It can be done by using a 22 gauge spinal needle using Pancuronium/ Vecuronium (0.1mg/kg EFW; Atracurium-0.4mg/Kg)
   - Setting up the transfusion Line- Pre-heparinised 10 ml syringes 6-7 in number

must be kept ready to be filled by blood. Priming the syringes and connecter with heparin or sodium citrate solution will prevent clot formation.

- Needle Entry (Figure 6)

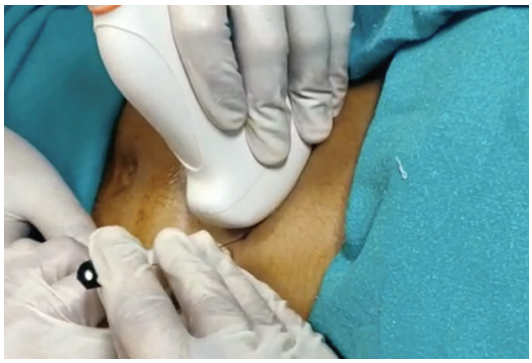


Figure 6: Technique of Needle entry

- On entering the umbilical cord, stylet is removed.
- If flow is immediate, obtain 1 ml blood sample in syringe to see the Haematocrit and send the remaining blood to the laboratory.
- If flow is not immediate and you you are in Wharton's jelly, slowly and carefully reposition the needle to enter into the vein
- Through the same access, inject a paralytic agent.
- However, if the placenta is posterior, trans amniotic approach is used, and the paralytic agent is injected intramuscularly into the thigh of the fetus with a 22-gauge spinal needle.
- Wait for 5-10 minutes and then proceed with IUT at the rate of 3-4 ml/min, through the 10 ml syringes connected to the three-way connector.
- Watch the segment of umbilical cord to see if blood is flowing through umbilical vein. (Figure 7)

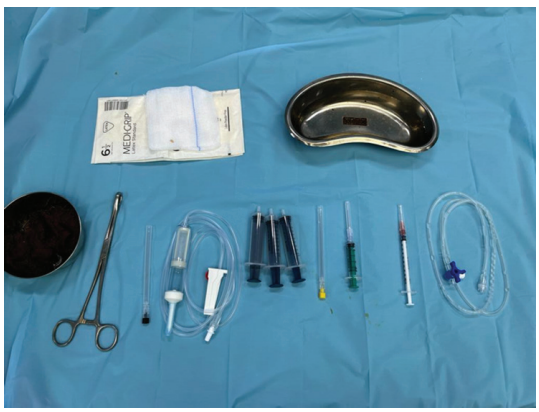


Figure 7: showing blood flow in the umbilical vein

viii. Post transfusion monitoring

- Once the blood is transfused, withdraw 1 ml of blood and discard. Next, withdraw another 1ml of blood to check the post-transfusion haematocrit.
- After the transfusion is complete and the needle is removed, watch the puncture site for streaming and check the fetal heart rate for bradycardia.
- All cases >28 gestational weeks should be monitored with fetal heart rate tracing for one hour following the procedure.

ix. Planning subsequent transfusions

- Based on the expected fall in haematocrit(Hct), which is equivalent to a 1% fall per day.
- MCA PSV is not very reliable for subsequent transfusions.

Complications of IUT

- Fetal bleeding- Most common complication of Cordocentesis /IUT (20-30%)
- Significant feto-maternal bleeding occurs in about 40%. It is common with procedures involving anterior placenta, with procedure lasting longer than 3 minutes, those requiring two or more needle insertions.
- Bradycardia- Transient fetal bradycardia occurs in 5-10%

  Most cases of fetal bradycardia resolve without intervention within five minutes. A higher rate of bradycardia occurs in growth-restricted foetuses (17% versus 4%) or in foetuses with absent diastolic flow (21% versus 5%)
- Infection: 1% cases
- Fetal loss- 1.4-2%.

  (Fetal loss is more if gestational age <24 weeks or the fetus has hydrops)

3. Planning Delivery: The timing of delivery depends on the gestational age, severity of fetal anaemia, and fetal maturity. When fetal surveillance has reassuring signs, labor can often be induced between 37 and 38 weeks. This approach has the advantage of allowing for attempted vaginal delivery. However, delivery by caesarean section is common when the fetal condition requires delivery before 34 weeks. If delivery is expected before 36 weeks, it is recommended to use steroids for fetal lung maturation.

   Intrauterine transfusions are generally performed until the 34th week of gestation. After 34 weeks, the risks of the procedure outweigh the benefits, and elective delivery is preferable when severe anemia

is suspected. Therefore, delivery can be planned at gestation > 35 weeks. (Box 5)

BOX 5

Care at Delivery

During delivery, these measures should be followed:

- Keep cross-matched blood ready before induction of labour in case of fetal anemia
- Clamp cord immediately.
- Keep cord long for possible catheterisation.
- Collect cord blood for blood group, bilirubin and direct agglutinin test. Exchange transfusions may be needed soon after birth.

Key Points

- Common red cell antigens that can cause significant fetal anaemia are D, c, E, e, Kell
- Invasive testing is not contraindicated if alloimmunisation has occurred.
- Anti-D prophylaxis should be given to prevent potential sensitisation after invasive testing prvided the mother is rhesus D (RhD) negative and is not sensitised.
- Anti-D prophylaxis should be given as early as possible after delivery within 72 hours but can be given even up to 10 days.
- The aim of management is to prevent sensitisation in an Rh negative unimmunised woman and detect fetal anemia and initiate early treatment in an Rh negative alloimmunised woman.

Conclusion

In conclusion, Rh alloimmunization is a complex condition that requires a comprehensive understanding of its pathophysiology, accurate diagnosis, and tailored management strategies to optimize fetal and neonatal outcomes.

