AOGD BULLETIN

Volume 20 I March 2021 I Monthly Issue 11

Price ₹30 Only

CARING FOR WOMEN'S HEALTH : EVIDENCE, ATTITUDE & PRACTICE

Dedicated Issue:
Reproductive Genetics and Fetal Medicine

AOGD SECRETARIAT
Institute of Obstetrics & Gynaecology,
Sir Ganga Ram Hospital
Sarhadi Gandhi Marg, Old Rajinder Nagar, New Delhi-110060
Tel.: 011-42251768, 1789
E-mail: secretaryaogdsgrh2020@gmail.com

Website: www.aogd.org

AOGD Office-Bearers 2020-21

Dr Mala Srivastava
President

Dr Kanika Jain
Vice President

Dr Mamta Dagar
Hon. Secretary

Dr Neeti Tiwari

Dr Sunita Kumar

Joint Secretary

Dr Shweta Mittal Gupta
Treasurer

Dr Tarun Das
Co-Treasurer

Dr Debasis Dutta

Dr Geeta Mediratta

Dr Chandra Mansukhani

Dr Punita Bhardwaj

Scientific Advisors

Editorial Board

Dr Geeta Mediratta

Dr Chandra Mansukhani

Editors

Dr Ruma Satwik

Dr Sharmistha Garg

Dr Sakshi Nayar

Dr Ila Sharma

Co-Editors

Dr Debasis Dutta

Dr Gaurav Majumdar

Dr Ankta Srivastava

Web Editors

AOGD Bulletin

Volume 20 • Monthly Issue 11 • March 2021

AOGD Secretariat
Institute of Obstetrics and Gynecology, Sir Ganga Ram Hospital
Sarhadi Gandhi Marg, Old Rajinder Nagar, New Delhi-110060
Tel.: 011-42251768, 1789
E-mail: secretaryaogdsgrh2020@gmail.com | www.aogd.org

Contents

Publisher/Printer/Editor
Dr Geeta Mediratta and Dr Chandra Mansukhani on behalf of Association of Obstetricians & Gynecologists of Delhi.

Printed at
Process & Spot C-112/3, Naraina Industrial Area, Phase-1, New Delhi 110 028

Published from
Institute of Obstetrics & Gynaecology
Sir Ganga Ram Hospital, Sarhadi Gandhi Marg, Old Rajinder Nagar, New Delhi-110 060

Editors
Dr Geeta Mediratta, Dr Chandra Mansukhani
Ph. No. 011-42251768, 1789; Email: secretaryaogdsgrh2020@gmail.com

From the President's Pen

Greetings to all the member of AOGD!!

I hope all our AOGD members are now enjoying the atmosphere where corona pandemic has slowed down and the vaccinations are on. The environment is opening up, but the threat of the corona pandemic is not yet gone. We still have to maintain our social distancing, mask use and frequent hand washing. We still have to keep guards on against the infection so as to prevent its re-surgence.

With all the precautions we did manage to have the first hybrid executive meeting at the auditorium of Sir Ganga Ram Hospital. It had been a very successful hybrid executive meet. We had Patrons like Dr. S. N. Mukherjee, Dr. Kamal Buckshee, Dr. Swaraj Batra, Dr. Neera Agarwal and Dr. Reva Tripathi to bless us. It was a pleasure to see all our seniors and colleagues in person after so many months. We took the opportunity to extend our thanks to our Patrons, Advisors, Executive members and Chairpersons of the various Sub-Committees of AOGD. We are highly grateful to all of them as they have stood with us during these challenging times. The year 2020 had brought many problems, difficulties, and restrictions with it. But with the help of all our AOGD members we were able to overcome these hurdles. From the deepest bottom of our heart we thank each and every member of AOGD who stood with us in solidarity during these difficult challenging Corona times. These moments will always be cherished as loving memories for many many years to come.

I am really also indebted to my entire team of Sir Ganga Ram hospital who gave me unconditional support throughout this year. As a result the activities of the society were nicely streamlined and executed perfectly.

This edition of the bulletin is on Reproductive genetics and Fetal medicine. We have collected articles of all the stalwart in Fetal medicine for this edition

FOGSI has initiated cervical cancer and breast cancer screening module for the International Women's Day on the 8th March 2021. We also request all our members to screen at least ten women on that day either in your hospital or your private clinic. So that mission of FOGSI is accomplished with the help of our large society. We should prove that when a society like AOGD works together we can achieve anything. The mission is for women's health, and we are all for it, for the benefit of the health of our women.

Long Live AOGD!

Dr Mala Srivastava
President, AOGD

From the Vice President's Pen

Warm Greetings to all members of the association !

We are now into the last month of this much eventful and challenging AOGD tenure of our's for the year 2020-21.

I hope we, at the AOGD Secretariat at SGRH have been able to live up-to the expectations of all AOGDians. We've tried to put our best efforts possible despite the restrictions during this CoViD-19 Pandemic, to continue the journey of learning.

As is said: **"Necessity is the mother of Inventions"**

As the challenges posed in front of us were new and unexplored, we used technology based innovative ideas to fulfil our objectives of continuing medical education in the field of Obstetrics and Gynaecology.

We held a first of it's kind **Hybrid** (Physical and Virtual) **meeting for executive members** on 18th February. In this, important discussions took place and decisions were made. Our Patrons were honoured by AOGD and then they presented Certificates of appreciation to all the Advisors, Senior Executive members, AOGD team of SGRH and Sub-Committee Chairpersons. Without their unconditional support and help, we would not have been able to hold the AOGD torch high during these difficult times.

We **congratulate all the new AOGD sub-committee Chairpersons** elected by the Executive members for the years 2021-23.

Our Editorial team has dedicated this month's bulletin to **'Reproductive genetics in Fetal medicine'**. I'm sure this exclusive Bulletin with write-ups from experts would be of great interest to the readers.

I would like to take this opportunity to express my heart-felt gratitude and thanks to Dr I. Ganguli, Dr K. Gujral, my Seniors at SGRH, all the Executive members of AOGD, and Dr Mala Srivastava for bestowing their trust upon me and for giving me the privilege to be associated with AOGD as a Vice-President. I am genuinely grateful to Dr Mamta and our SGRH team who helped me fulfil my duties and obligations towards AOGD.

I wish all our members a very **"Happy Holi !**

May this Spring bring new hopes and newer opportunities for every one!!

Regards,

Dr Kanika Jain
Vice President, AOGD

From the Secretary's Desk

Greetings to all !

Hope you all are keeping safe and healthy.

Though my journey as AOGD Secretary has been relatively ephemeral, my learnings cannot be capped. My responsibilities and interactions with the beloved AOGDIANS have metamorphosed me into a more compassionate, astute and meticulous person. I have grown and I hope I have shared this growth with you too.

FOGSI Screening Camps and Awareness Drive on **Preventable Cervical & Breast Cancers** from **6-8 March, 2021** on the occasion of **International Women's Day** were successfully organised by all AOGDIANS with high spirits and enthusiasm.

Our editorial team has brought the AOGD E-bulletin March version dedicated to **Reproductive Genetics in Fetal Medicine**, which should be of great interest and immense use to our readers.

Looking forward to your continued support.

Yesterday has gone. Tomorrow has not yet come. We have only today. Let us begin. – Mother Theresa

Warm Regards

Dr Mamta Dagar
Hon. Secretary

Monthly Clinical Meeting

AOGD Monthly Virtual Clinical Meet will be organised by Lady Hardinge Medical College, New Delhi on 26th March, 2021 from 04:00pm to 05:00pm.

From the Editor's Desk

Dr Geeta Mediratta
Chief Editor

Dr Chandra Mansukhani
Co-Editor

Dear Readers,

Welcome to the spring Edition of the AOGD Bulletin!!!

In this bulletin dedicated to **Reproductive Genetics and Fetal Medicine**, we bring you a variety of academic bonanza.

Dr. Shubha Phadke Professor & Head SGPIMS who is a renowned authority on Reproductive Genetics has penned a very informative article on topic **"Pedigree Analysis: The First Step of Genetic Testing"**. This will help our understanding of basics of Genetics tremendously.

Dr. Ratna Dua Puri has lucidly described **"Various clinical Vignettes in Genetics"** which illustrate the importance of genetics history taking and meticulous workup so as to reach a diagnosis.