Suggested Reading

1. Shah, P., Pawar, S.H., Naik, S.N., Sivjyothi, T., Rao, A. and Kakkar, A., 2023. A Real-world Prospective Study to Evaluate the Geographical Distribution, Isoimmunization Rate, and Utilization of Prophylactic Treatment of Rh-negative Pregnant Women in India (RhYTHM Study). Journal of South Asian Federation of Obstetrics and Gynaecology, 15(5), pp.594-600.
2. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus. 2011 Oct;9(4):388-93. doi: 10.2450/2011.0050-10. Epub 2011 May 31. PMID: 21839025; PMCID: PMC3200407.
3. Shaz BH. Perinatal Transfusion Medicine. In Transfusion Medicine and Hemostasis 2009 Jan 1 (pp. 241-249). Academic Press.
4. Kim YA, Makar RS. Detection of fetomaternal hemorrhage. Am J Hematol. 2012 Apr;87(4):417–23.
5. Norton ME, Stone J, Berghella V, Sciscione AC, Tate D, Schenone MH. Society for Maternal-Fetal Medicine (SMFM) Clinical Guideline #8: the fetus at risk for anemia--diagnosis and management. Am J Obstet Gynecol. 2015 Jun;212(6):697-710. doi: 10.1016/j.ajog.2015.01.059. Epub 2015 Mar 27. PMID: 25824811.
6. Practice Bulletin No. 181: Prevention of Rh D Alloimmunization. Obstetrics & Gynecology 130(2):p e57-e70, August 2017. | DOI: 10.1097/AOG.0000000000002232
7. Argoti P, Angarita AM, and Mari G. Hemolytic Diseases of the Fetus and Newborn. In Maternal-Fetal Evidence Based Guidelines. 2022 4th Ed. Editors: Vincenzo Berghella, Chapter 55 (pp.532-551) CRC press, NewYork.
8. Bhide A, Acharya G, Baschat A, Bilardo CM, Brezinka C, Cafici D, et al. ISUOG Practice Guidelines (updated): use of Doppler velocimetry in obstetrics. Ultrasound Obstet Gynecol. 2021 Aug;58(2):331-339. doi: 10.1002/uog.23698. Epub 2021 Jul 19. PMID: 34278615.
9. Martinez-Portilla RJ, Lopez-Felix J, Hawkins-Villareal A, Villafan-Bernal JR, Paz Y Miño F, Figueras F, Borrell A. Performance of fetal middle cerebral artery peak systolic velocity for prediction of anemia in untransfused and transfused fetuses: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2019 Dec;54(6):722-731. doi: 10.1002/uog.20273. PMID: 30932276.

Complicated Monochorionic Pregnancy: Mastering the Care

Shradha Agrawal*, K Aparna Sharma**
*Junior resident, **Professor
Department of Obstetrics and Gynaecology, AIIMS, New Delhi

Introduction

In a monochorionic twin pregnancy, both the fetuses are dependent on a single, shared placenta. If zygote divides after the first 72 hours, a monochorionic twin pregnancy results. These twins often have significantly more complications than their dichorionic counterparts and this should be included in the antenatal counselling of the mothers.

Diagnosis of Monochorionic Twins

All women with a twin pregnancy should be offered an ultrasound examination between 11^{+0} to 13^{+6} weeks gestation to assess fetal viability, gestational age, chorionicity and to exclude major congenital malformations.

Gestational age in twin pregnancies conceived spontaneously should be determined at first trimester scan by using the crown-rump length of the larger fetus to avoid the risk of estimating it from a baby with early growth pathology.

Chorionicity can be determined based upon the number of placental masses, presence of an intervening membrane and its thickness. This scan is best performed before 14 weeks gestation. Two separate placenta usually, but not always,a thick dividing membrane, generally ≥2 mm supports dichorionicity. The twin peak sign, also called lambda or delta sign, due to the placenta insinuating between the amniochorion layers is seen in dichorionic pregnancy. In contrast, a thin membrane (generally <2 mm) and T sign is seen in monochrionic diamniotic pregnancy.

Early diagnosis can help with management of the associated risks posed by twins.

Also, a careful visual examination of the placenta and membranes after delivery can establish chorionicity in many cases.

Aneuploidy Screening in Monochorionic Twin Pregnancy

Aneuploidy screening- Similar to that in singleton pregnancies, using the combined test (NT, beta hcg and PAPP-A). Noninvasive prenatal screening using cfDNA is also acceptable, having lower detection rates in multifetal pregnancies than singleton pregnancies. However, all these tests should be interpreted with value threshold set for multifetal pregnancies.

Screening for structural anomalies- Routine detailed ultrasound scan between 18-22 weeks for fetal anomaly as well as extended views of fetal heart for any anomaly. Monochorionic twins should undergo echocardiography because of an increased risk for cardiac anomalies.

Optimum ultrasound regimen for uncomplicated monochorionic pregnancies- Every 2 weeks from 16 weeks onwards until delivery.