Dr. Sunita Bijarnia Mahay has written interesting scenarios in her article **"Genetic Counseling- Scenarios of the usual and the unusual cases in fetal medicine and reproductive genetics".**

Fetal medicine section features excellent and very informative articles by the doyens of fetal medicine and ultrasound, Dr. Varun Duggal and Dr. Ashok Khurana on **"Nuchal translucency and the first trimester anomaly scan" and on "Cervical Length: Predicting Preterm Birth and Beyond"** respectively. Both articles are extensive expositions on the topics and will enhance the knowledge of the reader.

Dr. Anita Kaul has elaborated on "**Twin to Twin Transfusion Syndrome (TTTS)"** in a very illuminating manner and describing the management.

Dr. Nandita Dimiri has showcased her journey in her article entitled "**Experience of intrauterine transfusion in Rh isoimmunised pregnancies"** in Sir Ganga Ram Hospital. She has discussed important practical points so as to improve the outcome of IUT.

Dr. Tina Verma has dealt with the exciting field of fetal therapy and its importance in her article entitled **"Fetal Therapy- An overview of medical management of the unborn"**.

Wish you a Happy Holi!!

Editorial Team

Pedigree Analysis: The First Step of Genetic Testing

Shubha Phadke
MD [Pediatrics], DM [Medical Genetics], Professor & Head, Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow-226014, India

Gene, genome and genetic disorders are integral parts of biology and medicine. With developments during the last 3-4 decades, a lot of genetics has come from research laboratories to clinics. Genomic diagnosis and gene therapy are now realities. Though the specialized clinicians for the diagnosis and management of patients and families with genetic disorders have become available, all clinicians need to understand the principles of basic genetics and look at the patients with perspective of genetics. The perspective of genetics means to consider genetic disorders in appropriate clinical scenarios and evaluate them accordingly. The first step of evaluation of a case with a likely genetic disorder is to take the family history systematically by way of drawing a pedigree. Pedigree is a pictorial representation of family members with and without illness and their relationships. The website of Iowa Institute of Human Genetics shows how to draw a pedigree and the symbols used [https://medicine.uiowa.edu/humangenetics/resources/how-draw-pedigree]. The points to remember while considering a genetic etiology are: [i] Every genetic disorder need not have family history (similarly affected family member) [ii] Each genetic disorder is not congenital though many congenital disorders may have genetic etiology.

The objectives of drawing a pedigree are mainly two. The first is to document relationship of affected family members with each other and to know if the family has consanguineous marriages especially the parents of the child or individual with disease. The second objective is to interpret the data of pedigree to decipher the mode of disorder in concern and to find out the risk of recurrence in the family. The information obtained from a pedigree is needed for genetic counseling for prenatal diagnosis, and testing of possible carriers. Genetic disorders are divided into chromosomal, monogenic or multifactorial. The following case scenarios depict traditional modes of inheritances for monogenic disorders (also known as single gene disorders) which follow Mendelian principles. The case scenarios will highlight the basic principles of modes of inheritance and the issues involved in genetic testing and genetic counseling.

Autosomal Dominant Inheritance

Case Scenario 1

A third gravida, para 2 woman comes for genetic counseling. She has one normal son and the second offspring is a three year old girl with short upper limbs and oligodactyly (Fig 1 A & B). The family member affected with a disorder who brings the family to notice, is known as the proband and is depicted by 'P' in the pedigree. The mother on evaluation was found to have reduction defects of forearms with anomalies of digits including absent thumbs. The radial side defects of upper limb associated usually with cardiac anomalies are characteristic features of Holt-Oram syndrome which is inherited in an autosomal dominant manner. Holt-Oram syndrome is characterized by variable severity of manifestations in family members as seen in this case where the child has severe limb reduction defects as compared to the mother. As cardiac malformations are commonly seen in patients with Holt-Oram syndrome, echocardiograms were done and both the mother and daughter were found to have atrial septal defects. As shown in fig 1 C, one copy of a gene for Holt-Oram Syndrome is defective (i.e. harbours a disease-causing variation, which also used to be called a mutation). The causative gene is *TBX5* (https://www.omim.org/entry/142900). As we can see, the possibility of the defective gene being transmitted to the offspring is 50% (Fig 1 D). The chance that the offspring will receive a normal copy of the gene from the mother and will not have Holt-Oram syndrome is 50% or one in two.

It is usually preferable to draw a three generation pedigree and get information about the other relatives by leading questions, clinical evaluation (if possible) and photographs or other investigation records.

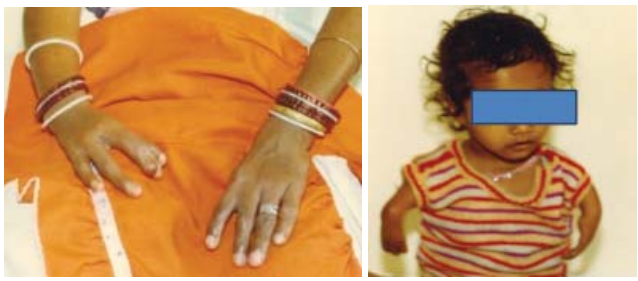


Fig 1 A & B: Mother and daughter with shortening of forearms and defects of digits including absent thumbs.

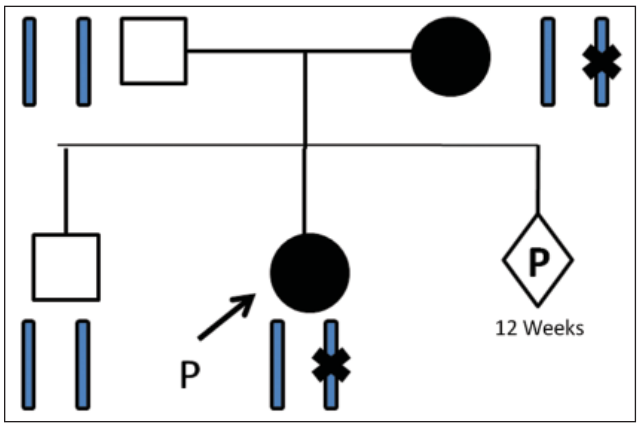


Fig 1 C: Pedigree of the family showing vertical transmission from mother to the daughter. Both of them have similar type of limb anomalies (but of variable severity) and hence are shown by shaded circles. Males in the family are depicted by squares. The two bars shown by the side of an each individual depict the two copies of the gene. The copy with a mutation is shown by a cross (X) across the gene.

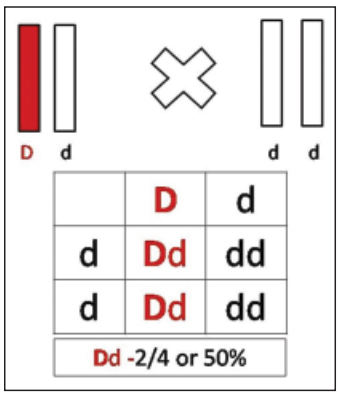


Fig 1 D: Punnett square showing transmission of alleles to the next generation. One parent has two alleles of the gene for a disease; one being normal 'd' and the other is disease causing 'D'. In the other parent both copies of the same gene are normal. Offspring with the genotype Dd will be affected with the disease.

Counseling: The possibility that the fetus in case 1 will have Holt-Oram syndrome is 50% or 1 in 2 in each pregnancy. However, the chance does not have memory and consecutive 2 or 3 offspring may be affected or normal. In this case the limb anomalies and cardiac anomalies can be detected by prenatal ultrasonography and if they are not detected, the fetal medicine specialist may reassure the family that the fetus does not have Holt-Oram syndrome. However, this may not be true. As seen in this family the severity of anomalies may vary from affected individuals in the family (variable expression) and milder defects may not be detected ultrasonographically. Also some anomalies which are seen in Holt-Oram syndrome like absent pectoralis major and pectus excavatum may not be prenatally detectable.

Case Scenario 2

A woman gave birth to a boy who developed respiratory problems at birth and needed admission in the neonatal intensive care unit for 15 days. At three months, the child was hypotonic, and did not achieve neck control or social smile. The causes of hypotonia and developmental delay in an infant are many. In such situations unless the etiology of the disease in the child can be identified, the risk of recurrence cannot be provided to the family. While evaluating the child, the pediatrician noted that the mother's face is expressionless and myopathic. Hence, the mother was asked to hold the doctor's hand and release immediately. She could not release easily and the fingers, especially thumbs remained in flexed position for quite some time. This suggested (and later confirmed) that the mother had myotonic dystrophy. A mother affected with myotonic dystrophy may have mild signs and symptoms and may remain undiagnosed. But this disease being an autosomal dominant disorder, she can transmit the disease to her offspring who can be severely affected with severe hypotonia and neonatal respiratory problems. On drawing the pedigree, she told that her brother has muscle weakness, difficulty in walking and her father has baldness and early onset cataract. Both these family members have manifestations of varying severity of myotonic dystrophy.

After confirmation of genetic defects for myotonic dystrophy in all the family members, the woman was told that during the next pregnancy she can get prenatal diagnosis by DNA test for myotonic dystrophy in chorionic villus sample. This case illustrates the marked variation in the expression of the disease in the family members and the need to take family history during pre-conception visit or in

the first antenatal visit, for timely identification of the families at high risk of genetic disorders.

Case Scenario 3

Ultrasonography at 18 weeks of pregnancy detected alobar holoprosencephaly in the fetus. The genetic etiologies of holoprosencephaly can be many. Fetal karyotype did not show trisomy 13 or any other chromosomal abnormality. After termination of the pregnancy, the fetal sample was sent for exome sequencing by a molecular technique known as Next Generation Sequencing (NGS). Exome sequencing studies the important (protein coding) regions of genes. NGS identified a known causative DNA variation in *SHH* gene. The same DNA variation was seen in the father who was clinically normal. On careful examination, he was found to have a single central incisor which is a microform of holoprosencephaly. Hence the risk of recurrence of holoprosencephaly will be 50% in this family; though the fetus with the same genetic variation may or may not have features of classic holoprosencephaly. This case again highlights variable expression of autosomal dominant disorders. If father and mother are both not harbouring the genetic variation found in the fetus, the risk of recurrence is almost nil as in such situations the disease causing mutation is usually de novo and not inherited.

Case Scenario 4

Fig 2 shows a pedigree of a family with two children with achondroplasia born to normal parents. Achondroplasia is the commonest type of genetic bone disorder leading to short stature. If one of the parents is a case of achondroplasia, the possibility of achondroplasia in his or her children is 50%. If both the parents of a child with achondroplasia are normal, we consider that this is due to de novo mutation occurring during gametogenesis. Rarely, recurrences in such families with de novo mutations are reported due to germline mosaicism. This suggests the existence of germline mosaicism. Germline mosaicism means existence of different cell populations with and without a DNA mutation in gonads of a clinically normal individual. Data shows that the empiric risk of recurrence after the birth of one child with achondroplasia is 1 in 400. Hence, all such families should be offered prenatal diagnosis by mutation testing in the chorionic villus sample. It should be noted that the shortening of long bones by ultrasonography in a fetus with achondroplasia does not become obvious till late second trimester.

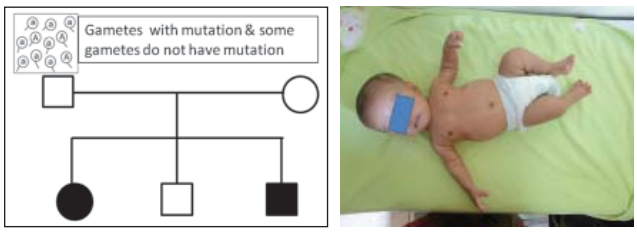


Fig 2 A & B: [A] Pedigree showing two children with achondroplasia (shown by dark squares) born to a couple who are normal [B] An infant with achondroplasia

Case Scenario 5

Another point about the genetics of achondroplasia that needs mention is homozygosity for achondroplasia mutation. It is not rare that two individuals with achondroplasia marry because of short stature. The possibility that they have offspring with achondroplasia is 50% and the possibility that the offspring inherits normal allele from both the parents and is normal is 25%. In addition, they have 25% risk of having an offspring who is homozygous for achondroplasia, which has a severe phenotype of lethal skeletal dysplasia (Fig 3).

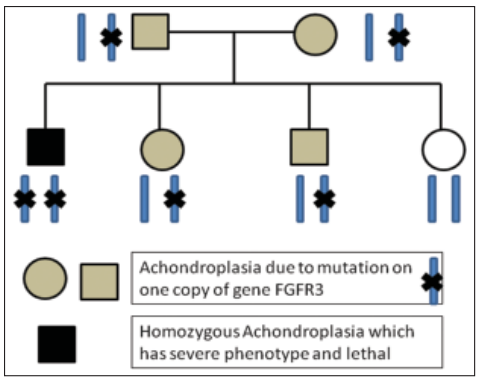


Fig 3: Homozygosity for an autosomal dominant disorder, achondroplasia. When both the parents have achondroplasia, the possibility that their offspring will have achondroplasia is 75% (50% risk of classic heterozygous achondroplasia and 25% risk of severe perinatal lethal type of homozygous achondroplasia)

Messages about Autosomal Dominant Inheritance

- Drawing pedigree during the first antenatal visit can identify pregnancies at risk of genetic disorders.
- Marfan syndrome, neurofibromatosis I, achondroplasia, hereditary spherocytosis are

some examples of autosomal dominantly inherited disorders. These disorders manifest when one of the two copies of the gene is defective / mutated. Evaluation of affected family members by appropriate genetic tests can provide timely genetic counseling and prenatal diagnosis for disorders with severe consequences

- Examination of family members is essential Online Mendelian Inheritance in Man (OMIM) is a free website to know the latest and complete information about genetic diseases and the genes
- Cause of recurrence in the family with one child affected with an autosomal dominant disorder when both parents are normal, can be germline mosaicism
- While counseling for autosomal dominant disorders confirmation of diagnosis, evaluation of parents for mild manifestations of the disease (variable severity, variable age of onset), and non- penetrance (Fig 4) need to be considered.
- Familial cancers like Retinoblastoma and cancer prone syndromes like von Hippel Lindau syndrome, Multiple endocrine neoplasia are inherited in autosomal dominant fashion.

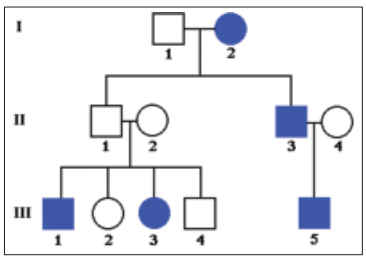


Fig 4: A pedigree showing skipped generation suggesting incomplete penetrance for the disorder in the family. Individual II-2 must be a carrier of genetic mutation as his mother (I–2), a son (III-1) and a daughter (III-3) are affected with the disorder. Hence individuals III-2 and III-4 may be carriers of the disease causing mutation even if they are not showing disease phenotype.

Autosomal Recessive Inheritance

Case Scenario 6

Beta thalassemia major is the commonest monogenic disorder in India. The disease manifests only when both the copies of the gene for beta globin (*HBB* gene) are mutated or defective (Fig 5 A). Obviously, both the parents of such an affected individual are (obligate) carriers of the disorders; i.e. they carry one mutated/ defective copy of the gene. Such mode of inheritance is known as autosomal recessive mode of inheritance. In the current era, with the availability of prenatal diagnosis for beta thalassemia, the recurrence of the disease in the family as shown in Fig 5 A, could have been easily avoided.

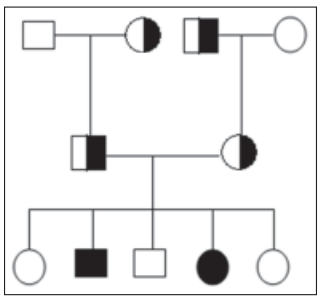


Fig 5 A: Pedigree of beta thalassemia major, a disorder showing autosomal recessive inheritance. The parents and grandparents of the individuals affected with the disease (Full shaded) are carriers (half shaded). The carriers are clinically unaffected but can be detected by increased levels of haemoglobin A2 by high performance liquid chromatography assay of hemoglobin (Hb HPLC) and by mutation testing by *HBB* gene sequencing

As soon as the diagnosis of thalassemia major or any genetic disease is done; it is the responsibility of the clinician to order genetic test for mutation analysis and provide the family information about the risk of recurrence in the next pregnancy. The importance of mutation testing for prenatal diagnosis should be conveyed to the family. Every child with beta thalassemia will have different mutations and without the report of mutations in the proband or carrier parents, prenatal diagnosis cannot be provided.

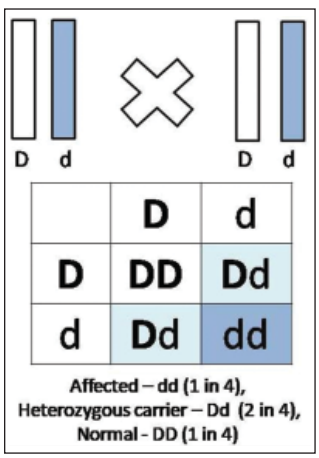


Fig 5 B: Punnett square showing gametes (D – Normal allele; d – Allele with disease causing DNA sequence variation or a mutation) and possible zygotes of a couple where both of them are carriers of an autosomal recessive disorder. The zygotes with genotype of dd will be suffering from the disease and the possibility of such conceptus is 1 in 4. DD is a normal genotype while Dd (2 in 4) will be carriers of the disorder like the parents.