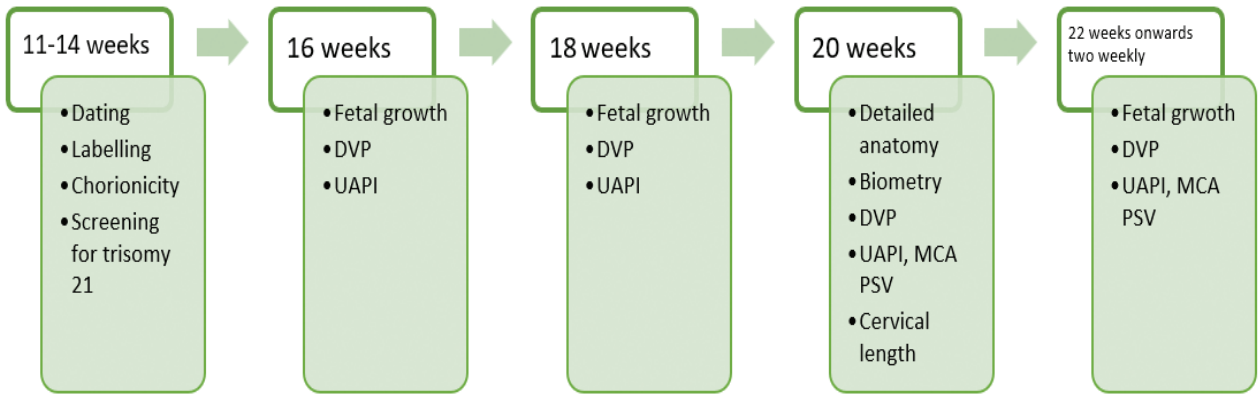
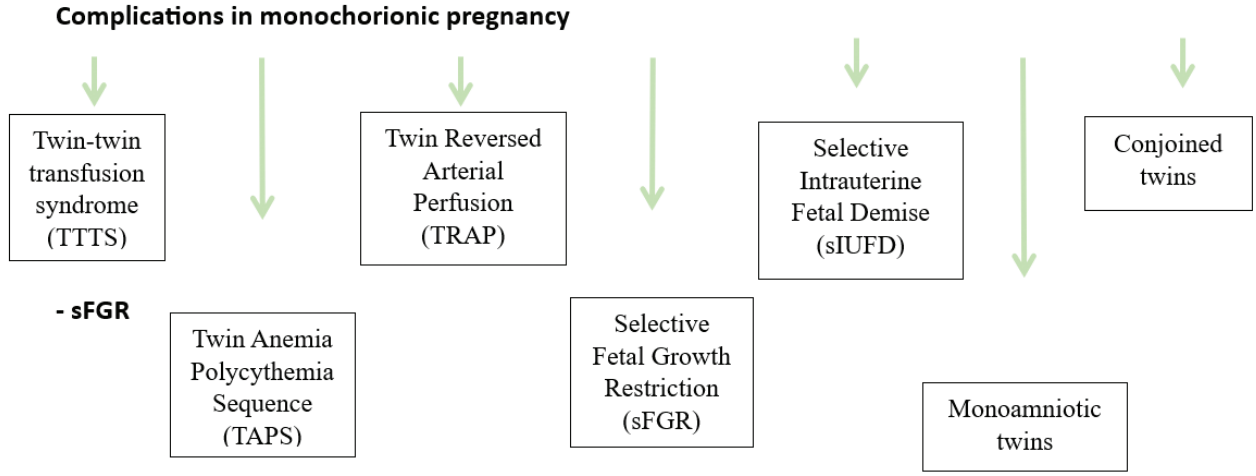


Figure 1: Ultrasound surveillance in uncomplicated monochorionic pregnancy

Implications of discordance in Nuchal translucency (NT) or Crown rump length (CRL) in the first trimester

NT discordance of ≥20% is associated with more than 30% risk of early intrauterine death or development of severe TTTS. However, the predictive value of discordance in NT or CRL in the development of TTTS is poor. Risk of complications is less than 10% if the NT discordance is <20%. CRL discordance of ≥10% or NT discordance ≥20% is an indication of detailed ultrasound assessment and testing for karyotypic abnormalities.



All monochorionic placentas share some anastomotic connections. They may be artery-to-artery, vein-to-vein, or artery-to-vein connections; may be superficial or deep. The degree to which these anastomoses are dangerous to either twin depends on the intertwin pressure gradient .

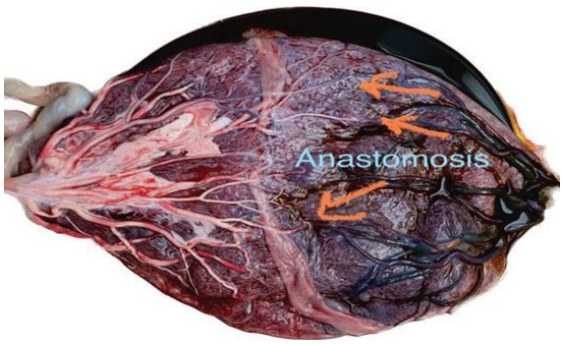


Figure 2 showing anastomosis in monochorionic placenta

Twin-Twin Transfusion Syndrome

Pathophysiology- TTTS complicates 10-15% of monochorionic twins. In this syndrome, blood gets transfused from donor twin to its recipient sibling such that the donor becomes anemic and eventually growth restricted. In contrast, the recipient becomes polycythemic and develops circulatory overload with heart failure and hydrops and may suffer from hyperviscosity and occlusive complications. TTTS typically presents in midpregnancy when the donor becomes oliguric and develops oligohydramnios and the recipient fetus develops polyhydramnios. Cerebral abnormalities and neurodevelopmental disability are some serious complications and can occur in both donor and recipient twins.

Surveillance begins at 16 weeks gestation and studies performed every 2 weeks noting and recording fetal biometry and liqour volumes, deepest vertical pocket (DVP). Fetal bladder should also be visualised.

Diagnosis is based on sonographic findings which are staged using the Quintero system. Growth discordance may be found with TTTS but are not considered diagnostic criteria.

Prognosis is related to the Quintero stage and gestational age at presentation. Some stage I cases remain stable or even regress without intervention. However, 60% cases progress. Conversely, stage III or higher have perinatal loss rate of 70 to 100% without intervention.

Management options include laser ablation of anastomoses, septostomy, amnioreduction or selective feticide.