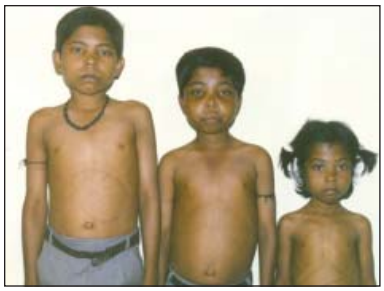


Fig 5 C: Three siblings affected with homozygous beta thalassemia

Risk of Recurrence in Siblings

The examples of autosomal recessive disorders are spinal muscular atrophy, most of the inborn errors of metabolism like Gaucher disease, lethal skeletal dysplasias like short rib polydactyly syndrome, etc. If you see the pedigrees of the families with these disorders, it will be observed that the previous generations in the family of patients do not show any individual affected with the disorder. In a family one or more offspring of normal parents are affected. The risk of recurrence of the same disorder in the siblings of an affected child is 25% or 1 in 4 (Fig 5 B). However, chance has no memory and in some families consecutive 2, 3 or rarely more children affected with an autosomal recessive disorder may be born (Fig 5 C)

Genetic counseling of families with one child with an autosomal recessive disorder before planning the next pregnancy is of utmost importance. This requires timely diagnosis of the proband (affected individual in the family) and whenever possible DNA based studies should be done as prenatal diagnosis based on causative DNA sequence variation has minimum error rate. Some phenotypes which can be due to autosomal recessive genetic disorders are developmental delay, an infant with hypotonia, retinitis pigmentosa, prelingual deafness, stillbirths, acute sickness during neonatal illness etc. Such illnesses are often mislabelled as septicemia, cerebral palsy (Fig 6), etc. and are not investigated thoroughly. Children with intellectual disability are labelled as cerebral palsy and are not investigated for genetic etiologies. Many genetic disorders like structural malformations of brain, neurodegenerative disorders, inborn errors of metabolism (e.g. Metachromatic leukodystrophy) have presentations like developmental delay and mental retardation. These may superficially look like cerebral palsy but are progressive and may have increased risk of recurrence in the family. For lack of suspicion of the possibility of genetic disorder, the family may suffer due to recurrences. Every effort should be made to reach an accurate diagnosis in the proband (Fig 7).

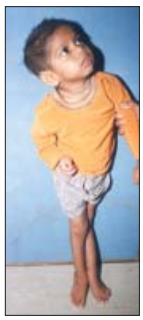


Fig 6 A: Fifteen month old child with metachromatic leukodystrophy labelled as cerebral palsy due to spasticity in lower limbs.

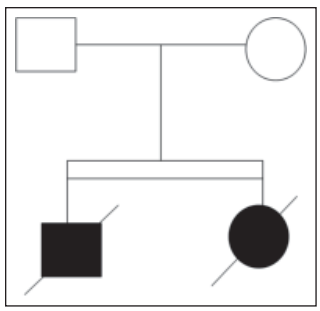


Fig 6 B: A consanguineously married couple had 2 offspring who had died in the neonatal period following an acute illness from day 4 of life. The cause of neonatal deaths in this case is likely to be an autosomal recessive disorder such as an inborn error of metabolism

Case Scenario 7

A fetus had cord around his neck with meconium aspiration syndrome which resulted in neonatal demise. The family was referred for genetic counseling. The parents were second cousins. The family history showed that the previous pregnancy was complicated by polyhydramnios and resulted in stillbirth of a male who had talipo-equinovarus. Both the neonatal deaths were attributed to birth asphyxia. Obviously, the disorder in concern is likely to be an autosomal recessive disorder. But unfortunately, neither the child nor any blood sample was available for testing. Hence, the parents were subjected to exome sequencing from the DNA extracted from the blood. It showed pathogenic sequence variation in *LMOD3* gene in heterozygous form in both the parents. This confirms that they are carriers of this mutation in *LMOD3* gene. *LMOD3* gene is the gene for Nemaline myopathy.

Nemaline myopathy presents with decreased fetal movements, polyhydramnios, neonatal respiratory problems, and congenital joint contractures. Most die of respiratory failure in early infancy. All patients have severe generalized hypotonia and weakness at birth, respiratory insufficiency, and feeding difficulties. Thus in these children the neonatal respiratory problems were due to congenital myopathy and not due to perinatal problems. The risk of recurrence will be 25% and in subsequent pregnancies prenatal testing by DNA testing on chorionic villus sampling at 12 weeks is indicated. It would have been very useful if one or 2 ml of blood of the children in EDTA vial or a piece of umbilical cord was saved for DNA analysis in future.

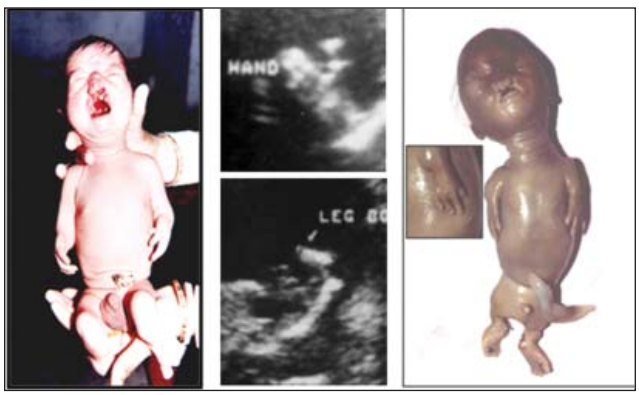


Fig 7 A, B & C: [A] A family showed the picture of their first child who died at two months of age. The picture shows facial cleft with mesomelic shortening of limbs which is characteristic of Roberts syndrome. [B] Hence, USG based prenatal diagnosis could be done in the next pregnancy of the mother. Fig shows short tibia and fibula with oligodactyly [C] Fetus with Roberts syndrome terminated after prenatal diagnosis.

Consanguinity and autosomal recessive disorders: Presence of consanguinity in parents supports the autosomal recessive mode of inheritance of the disorder in the family. For example, if a consanguineously married couple with no disorder has a child with microcephaly or deafness, the risk of recurrence in their next offspring is likely to be 25% or 1 in 4. Also, the other individuals in the family may be carriers of the disorder and consanguineous marriages in the family may be at risk of birth of a child with the genetic disorder in concern in the family. For rare autosomal recessive disorders, the possibility that the parents are consanguineous increases though a child with an autosomal recessive disorder may be born in any family.

Extended family screening: For autosomal recessive disorders like thalassemia, sickle cell disease, spinal muscular atrophy, it is recommended that the members of the extended family like cousins, uncle, aunt, nieces and nephews be informed about the disorders, offered carrier testing and genetic counseling. Another issue in common autosomal recessive disorders is marriage between affected person and a carrier. This is observed for deafness and sickle cell disease in the general population and for rare disorders in consanguineous families. The pedigree of such a family looks like a pedigree of an autosomal dominant inheritance as the carrier individual is clinically normal (Fig 8).

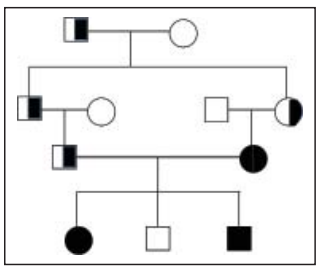


Fig 8: A pedigree of sickle cell disease showing pseudo-dominance

Occurrence of two offspring with a disease phenotype to clinically normal parents suggests a possibility of autosomal recessive mode of inheritance for the disorder in concern. Other genetic etiologies in such situation can be chromosomal imbalances due to a balanced chromosomal rearrangement in a parent, intake of teratogenic drugs (e. g. antiepileptics) by mother, germline mosaicism, X linked disorder, etc.

Messages

- The disorders inherited in an autosomal recessive fashion include many serious disorders presenting during childhood. Many of them warrant prenatal diagnosis.
- Both males and females are affected.
- The severity of presentation is usually similar in the affected siblings.
- Usually in a family the affected individuals may be seen in only one generation and mostly, there is only one affected child.
- Fetuses with malformations, stillbirths, intrauterine fetal deaths, and neonatal deaths should be evaluated to identify the etiology. Many of them may be due to autosomal recessively inherited genetic disorders, for which there would be a risk of recurrence in the family.

- For common disorders namely beta thalassemia and spinal muscular atrophy, screening of all couples pre-conceptionally or early in pregnancy is indicated.
- Presence of consanguinity in the parents suggests possibility of an autosomal recessive disorder but autosomal recessive disorders occur in nonconsanguineous families as well. Also, chromosomal, autosomal dominant or X-linked disorders may occur in consanguineous families.
- Genetic counseling may be offered to the extended family members of a patient with autosomal recessive disorders. Consanguinity may be avoided in families with autosomal recessive disorders.

X-Linked Recessive Inheritance

Case scenario 8

See the 3 generation pedigree of a family with haemophilia A (Fig 9 A). The proband is shown by an arrow and 'P' [III -2]. His sister [III-3] wishes to find out the possibility of her giving birth to a child with haemophilia A. Also, his maternal aunt's daughter [III-1] wishes to know the possibility of her next child being affected with haemophilia. The pedigree shows the characteristic X-linked recessive mode of inheritance where only males are affected with the disease and the affected males are related through carrier females. It should be noted that III-1 has a son with haemophilia and even without testing one can tell that the risk of hemophilia in her sons is 50% and that her daughters will not be affected.

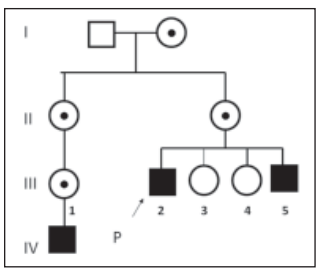


Fig 9 A: A pedigree showing characteristic X linked recessive inheritance (Carrier females are shown by a dot in the centre). If the blood sample of the affected boy is not available for mutation testing, mutation testing can be done using the blood sample of any of the obligate carrier females.

The woman III-3 may or may not be a carrier of haemophilia A. The possibility is 50% each. She wants to get prenatal diagnosis done when she conceives. To test her for carrier status for haemophilia A we need to find out the causative DNA variation / mutation in her brother by testing him. The mutation identified in any affected member of the family can be used for carrier testing and prenatal diagnosis. However, knowledge of mutation is not necessary for counselling III-1. III-2 was tested and found to carry an inversion mutation in the factor VIII gene. Then the sister III-3 was tested and was found to be a carrier. After genetic counseling, III-3 decided to go ahead with prenatal diagnosis when she conceives while III-1 opted not to go for prenatal diagnosis.

One would wonder about the different decisions in different members of the family. The reasons could be their personal experience with the disorder. The brothers of III-3 were older and during their childhood had not received an optimal therapy with coagulant factors due to cost constraints. They had developed joint contractures and some physical disabilities also. III-3 had seen some life-threatening complications in them as well. Hence, she was not ready to take the risk of giving birth to a child with haemophilia. On the other hand, the child of III-1 is young and because of timely diagnosis is getting prophylactic therapy with coagulant factors. So his mother feels that haemophilia is a treatable disease and does not justify prenatal diagnosis and termination of pregnancy if the fetus is found to be affected.

Issues in Genetic Counseling for X-linked Recessive Disorders

This family illustrates most of the issues in genetic counseling for X-linked recessive disorders. X-linked inheritance is seen in the the disorders caused by mutations in genes on the X chromosome. It came into prominence due to Hemophilia which was present in the British and Russian royal families. Other than hemophilia A and B, Duchenne muscular dystrophy and retinitis pigmentosa are other disorders well-known for their X-linked inheritance. In the X-linked recessive mode of inheritance, carrier females are clinically normal and affected males are related through the carrier females. Over the last few decades many X-linked genes for intellectual disability have been identified, *FMR1* being the commonest cause of familial mental retardation / intellectual disability. *FMR1* gene is related to fragile X mental retardation. The

boys with fragile X mental retardation usually do not have a very obvious phenotype and clinical diagnosis is difficult (Fig 9 B). Hence, all boys with intellectual disability or developmental delay need to be investigated for fragile X mental retardation syndrome by DNA based testing.

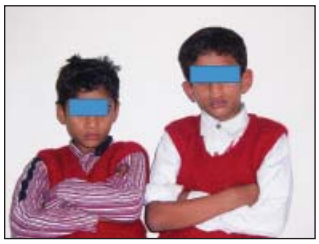


Fig 9 B: Two brothers with fragile X syndrome – Note that they do not have any clinically obvious phenotype

The presence of a disorder in a son and a brother (or son of a sister) of a woman confirms an X-linked mode of inheritance of the disorder in the family. However, if a woman has two sons with a disorder for which the mode of inheritance is not known, then the mode of inheritance can be X-linked, autosomal recessive or even autosomal dominant with germline mosaicism or incomplete penetrance.

The risk of recurrence of an X-linked recessive disorder depends on the carrier status of the mother as in some families with one child with Duchenne muscular dystrophy or haemophilia, the mother may not be a carrier on testing. Hence the risk of recurrence may not be high. However, like autosomal dominant disorders, germline mosaicism is documented for X-linked disorders as well. Table I shows the risk of recurrence for X-linked recessive disorders in various scenarios.

X-Linked Disorders Manifesting in Females

Characteristically most of the X linked disorders do not manifest in carrier females as they have another copy of X chromosome which carries a normal copy of the gene in concern. However, due to non-random Lyonization and some other reasons, manifestations in carrier females are not uncommon. Some hemophilia carrier females may have factor levels on the lower side and may bleed profusely after delivery or surgery. The other causes of manifestations of an X-linked disorder in females are homozygosity (as mentioned in table I), X- autosome translocation, a carrier female with Turner syndrome (45,X) or a female with 46,XY karyotype.

The manifesting females are quite common with fragile X syndrome. They may have long characteristic face with learning disabilities or mild intellectual disability. Manifestations of milder severity are seen in about 30% of female

Table 1: Risk of recurrence for X-linked recessive disorders in various scenarios

Scenario	Carrier status of the mother	Risk of recurrence in offspring	Carrier status in daughters	Comment
One son affected with the disorder	May or may not be carrier	50% of sons if the mother is carrier. Less if mother is not found to be a carrier	50% if the mother is carrier.	Blood test for carrier status cannot rule out germline mosaicism
One son and one maternal uncle or son of a sister is affected	Mother is an obligate carrier	50% of sons	50% of daughters	Mutation testing of the affected patient or carrier mother is required for prenatal diagnosis
Husband is affected	Wife needs to be tested if the family is consanguineous	Nil	All daughters will be carriers	If the wife is a carrier, the counseling will differ.
Husband is affected and the wife is a carrier	Wife is a confirmed carrier by a DNA test	50% of sons	50% of daughters will be homozygous and affected*. 50% of daughters will be carriers.	This is a rare situation
Brother of wife is affected	Wife (mother) may or not be carrier, but needs to be tested by a DNA based test		Mutation testing of the affected individual in the family is essential & is the first step	

Note: *Affected females due to homozygosity for the mutation can occur for disorders like haemophilia, G6PD deficiency, retinitis pigmentosa, color blindness where affected males have reproductive fitness; i. e. they can survive to adulthood and reproduce.

carriers of fragile X syndrome. Hence the mode of inheritance for fragile X syndrome is described as an X-linked semi –dominant or X-linked dominant with incomplete penetrance.

X-Linked Dominant Disorders

Some X-linked disorders manifest in all females with mutation and are hence labelled as X linked dominant. The classic example is X-linked hypophosphatemic rickets. The pedigree shows that there is no male-to-male transmission and the number of affected females is more than the affected males. However, the severity of disorder is less in females as compared to that in males. For the same reason, some disorders like Rett syndrome due to mutation in *MECP2* gene is always seen in females and it was considered that the disorder is lethal in males. However, mutation proved males with *MECP2* related neuro-developmental disorder are getting identified. Thus the compartments of X-linked recessive and X-linked dominant are no longer watertight.

Messages

- The risk calculations for X-linked disorders are simply based on the pedigree.
- The pedigree can give conclusion about the mode of inheritance if it is not known for the phenotype in concern.
- The pedigree of an X-linked disorder can guide the counsellor about probable carriers in the family. Those females can be offered genetic counseling and carrier testing.
- Mutation testing of an affected male or an obligate carrier female is essential for prenatal testing and carrier testing.
- In India, sometimes X-linked inheritance may have psycho-social issues and while counseling one should take care that the carrier woman is not made to feel guilty or is not stigmatized by the family. However, most of the families are comfortable even if the inheritance from mother's side is obvious.
- Like autosomal dominant disorders, germline mosaicism is reported for X-linked disorders. Even if there is only one child with hemophilia or Duchenne muscular dystrophy in the family and the mother is not carrier, prenatal diagnosis should be offered as the possibility of germline mosaicism cannot be ruled out.
- The need and acceptance of prenatal testing may vary from person to person and disease to disease. It will depend not only on the risk of recurrence but the family's experience of the disease and perceived burden.
- Manifestations of X-linked disorders in females are not uncommon, but are usually less severe than those in males.
- Counseling for X-linked dominant disorders is different from that of X-linked recessive disorders.