TTTS stage I-

- Normal cervical length and women without any symptoms- Expectant management- Weekly DVP, Umbilical artery Pulsatility index (UAPI) and Middle cerebral artery peak systolic velocity(MCA PSV); 3-4 weekly growth parameters.
- Maternal discomfort, worsening polyhydramnios or short cervical length (<25mm)-

<26 weeks- Fetoscopic laser ablation

>26 weeks- Amnioreduction due to technical limitations with advanced gestation

TTTS stage II or more- Fetoscopic laser ablation

Amnioreduction should be reserved when TTTS is diagnosed after 26 weeks or in situations where expertise for laser coagulation is not available or for immediate relief of symptoms due to polyhydramnios.

Following laser treatment, recurrence rate is up to 14% and ultrasound surveillance should be performed weekly initially for 2 weeks or till clinical resolution occurs followed by 2 weekly till termination. Each scan should assess fetal growth, amniotic fluid volume and doppler (UAPI, MCA PSV and ductus venosus doppler) along with examination of the fetal brain, heart and limbs.[3] Risk of neurodevelopmental impairment in TTTS survivors treated with laser therapy is 14% with a higher risk in patients developing TTTS at later gestations.[5]

Monochorionic twin pregnancies complicated by TTTS and treated should be delivered between 34-37 weeks gestation.[1]

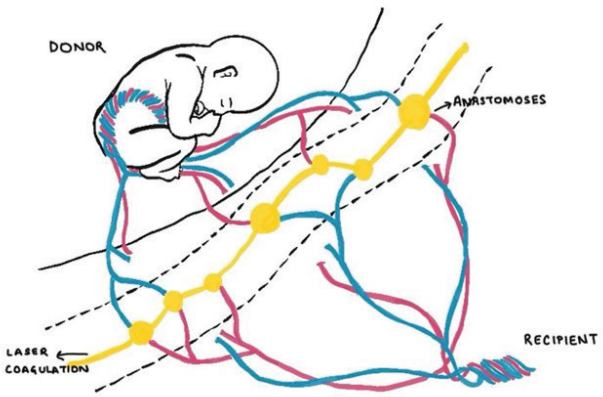


Figure 3 demonstrating fetoscopic laser coagulation

Twin Anemia-Polycythemia Sequence

Chronic fetofetal transfusion due to 'miniscule' artery-vein anastomoses underlies this form, which is characterised by significant haemoglobin differences between donor and recipient twins. Discordancy in MCA PSV values in multiple of median(MoM) between the twins (> 1.5 MoM in donor and <1 MoM in the recipient twin) without significant oligohydramnios/ polyhydramnios is diagnostic.

TAPS can occur spontaneously in upto 2% of monochorionic twins and in 13% following fetoscopic laser ablation. Hence close sonographic surveillance is needed even after laser therapy.

Routine screening for TAPS is not recommended and is confined to complicated monochorionic twin pregnancy where risk of TAPS is high (such as TTTS or sFGR)

Twin Reversed-Arterial-Perfusion Sequence

The twin pair is a normally formed donor twin and a grossly malformed recipient twin that lacks a heart. It is caused by a large artery-to-artery placental shunt and is characterised by a unidirectional flow of blood from donor to recipient (acardiac) twin. This results in cardiomegaly and high output cardiac failure in the donor twin and a malformed recipient twin. Radiofrequency ablation of the umbilical cord is the preferred modality of choice.

Selective Fetal Growth Restriction

Slower growth of twins compared to singletons is a physiologic phenomenon due to epigenetic and hormonal mechanisms. Monochorionic twins have a higher discordancy rates than dichorionic twins.

Diagnosis- At each scan from 20 weeks gestation onwards, EFW discordance is calculated. Discordance is calculated using the formula:

([Larger twin EFW - Smaller twin EFW] / larger twin EFW) x 100

sFGR is defined as an EFW discordance of more than 20%, and is associated with an increase in perinatal risk.

Liquor volumes should be measured to differentiate from TTTS. In isolated sFGR, there is commonly oligohydramnios in one sac and normal liquor in the other sac.

Classification and prognosis- Umbilical artery doppler evaluation in sFGR allows definition of prognosis and perinatal morbidity. Hence, the classification of sFGR depends on the pattern of end-diastolic velocity in the umbilical artery. In Type I, the umbilical artery doppler waveform has positive end-diastolic flow. In Type II, there is absent or reversed end diastolic flow (AREDF). In Type III, there is cyclical/ intermittent pattern of AREDF.

Survival rate in Type I is greater than 90%. Type II sFGR is associated with a high risk of IUD (29%) of the growth restricted twin and associated risk of neurodevelopmental delay if the other twin survives. Type III sFGR is associated with 10-20% risk of sudden death of the growth restricted fetus which is unpredictable (even when ultrasound features have been stable) and 10-20% risk of neurological injury in the larger twin.

Surveillance- Once sFGR is diagnosed, at least weekly monitoring of fetal well being with doppler assessment (UA and MCA) and 2 weekly growth parameters is recommended.

Termination of pregnancy- In type I sFGR, delivery should be planned by 34-36 weeks gestation. In type II and III sFGR, planned delivery should be considered by 32 weeks, unless fetal growth velocity is significantly abnormal or there is worsening of fetal doppler assessment.

Selective Intrauterine Fetal Demise (sIUFD)

Pathophysiology- In monochorionic twins, there is placental vascular anastomosis and hence a risk of 'acute inter-twin transfusional events' causing sudden fetal death. This can occur in apparently uncomplicated monochorionic twins but is more prevalent in monochorionic twins complicated by sFGR and even treated TTTS. Damage to the surviving twin is believed to be caused by loss of its circulating volume into the circulation of the dying twin leading to ischemic organ damage.