Mitochondrial Inheritance

As it is well known, the mitochondria are always inherited from the mother and they have their own DNA. The function of mitochondria is production of energy and any abnormality in mitochondrial function leads to manifestations involving brain, heart, eyes and any other system. Hence, clinical presentations with multisystem involvement suggest the possibility of mitochondrial disorder. Some genes for structural proteins and enzymes in the mitochondria are in the nuclear genome and follow autosomal recessive inheritance. But the disorders caused by mutations in genes in the mitochondrial genome follow classical mitochondrial mode of inheritance. It means the disorder can only be transmitted by the mother. However, the disease manifestations depend on the load of mutated mitochondria and their variations in various cells and tissues. The risk in the offspring cannot be definitely predicted as it depends on the number of mutated mitochondria in the ovum. The prenatal diagnosis for mitochondrial disorders, though technically feasible has limitations due to inability to predict phenotype and mutation load. For this reason, if mitochondrial disorder is confirmed in the proband, the family may be offered an option of ova donor. In vitro fertilization with replacement of mitochondria from a normal female has been done to avoid the transmission of mitochondrial disorders.

Other Non-Mendelian Modes of Inheritances

Like mitochondrial inheritance, there are other genetic phenomena which do not follow Mendelian laws. The molecular pathogeneses behind them have been identified. The genetics of these disorders is complex and genetic counseling should address these issues. Table II provides brief insights into

Table 2: Non- Mendelian inheritance- Explained in brief

Mode of inheritance	Mechanism	Representative Disorder	Comment
Dynamic mutations	Increase in the number of triplet repeats in the coding or non-coding region of the gene	Fragile X syndrome*, Spinocerebellar ataxia, Huntington disease, Myotonic dystrophy, Friedreich ataxia**	The severity of manifestations, especially age of onset may depend on the number of repeats
Paternal imprinting	The copy of the gene inherited from the father is shut off even if there is no DNA sequence variation	Angelman syndrome	The disorder occurs if the maternal copy of the gene is deleted.
Maternal imprinting	The copy of the gene inherited from the mother is shut off even if there is no DNA sequence variation	Prader-Willi syndrome	The disorder occurs if the paternal copy of the gene is deleted.
Uniparental disomy	Both copies of a gene or homologous chromosome are from one parent only	Angelman syndrome – if both the copies of chromosome 15 are from father and Prader-Willi syndrome if both copies are from mother	

the non-Mendelian modes of inheritances other than mitochondrial inheritance. These unusual situations occur only in a few genes. In some genes there are triplets of nucleotides like CGG (in *FMR1* gene) and increase in the number of these triplets causes the disease. For some genes, the behaviour / expression of genes varies based on whether it is inherited from the mother or the father. This phenomenon is known as imprinting. Rarely both copies of such genes may be inherited from mother or father. This is known as uniparental disomy and may lead to the manifestations of disorders caused by imprinted genes even if the gene sequence is normal and two copies of the gene are present.

Messages

- Taking a three generation pedigree in every case during preconception or prenatal visit is essential to identify if the family / current pregnancy is at risk of a genetic disorder.
- Pedigree can help to decipher the mode of inheritance of the disorder in the family and /or to identify candidates for genetic counselling and carrier detection testing.
- All probands with genetic disorders or phenotypes suggesting the possibility of genetic disorder should be evaluated preferably before planning pregnancy.
- The evaluation of fetuses with malformations, intrauterine death, stillbirth and neonatal death should be evaluated for etiology by autopsy and genetic testing. This is essential for providing information regarding prevention of recurrences.
- Storage of sample (one ml of blood in EDTA vial or one cm piece of umbilical cord) can go a long way in providing accurate diagnosis of the proband even after death and provide prenatal diagnosis to the family.

References

1. Amberger JS, Hamosh A. Searching Online Mendelian Inheritance in Man (OMIM): A Knowledgebase of Human Genes and Genetic Phenotypes. *Curr Protoc Bioinformatics*. 2017;58:1.2.1-1.2.12. Published 2017 Jun 27. doi:10.1002/cpbi.27
2. Bird TD. Myotonic Dystrophy Type 1. 1999 Sep 17 [Updated 2019 Oct 3]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1165/
3. Narayanan DL, Girisha KM. Genomic Testing for Diagnosis of Genetic Disorders in Children: Chromosomal Microarray and Next-Generation Sequencing. *Indian Pediatr*. 2020;57(6):549-554.
4. Phadke SR, Puri RD, Ranganath P. Prenatal screening for genetic disorders: Suggested guidelines for the Indian Scenario. *Indian J Med Res*. 2017;146(6):689-699. doi:10.4103/ijmr.IJMR_1788_15
5. Wagstaff J. Genetics beyond Mendel. Understanding nontraditional inheritance patterns. *Postgrad Med*. 2000;108(3):131-138. doi:10.3810/pgm.2000.09.1.1210
6. Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet*. 2019;393(10173):747-757. doi:10.1016/S0140-6736(18)31940-8
7. Daum H, Meiner V, Elpeleg O, Harel T; collaborating authors. Fetal exome sequencing: yield and limitations in a tertiary referral center. *Ultrasound Obstet Gynecol*. 2019;53(1):80-86. doi:10.1002/uog.19168
8. Crotti L, Tester DJ, White WM, et al. Long QT syndrome-associated mutations in intrauterine fetal death. *JAMA*. 2013;309(14):1473-1482. doi:10.1001/jama.2013.3219

Clinical Vignettes: Genetics - Bench to Bedside in Obstetrics care

Ratna Dua Puri
MD [Pediatrics], DM [Medical Genetics], Professor & Chairperson,, Institute of Medical Genetics and Genomics
Ganga Ram Institute of Postgraduate Medical Education and Research, Sir Ganga Ram Hospital, New Delhi, India

Introduction

The foundation of genetics was laid through the pea plant experiments by Gregor Johann Mendel. He established the principles of hereditary and "recessive" and "dominant" through his work. From the 19th century, to the turn of the 20th century when the significance of his work was recognized, the science of genetics has grown, and immense progress made. Identification of the DNA structure ushered in the age of molecular biology, and almost simultaneously identification of the chromosome structure heralded the era of cytogenetics. Thereafter technological advances enabled determination of the DNA code through Sanger sequencing in the 1970s and subsequent automation allowed for sequencing of the human genome through the Human Genome Project.

Genetics has an important role in the clinical practice of obstetrics and gynecology with relevance to antenatal care within the ambit of diagnosis and prevention of genetic disorders in the fetus. There are two scenarios that are commonly encountered in antenatal care

1. Identification of a fetal abnormality in the absence of a significant family history
2. A high-risk pregnancy due to the presence of a positive family history of a genetic disorder

The subsequent case vignettes will illustrate the approach to suspicion of a possible genetic etiology and diagnosis and counseling of families in these situations:

Case Scenario 1

Situation

A low risk primigravida had a nuchal translucency (NT) of 4.5 mm at a CRL of 63mm. This NT was > 99th centile for the CRL of the fetus. The nasal bone was present and the other first trimester markers were normal. No other malformations were present.

Pretest Counseling

The couple was counselled of the implications of an increased nuchal translucency. The risk of chromosomal disorders is about 20% but increases proportional to the NT increase. With a NT > 3.5 mm, the risk of chromosomal disorders increases upto 50%. Structural malformations account for 2.5 -5% and other risks include single gene disorders, especially noted are the Noonan syndrome and Rasopathies.

Genetic Test Performed

For this patient, the ultrasound did not show any major malformations. A chorionic villus sampling (CVS) was offered to evaluate the fetal chromosomes. The sample to be tested are the chorionic villi. A pre- requisite is to check for maternal cell contamination and maternal blood must be provided for this alongwith the CVS sample.

There are two options to examine the 23 pairs of chromosomes, karyotype and chromosomal microarray. The karyotype gives information of abnormalities of number of all chromosomes but only larger structural abnormalities greater than 5-10 Mb size.