Why is screening required? When it occurs in the first trimester, it manifests as a vanishing twin. For the survivor, risk of death does not increase and no additional surveillance is required. sIUFD occurring in later gestations poses risk of death, preterm birth and neurological injury in the survivor twin. Monochorionic diamniotic twins with sIUFD had 15% risk of co-twin demise. sIUFD occurring before 34 weeks was associated with 29% risk of neurodevelopmental morbidity in monochorionic diamniotic twins (threefold higher than in dichorionic gestations) whereas in sIUFD occurring after 34 weeks, likelihood of neurological deficits was essentially the same between both monochorionic and dichorionic twins.

Monitoring- Survivor twin should be monitored for fetal anemia by serial MCA PSV monitoring. Fetal biometry and assessment of umbilical artery and MCA doppler should be scheduled every 2-4 weeks. Fetal Magnetic resonance imaging(MRI) of the brain may be performed 4-6 weeks after co-twin demise to detect neurological abnormality in the survivor twin and neurodevelopmental assessment should take place at 2 years age.

Management- Conservative management (i.e. continuing the pregnancy) is often the most appropriate course of action in preterm sIUFD. In preterm sIUFD continuing the pregnancy is often the most appropriate course of action because neurological harm to the surviving twin, if any, has already happened by the time death was diagnosed and preterm termination will only increase the neurological morbidity.

Monoamniotic Twins

These twins make up for <1% of all twin pregnancies. They have higher risk of congenital anomalies and fetal echo is strongly recommended. Twin-twin transfusion syndrome rates are however lower due to the near universal presence of protective arterioarterial anastomoses. Umbilical cord entanglement is almost always present in MCMA twins but does not contribute to morbidity and mortality. Monitoring done 2 weekly starting from 16 weeks, admission at 26-28 weeks, steroid cover, twice weekly biophysical profile and deliver by cesarean section at 32-34 weeks.

Selective Fetal Reduction

Monochorionic twins carry a higher risk of complications due to vascular anastomoses and discordance for structural anomalies. These conditions may lead to fetal demise in one of the twins which are associated with cotwin demise or severe cerebral injury. In dichorionic twins, conservative management is preferred whereas for monochorionic twins, this warrants intervention to protect the healthy cotwin against the adverse effects of spontaneous demise of the other.

Indications for selective fetal reduction- Selective reduction should be considered in all higher order pregnancies including triplets and these are typically done in late first trimester. Other indications are complications of monochorionic twin pregnancy such as advanced TTTS, TRAP, sFGR (type 2 and 3) . In case of twins discordant for anomalies, candidates for selective termination are those where one twin is at risk of demise posing a risk to the cotwin, or when the anomaly carries risk of significant morbidity after birth. Non lethal or surgically correctable abnormalities may not be offered reduction as it poses a significant risk to the other fetus.

Timing of selective termination- Usually offered between 16-27 weeks gestation. Beyond 28 weeks, the risk due to the procedure may be greater and there are reasonable chances of survival even without the procedure.

Techniques available- Aim is to occlude the umbilical cord which can be by intrafetal procedures such as interstitial laser coagulation (ILC) and radiofrequency ablation (RFA), or coagulation of the umbilical cord- fetoscopic laser coagulation (FLC) and bipolar cord coagulation (BCC). RFA generally has fewer

complications but has unfavorable outcomes in advanced gestations and technically difficult in fetuses with congenital abnormalities. In monoamniotic twins where cord transection is needed, BCC or FLC are preferred.[7] In case of an anomaly causing polyhydramnios and affecting the course of pregnancy, amnioreduction followed by termination of the affected fetus can be done in the same sitting.

Complications- Selective fetal reductions are precarious procedures and carry risks of fetal demise, PPROM, preterm delivery and intrauterine infection. Risk of preterm birth prior to 32 weeks is 20%. Risk of adverse neurological sequelae in the surviving cotwin may also be increased compared with that in uncomplicated pregnancy.

Post procedure monitoring- Fetal cardiac activity should be checked the day following the procedure. If fetal anemia is suspected, MCA doppler should be performed followed by ultrasound examination to assess the fetal brain at 2 and 4 weeks after the procedure. In case of suspected cerebral injury, fetal MRI should be advised.

1. Labor and delivery

• Type of twin gestation	• Timing of delivery
• Uncomplicated dichorionic diamniotic twin	• 38 to 39 weeks
• Uncomplicated monochorionic diamniotic twins	• 34 to 37 weeks • After 36 weeks (RCOG 2016)
• Monochorionic monoamniotic twins	• 32 to 34 weeks
• Treated TTTS • With persisting abnormality • Resolved TTTS	• 34 weeks • By 37 weeks
• Type I SFGR	• 34 to 36 weeks
• Type II and III SFGR	• 32 weeks
• Preterm sIUFD	• 34 to 36 weeks
• Term sIUFD	• Immediate delivery

Continuous electronic fetal monitoring should be done during labour for all monochorionic twins.

Delayed cord clamping is not recommended in monochorionic twins as the risk of acute intertwin transfusion at birth outweighs the benefit of delayed cord clamping.

Delivery Route

First twin cephalic	
Cephalic-cephalic	Vaginal
Cephalic-non cephalic	Trial of labour can be given If second twin larger than the first twin by >20% or if <1500 gm or < 28 weeks, LSCS preferred.
First twin non cephalic	Cesarean

Suggested Reading

1. Kilby MD, Bricker L on behalf of the Royal College of Obstetricians and Gynaecologists. Management of monochorionic twin pregnancy. BJOG 2016; 124:e1–e45.
2. Cunningham F, Leveno KJ, Dashe JS, Hoffman BL, Spong CY, Casey BM. eds. Williams Obstetrics, 26e. McGraw Hill; 2022
3. Khalil A et al, ISUOG Practice Guidelines: role of ultrasound in twin pregnancy, 2016
4. Multifetal Gestations: Twin, Triplet, and Higher-Order Multifetal Pregnancies: ACOG Practice Bulletin, Number 231. Obstetrics & Gynecology 137(6):p e145-e162, June 2021
5. Hessami K et al, Perinatal risk factors of neurodevelopmental impairment after fetoscopic laser photocoagulation for twin-twin transfusion syndrome: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2021 Nov;58(5):658-668
6. Ghirardello S et al. Italian Recommendations for Placental Transfusion Strategies. Front Pediatr. 2018 Dec 3;6:372
7. Van Hoek et al, Perinatal outcome after selective fetal reduction in monochorionic twin pregnancies: A comparison of techniques over a 20-year period

Demystifying TORCH infection in Pregnancy: Case based review

***Suchandana Dasgupta, **Sumitra Bachani**
*FNB Maternal Fetal Medicine, ** Professor& Sr Specialist
Vardhman Mahavir Medical College & Safdarjung Hospital. New Delhi

Introduction

There is a group of viral, bacterial and protozoan infections which are transmitted trans-placentally via the chorionic villi and can lead to severe fetal anomalies or even fetal loss. They are collectively called TORCH infection which includes (TO)xoplasmosis, (R) ubella, (C)ytomegalovirus (CMV), and (H)erpes simplex virus. Sometimes the 'O' in the acronym is used variably to denote (O)ther agents such as syphilis, varicella zoster virus, parvovirus B-19, listeriosis, coxsackie virus, hepatitis and zika virus. The first exposure causes immunity in most infection, however re-infection is reported in some infections such as CMV. Gestational age of the fetus at the time of maternal infection influences the degree of severity. India has a high seroprevalence rate and routine screening is not recommended in national guidelines. Immunization is advisable for Rubella and Varicella in non-immune women of reproductive age group prior to conception. In this article different clinical cases and their interpretation along with management will be discussed.

Toxoplasmosis

Case: 26 years old G3A2 presented to Fetal medicine clinic at 18 weeks of gestation with fetal ascites, hepatomegaly and intracerebral calcification.

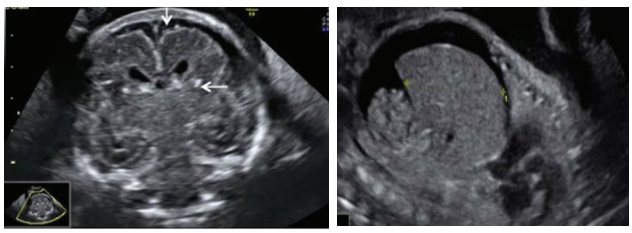


Fig 1: Ultrasound showing intracerebral calcification, ascites, hepatomegaly

Step wise Investigation and Management:

This raised the suspicion of TORCH infection, hence paired maternal serology was ordered. Toxoplasma IgM positive (IgM test value: 16.7 U/ml and Control value: 1 U/ml) and IgG negative (IgG test value: 0.13 U/ml and control value: 1 U/ml). Repeat serum tests after 4 weeks IgM was negative and IgG value raised to 32.8 U/ml. Rising IgG was suggestive of recent infection.

To prevent vertical transmission Spiramycin was started. After 6 weeks of the maternal infection and beyond 20 weeks of pregnancy, amniocentesis was performed and Toxoplasma PCR was done which was reported negative. Follow up ultrasound was done at regular interval. She delivered alive and healthy male child, 2.8 kgs at term. Follow up till 6 months did not show any sequalae suggestive of congenital infection.

Discussion

- There is no role of routine testing for TORCH infection in antenatal period unless there is history of definite maternal clinical illness or exposure as in Varicella or ultrasound features pointing towards possible infection. In this case the indication was presence of ascites, hepatomegaly and intracerebral calcification.
- The correct way to evaluate maternal infection is to order paired serology (IgM and IgG) at first presentation and repeating the same after 4 weeks. In this case, it was an acute infection as there was initial high IgM and negative IgG which after 4 weeks shows seroconversion i.e. negative IgM and more than 4-fold rise of IgG.
- Fetal infection to be confirmed by invasive testing for pathogen specific PCR after at least 6 weeks of maternal infection and after 18-20 weeks of gestation. In this case fetal infection was absent.
- Fetal infection and fetal affection are not same. Hence, follow up with regular ultrasound is recommended.
- To prevent vertical transmission after maternal toxoplasma infection during pregnancy, Spiramycin 1g tablet taken orally three times daily till delivery, in the absence of confirmed vertical transmission.
- No vaccine is available, preventive measures include hand-washing, avoiding consuming raw meat or vegetable and contact with cats.
- Long term follow up is needed to exclude congenital infection.

Rubella

Case: 26 years old Primigravida with Rh negative pregnancy presented in Fetal Medicine clinic at 14

weeks with report of high Rubella IgM. She had history of febrile illness at 8 weeks of pregnancy, without rash for which TORCH test was ordered.

The maternal Rubella serology at 10 weeks: IgM- 49.55 U/ml and IgG- 9.34 U/ml

Step wise investigation and management:

She presented after 4 weeks of initial report, hence a repeat paired serology was ordered along with IgG avidity. Maternal serology at 15 weeks: IgM- 0.18 U/ml, IgG- 28.8 U/ml, IgG avidity: 86.5%. High avidity suggested a past infection (more than 12 weeks) which indicates the periconceptional period in this case. Hence, invasive testing was done after 20 weeks for Rubella PCR which was negative.

Regular follow up done, antenatal period was uneventful otherwise. Pregnancy outcome was also good. Postnatal follow up at 6 months satisfactory.

Discussion:

- In this case maternal febrile illness in first trimester was the indication for TORCH testing.
- Correct interpretation of serology is utmost important. (Table 1) In current case, it was subacute infection before 3 months which is around the periconceptional period.