In contrast the microarray additionally provides information of much smaller structural abnormalities [20-200 kb] and give additional information of upto 3- 5% beyond a normal karyotype in patients with increased NT. For this patient a chromosomal microarray and FISH test for the five common aneuploidies was performed.

Genetic Test Result

FISH was normal for the five chromosomes [13, 18, 21 and sex chromosome aneuploidies] The array showed a 2.7 Mb genomic deletion at 22q11.21 consistent with the 22q microdeletion syndrome.

Post Test Counseling

Post-test counselling informed the couple of the postnatal outcomes of this deletion. In view of the

associated learning difficulties and wide range of malformations associated with this disorder, the couple opted to discontinue the pregnancy.

As the microdeletion can be inherited in 10% cases from the parents, both partners should be tested by FISH or MLPA for this mutation. If the parents are not carriers, then the recurrence risk is small though slightly higher than the general population in view of gonadal mosaicism. If one of the partners is a carrier, then the recurrence risk is 50% in each conception.

Learning Objectives

1. Increased NT is for gestational age measured as per standard guidelines.
2. Associations of increased NT include structural malformations, chromosomal disorders, monogenic syndromes.
3. Genetic counseling (pre-test and post-test) is essential to explain to the patient the genetic tests options in each case and the outcomes of the tests.
4. Chromosomal microarray is the preferred test to evaluate for abnormalities of number and structure of chromosomes.
5. Panel testing for Noonan syndrome and Rasopathies can be offered in defined cases of increased NT.

Case Scenario 2

Situation

A 39 years second gravida was referred for counseling for advanced maternal age. She had been offered noninvasive aneuploidy screening by cffDNA analysis to examine for her age-related risk of chromosomal aneuploidies.

History

However, her first child, 13 years old, had intellectual disability and polydactyly. The latter was a familial trait and present in two other asymptomatic first-degree relatives of the girl. The cause of the intellectual disability had not been evaluated.

Counseling and Tests performed

As the pregnancy was currently at 17 weeks gestation, an amniocentesis and chromosomal microarray was done after pretest counseling. The fetus had an unbalanced chromosomal trans location. The older affected girl child was identified to have the similar unbalance translocation explaining the etiology of the intellectual disability. The parents karyotype was asked for and the mother had a balanced translocation between chromosomes 5 and 6; 46,XX, t (5;6) (q33;q23)

Post Test Counselling

The couple were counselled about the chromosomal translocation and the need to do prenatal diagnosis in each pregnancy in view of a risk of upto 30% of an unbalanced chromosomal complement in the fetus. Majority of unbalanced chromosomal disorders are associated with intellectual disability. Testing of at – risk family members for a translocation carrier status was recommended.

Learning Objectives

1. A 3-generation family history should be the first step for each pregnant lady.
2. Identify the etiology for an affected member in the family.
3. Estimate the risk if any for the fetus.
4. cffDNA testing is not an appropriate option with a positive family history of a genetic disorder.

Case Scenario 3

Situation

A family have three affected members [father, paternal aunt and first child] with tuberous sclerosis due to a gene mutation in the TSC2 gene. All three members are intellectually normal and only have the cardiac and skin manifestations of TS. In the second pregnancy of the couple prenatal diagnosis at 11 weeks identified the fetus to harbour the familial mutation in the TSC2 gene. The couple were counselled that the fetus is affected with Tuberous sclerosis. They wanted to know if the child after birth would be similar to the other members in the family.

Variable Expression and Challenges in Counselling

Tuberous sclerosis is an autosomal dominant disorder with inter and intrafamilial variability of clinical findings. In addition to the cutaneous manifestations of TS, upto 50% patients have neurodevelopmental delay and behaviour

abnormalities. It is not possible to counsel as to who will develop intellectual disability in fetal life.

The families have to make a decision after counselling on to continue or discontinue the pregnancy. In this family the pregnancy was continued. The neonate had a rhabdomyoma that progressively decreased in size after birth. However a t 2 months age he developed seizures and had global developmental delay on follow up. This phenotype was different from other affected members with the same mutation in the family, exhibiting the concept of variable expression for autosomal dominant disorders.

Learning Objectives

1. Autosomal dominant disorders can have variable expression and penetrance in different members of the same family.
2. It is important to explain this to the family, especially where the genetic disorder is associated with mental retardation.

Case Scenario 4

Situation

Primigravida at the 13 weeks visit is identified to have a hemoglobin of 9 gm/dl. She is started on iron as it was considered that poor appetite and vomiting in the first trimester accounted for her hemoglobin. A subsequent visit after 2 months did not show an increase in the hemoglobin and then she was investigated for the etiology of anemia.

She was identified to be a beta thalassemia carrier based on low MCV (65fL) and MCH (18pg) and high RBC count (6 million/mm3). The HbA2 levels on the HPLC test was increased at 5%.

Her husband was then tested for his carrier status by HPLC HbA2 that included a complete blood count as well. He was also a beta thalassemia carrier. A family history now identified that his brother had died with beta thalassemia major at 10 years age.

Counseling

Through all these investigations the pregnancy had advanced to 22weeks gestation.

The couple were counselled of the 25% risk of beta thalassemia major in the fetus. This is a chronic blood disorder where the child is dependent on monthly transfusions to maintain the hemoglobin. The resulting iron overload later requires chelation therapy. The fetal testing could only be done once the mutation in the beta globin gene in both partners was identified. This process also requires atleast 2 weeks.

Outcome

For this couple the prenatal testing could not be offered in view of advanced gestation and the limitations posed by the PCPNDT act.

All this was a result of missing an important family history, as also an incomplete workup in the first antenatal visit.

Learning Objectives

1. Each pregnant couple must be tested for their beta thalassemia carrier status as this is significant and varies from 3 – 15% in different communities in India.
2. The modality of testing is by HPLC HbA2 levels that includes Hb, MCV, MCH and RBC count.
3. If both partners are identified to be carriers, mutation analysis in the HBB gene should be performed immediately at an accredited laboratory.
4. This is important as prenatal testing is best offered at 11 weeks gestation by CVS.
5. The fetal testing is by checking the familial mutation identified in the parents.
6. A delay at any step delays timely prenatal testing.

Case Scenario 5

Situation

The first child of a couple was diagnosed to have Spinal Muscular Atrophy (SMA) type 1. He died at 9 months age. The diagnosis was confirmed by mutation testing of the SMN gene and the couple were confirmed to be carriers for SMA. They were counselled of the 25% recurrence risk of SMA in each pregnancy as it is an autosomal disorder.

The couple were counselled, and prenatal diagnosis was performed by CVS at 11 weeks gestation in the second and third pregnancy of the family. Unfortunately both times the fetus was affected and the pregnancy was discontinued.

Genetic Misconceptions and Outcome

In the fourth pregnancy the couple did not undergo prenatal testing due to the misconception that in

this pregnancy the fetus will not be affected as the previous three pregnancies of the couple were affected. Unfortunately for the couple the child had SMA .

Learning Objectives

1. The correct Interpretation of a Genetic Risk:
2. It is essential to note that the recurrence risk of 25% is for each conception and the next pregnancy has no memory of the outcome of the previous pregnancy.
3. Hence for all genetic disorders with recurrence risks, prenatal testing should be offered in each conception after pretest counseling of the couple.

Case Scenario 6

Situation

A second gravida is booked and gives a history that her first 4 years old child has a genetic disorder diagnosed to be epidermolysis bullosa (EB). There is no family history of EB or any other genetic disorder.

The lady is referred for prenatal diagnosis of EB at 12 weeks pregnancy. In this family no molecular tests has been done in the affected child to ascertain what type of EB he has and the gene mutation that is responsible for his condition. The carrier status of the parents is also not known.

Pretest Counselling

In this scenario the couple are counselled about the different types of EB and the multiple number of genes that can be associated with EB. They are asked to test the affected child as the gene and the mutation in one of the EB genes has to be identified before prenatal testing. Thereafter parental testing for the carrier status and fetal testing can be performed.

The couple are not keen to have their first child tested as they have been told that there is no cure for EB and that they should test the fetus in the second pregnancy to ensure that the disease does not recur in the family.