Table 1: INTERPRETATION OF MATERNAL SEROLOGY

IgM	IgG	IgG avidity	Interpretation
-ve	-ve		Susceptible
+ve	-ve		Acute infection*
-ve	+ve		Past infection**
+ve	+ve		Subacute infection***
+ve	+ve	Low avidity	Infection within 3 months
+ve	+ve	High avidity	Infection before 3 months

*to confirm with repeat serology after 4 weeks
**Avidity helps in deciding time of infection
***Avidity, Detailed history, Ultrasound to be used for deciding time of infection

- The false positive rate of Rubella IgM is 10-15%. Confirmation of fetal infection to be done by virus specific PCR by invasive testing at least after 6 weeks of maternal infection or delayed until after 18–20 weeks' gestation, when fetal urination is established; else risk of false negative report is high.
- Rubella is the most teratogenic virus, when infection occurs during the first 12 weeks of pregnancy the risk of fetal infection and fetal affection (Congenital Rubella Syndrome) is 90% and 97% respectively. The four most common anomalies in CRS are sensorineural deafness, eye defects (commonly cataract),central nervous system(CNS) defects and Cardiac defects.
- In proven infection Medical Termination of Pregnancy can be offered to the couple after proper counselling.
- Fortunately, vaccination is available against Rubella infection. The trivalent MMR vaccine is a part of the national immunization programme. It should be administered to all children between 12-15 months, 2nd dose to be administered at least 1 month after 1st dose but before 6 years. Susceptible adolescent girls and all women of reproductive age group should receive vaccine (RA 27/3 vaccine, subcutaneously, 0.5ml). During pre-conceptional counselling, Rubella IgG should be advised when definitive vaccination history is not known and if negative vaccination should be offered. Overt clinical symptoms manifest only in 50-75% women, hence clinical history may not always suggest prior illness. Pregnancy should be deferred for 1 month after the vaccine however in case of conception within a month of vaccination pregnancy should not be terminated. Re-infection is rare and the chance of CRS is <5%.

Cytomegalovirus

Case: 27 year old G2P2L0 at 18 weeks 6 days of gestation presented to Fetal Medicine Clinic with history of two early neonatal death in which both babies were diagnosed with neonatal CMV infection.

First pregnancy was a spontaneous conception, no antenatal or intrapartum complications. She delivered a 3.5kg boy baby vaginally at term. The baby cried immediately after birth and was discharged on day 2 of life. Exclusively breastfed and received immunisation at birth. On day 11 of life, the baby had vomiting, loose stool, respiratory distress and rash all over body. After hospitalisation he was diagnosed with pneumonia. Lumber puncture revealed CMV meningitis Expired on day 15 of life.

In next pregnancy, similar events were there, on postnatal day 2 baby developed fever and respiratory distress. Lumber punctured confirmed CMV infection which was treated with acyclovir. Unfortunately, the baby expired on day 22 of life.

Step wise investigation and management:

The couple explained about recurrent CMV infection during pregnancy. There is 1-2% chance of re-infection in present pregnancy and they were offered invasive testing. Anomaly scan was done which was normal. Amniocentesis was done and CMV PCR was ordered which was negative. She was counselled and advised Tab Valacyclovir 8gm/day throughout the pregnancy

to prevent vertical transmission later in the pregnancy. Antenatal, intrapartum and postpartum period uneventful. Regular follow up done till 12 months of age and the baby is currently doing fine.

Discussion

- Non-primary or recurrent infection results from reactivation of prior infection or reinfection with a different strain of the virus. The risk of congenital infection is 1-2% following a non-primary infection. Symptomatic congenital CMV occurs in ≤ 1% and asymptomatic in rest of cases. Risk of sequalae is ≤ 10%.
- In this case as it is not a primary infection, serology was not ordered and directly invasive testing was done to diagnose fetal infection.
- Valacyclovir in a high dose is proven to reduce the mother to child transmission.
- Young children are mostly affected by CMV infection and pregnant women who have child of age less than 3 years should practice avoiding exposure body fluid or saliva, kissing on lips and washing hands thoroughly after being in contact with their bodily fluid. No vaccine has been approved till date.

Herpes Simplex Virus:

Case: 30 years old G3P1L0A1 presented at 12 weeks 5 days at Fetal Medicine Clinic with positive HSV IgM report and she had similar previous history. In the first pregnancy at around 3 months gestation, she had herpes labialis and herpes genitalis which was not treated then. Clinical symptoms continued on and off during pregnancy. She had an intra-uterine demise at 34 weeks and the fetus had fetal growth restriction and bilateral pleural effusion. After delivery she received valacyclovir and it was resolved. In next pregnancy, at 5 weeks she again had herpes labialis and genitalis. She had missed abortion at 11 weeks.

Step wise investigation and management:

She had herpes genitalis in this pregnancy when presented, Tab Acyclovir started and given for 5 days. Antenatal follow up done, no recurrence of symptoms. From 36 weeks, suppressive therapy with acyclovir given till delivery. She delivered vaginally at term, alive and healthy girl. Postnatal follow up at 6 months uneventful.

Discussion:

- Infection during 1st or 2nd trimester is not associated with increased risk of miscarriage or congenital anomaly. Fetal affection or Neonatal herpes infection is there when infection occurs during 3rd trimester.
- Primary infection before 30-34 weeks has 20-25% risk of transmission but in non-primary or recurrent infection the overall risk of transmission is <1%.
- Symptomatic recurrent infection should be treated with acyclovir or valacyclovir.
- Suppressive therapy from 36 weeks is indicated in women who had a genital lesion either primary or recurrent infection anytime during pregnancy.
- Mode of delivery is decided depending on presence of active lesions. When genital lesions are present and vaginal delivery is allowed, the risk of neonatal herpes is estimated to be 41%.
- Prevention by safe sexual practice is the key.

The other infections which have similar way of transmission, risk of fetal infection and affection are Varicella Zoster or Chicken pox, Parvovirus B19, Zika virus, Syphilis, Listeriosis, Coxsackie virus, Hepatitis virus. Detailed discussion about these is beyond the scope of this article. However, diagnosis of maternal infection using serology, diagnosis of fetal infection using virus specific PCR, regular follow up with ultrasound to detect fetal affection and evaluation at birth and later for diagnosis of neonatal or congenital infection are the key features of managing these infections.

Suggested Reading

1. Khalil A, Sotiriadis A, Chaoui R, da Silva Costa F, D'Antonio F, Heath P t., et al. ISUOG Practice Guidelines: role of ultrasound in congenital infection. Ultrasound in Obstetrics & Gynecology. 2020;56(1):128–51.
2. Screening and management of TORCH in pregnancy; FOGSI 2014. Available at https://www-fogsi-org.webpkgcache.com/doc/-/s/www.fogsi.org/wp-content/uploads/2015/11/smtp.pdf. Last accessed in June, 2023.
3. K Aparna Sharma, Deepika Deka. "TORCH Infections in Pregnancy." Management of High-Risk Pregnancy A Practical Approach. Subha Sagar Trivedi, Manju Puri, Swati Agrawal, Jaypee Brothers Medical Publishers (P) Ltd, 3rd Edition, 2022, Chapter 30, 347-355.
4. Stray-Pedersen B. Treatment of toxoplasmosis in the pregnant mother and newborn child. Scand J Infect Dis Suppl 1992;84:23–31.
5. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital CMV infection. Rev Med Virol 2009;17:253-276.
6. Pesch MH, Saunders NA, Abdelnabi S. Cytomegalovirus Infection in Pregnancy: Prevention, Presentation, Management and Neonatal Outcomes. J Midwifery Womens Health. 2021 May;66(3):397-402.

7. Rawlinson WD, et al. Lancet Infect Dis 2017;17:e177-e188.
8. Pregnancy and Rubella. Available at https://www.cdc.gov/rubella/pregnancy.html. Last accessed in June, 2023.
9. Management of genital herpes in pregnancy. Available at https://www.rcog.org.uk/guidance/browse-all-guidance/other-guidelines-and-reports/management-of-genital-herpes-in-pregnancy/. Last accessed in June, 2023.
10. Management of Genital Herpes in Pregnancy, May2020. ACOG practice bulletin, number 220.


Save the date
worldCongress of
NARCHI
19th to 21 September
2025
Rajasthan
international centre
JAIPUR

NARCHI Delhi Activities

April 2024 Handing Over Ceremony of NARCHI Delhi Office from Team LHMC to Team SGRH

Public Awareness Lectures and Camps Held in April, 2024

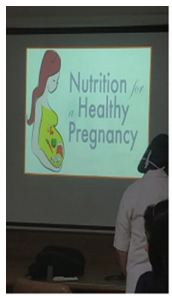
Public Awareness Lectures and Camps
On Thalassemia and Respectful Meaternity Care Held in May 2024

ANM Training on IUCD/PPIUCD Insertion in June, 24

Public Awareness Lectures and Camps on Thalassemia and Respectful Meaternity Care Held in June 2024

CME ON Cervical Cancer Prevention Held in June, 2024

Public Awareness Lectures and Camps on Respectful Maternity Care in July & August 2024

Public Awareness Program on Menstrual Health in Adolescents held in September, 2024





30th Annual Conference of NARCHI - Delhi Branch.
NARCHI Bulletin and Souvenir were released by Dr Ajay Swaroop, Dr Jayashree Sood and Esteemed Faculty

CTG Workshop

Endoscopy Workshop

Inferitility Workshop

Nurses Workshop

under the guidance of **Dr Veena Acharya** (Chairperson NARCHI ICMCH)

Awareness about cervical cancer in Public and paramedical staff

Release of Poster for awareness of Cervical Cancer 17 November 2023

Adolescent Health education in schools of Alwar 20 January 2024

Awareness about the women health organized by the Indian medical association at Alwar

Awareness calendar for cervical cancer prepared by Dr Veena Acharya. Presented to Hon'ble Governor and Health Minister of Raj. Respectively

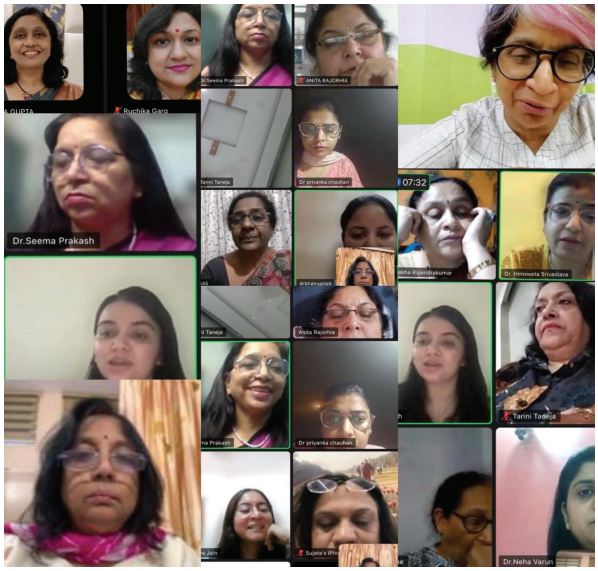
World Cancer Day in Renova Cancer Center

Workshop and CME Done by the NARCHI State Branch

- Prevention of PIH and PPH in pregnancy
- Stress management for health life style

Laparoscopy and hysteroscopy Workshop

NARCHI Rajasthan Activities



NARCHI Nagpur Activities

School Health and One Rural Health Check up Camp

NARCHI Punjab (Ludhiana & Jalandhar) Activities

This was for Punjab Page After First two Photos of Exchange Conference Photos

NARCHI Durgapur Activities