Molecular Testing

They agree after extensive counselling but three weeks are lost in the process. The method of testing for EB, a heterogeneous disorder that can occur due to mutations in one of multiple genes is clinical exome sequencing. The turnaround time is 4- 6 weeks. After the first report, checking the mutation significance identified in the report, parental testing all take time

Learning Objectives

1. An affected child / patient with a genetic disorder must be evaluated pre-pregnancy.
2. If they present for the first time in pregnancy refer them after the first visit to the geneticist for appropriate testing.
3. There is a turnaround time of 2-6 weeks depending on the type of genetic test required.
4. Prenatal testing is only possible after the specific gene and mutation is identified in the affected child.
5. The couple should be guided by the obstetrician that the affected child / patient will have to be tested before the fetus can be tested.
6. Direct genetic testing is not offered in the fetus without confirming the molecular mutation / chromosomal change in the affected person in the family.

Case Scenario 7

Situation

A couple is referred at 8 weeks of pregnancy as the first child had a history of loss of attained milestones, seizures and a poor neurological course before she died at 3 years of age. No definitive diagnosis had been made for the child and her DNA had not been stored. The couple were keen to ensure that they would not have another affected child.

Unfortunately medical records of the child had been destroyed at the time of her death.

What Can Be Done

The parents were very co-operative and step by step, starting from the family history, all the details of clinical history and examination findings of the affected child were elucidated.

A list of differential diagnosis was arrived at and molecular testing was planned in the parents as sample of the affected child was not available. A molecular test for the common deletion in the GALC gene for Krabbe disease was performed in

the couple. They were carriers for this deletion. Based on this a possibility of Krabbe disease was entertained in their first child although it could not be confirmed on her sample.

Outcome

As the genetic diagnosis was confirmed by testing a recurrence risk of 25% for Krabee disease, a neurodegenerative disorder without treatment was ascertained.

Prenatal diagnosis was performed for the family. The above deletion mutation for which the parents were tested to be carriers was tested in the CVS sample.

Learning Objectives

1. Early referral for families with history of a suspected genetic disorder is essential and can be fruitful to define the familial disorder for which the fetus is at risk.
2. Prenatal testing cannot be performed if the relevant gene mutation is not identified in the family.

Case Scenario 8

Situation

A couple were referred in the second pregnancy. The first pregnancy was discontinued in view of multiple malformations that were identified on the antenatal scan. No details of the malformations were noted and no fetal samples for testing were available.

In the second pregnancy craniosynostosis was suspected and there were associated limb anomalies. The gestation was advanced and the pregnancy was continued. This appeared to be an autosomal recessive disorder. The couple were counselled of the importance of a detailed morphology examination of the fetus and fetal autopsy. As they wanted to deliver at their parental home the fetal autopsy was a challenge.

The Best Way Forward

In view of this, a limited autopsy was discussed – detailed morphology examination of the fetus after delivery and each finding to be recorded. An antero -posterior and lateral radiograph of the fetus. As craniosynostosis was suspected antenatally, a CT Scan head with bone window was requested. Photographs of the neonate after delivery and collection of a blood sample in a heparin and EDTA vacutainer was asked for.

Making a Definite Diagnosis

Armed with all the above details the couple came for counselling. A clinical diagnosis of carpenter syndrome, an autosomal recessive craniosynostosis syndrome was made. The couple confirmed that the first child also had similar findings.

As the blood sample of the second affected were available, we were able to perform the molecular studies for Carpenter syndrome and confirm the clinical diagnosis.

Oucome

The couple now understood what had happened in the first two pregnancies. They were relieved that a diagnosis had been reached. They were able to plan future pregnancies based on the reproductive options discussed with them.

Learning Objectives

1. Each pregnancy that is discontinued for antenatally detected fetal malformations with adverse outcomes or intrauterine death must have a fetal autopsy.
2. If autopsy is not possible document each malformation and dysmorphism present.
3. Photographs speak volumes and should be taken after parental consent is signed.
4. Radiographs of the fetus should be taken.
5. An appropriate fetal sample–placental tissue from the fetal surface of the placenta, fetal skin sample, fetal blood in heparin and EDTA vacutainer must be stored for chromosomal and / or molecular genetic tests
6. Liase with a geneticist before a pregnancy is discontinued.

Case Scenario 9

Situation

A neonate was born at term to a low risk primigravida Her pregnancy had been uneventful and she did not have thyroid disease. There was no family history of agentic disorder.

At 9 months of age her infant was examined for developmental delay as he did not have good head

holding and did not recognize or respond to the parents. He has a slight coarse facies.

He was diagnosed to have hypothyroidism and replacement therapy was initiated. However he remained to have some intellectual deficits as a result of the delay in diagnosis of hypothyroidism

If new born screening (NBS) had been done for this neonate, hypothyroidism would have been identified at birth. Early initiation of this cheap therapy would have prevent intellectual disability in the infant.

Learning Objectives

1. While we wait for universal newborn screening through a public health funded program, it is imperative that we screen all newborns before discharge.
2. The disorders to be tested for can be decided upon as per the policy of the hospital, but common disorders like hypothyroidism and congenital adrenal hyperplasia must be tested, followed up with early institution of treatment if required.
3. The obstetrician must introduce the concept of NBS to the family during pregnancy so that they are prepared for it after the birth of their child.

Conclusion and Summary Messages

1. The tests for chromosomal disorders include karyotype, microarray and FISH tests. Microarray has the highest resolution to identify small abnormalities of chromosome structure. It can identify all abnormal chromosomal findings identified by a karyotype. It should be performed on the affected patient and is not a first line test for asymptomatic parents.
2. Karyotype is a good test to identify abnormalities of chromosomes number and to check for a balanced translocation in unaffected parents.
3. Single gene disorders occur due to mutations in one of the 20,000 genes present in the human genome.
4. Prenatal testing requires information of the involved gene and the mutation in the affected patient. It is important to explain to the families the need to test the affected patient before the fetal status can be tested.
5. Carrier screening for beta thalassemia at the first visit in each couple must be performed.
6. The recurrence risks for genetic disorders apply for each conception. Subsequent pregnancies do not have memory of the previous events.
7. Newborn screening must be discussed with the couple during pregnancy so that they are prepared when the child is born.

References

1. Sinajon P, Chitayat D, Roifman M, et al. Microarray and RASopathy-disorder testing in fetuses with increased nuchal translucency. *Ultrasound Obstet Gynecol*. 2020;55(3):383-390.
2. Carlson LM, Vora NL. Prenatal Diagnosis: Screening and Diagnostic Tools. *Obstet Gynecol Clin North Am*. 2017;44(2):245-256.
3. Issa MY, Chechlacz Z, Stanley V, et al. Molecular diagnosis in recessive pediatric neurogenetic disease can help reduce disease recurrence in families. *BMC Med Genomics*. 2020;13(1):68.
4. Gagnon A; GENETICS COMMITTEE. Evaluation of prenatally diagnosed structural congenital anomalies. *J Obstet Gynaecol Can*. 2009;31(9):875-881.
5. Modern genetics in obstetrics and gynecology. ACOG Technology Assessment in Obstetrics and Gynecology No. 14. American College of Obstetricians and Gynecologists. Obstet Gynecol 2018;132:e143–68.
6. Gray KJ, Wilkins-Haug LE, Herrig NJ, Vora NL. Fetal phenotypes emerge as genetic technologies become robust. Prenat Diagn. 2019;39(9):811-817
7. Committee on Genetics and the Society for Maternal-Fetal Medicine. Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol*. 2016;128(6):e262-e268
8. Christopher, R., Rama Devi, A.R., Kabra, M. et al. Newborn Screening for Congenital Hypothyroidism and Congenital Adrenal Hyperplasia. Indian J Pediatr 85, 935–940 (2018)
9. Puri RD, Kotecha U, Lall M, Dash P, Bijarnia-Mahay S, Verma IC. Is the diagnostic yield influenced by the indication for fetal autopsy?. *Am J Med Genet A*. 2016;170(8):2119-2126.

Genetic Counseling- Scenarios of the usual and the unusual cases in fetal medicine and reproductive genetics

Sunita Bijarnia-Mahay
Senior Consultant, Institute of Medical Genetics & Genomics, Sir Ganga Ram Hospital, New Delhi

As we stride ahead in the 21st century, the importance of our genetic makeup is progressively being realized in almost every medical field. Increasing awareness about genetic disorders among physicians and general public, assisted by advanced technology enabling accurate and fast genetic diagnosis has been at the helm of clinical practice. A changing scenario is being witnessed in all obstetric clinics, where more attention is being paid to a background history in self or family, or any issues in an ongoing pregnancy. We experienced these changing trends in prenatal diagnosis in the last decade, where testing is moving from genetics to genomics[1].

This article aims at updating the information about conditions requiring genetic counseling - related to fetal medicine and reproductive genetics, to the much aware clinician of today, and provides case based approaches in management of some challenging situations.

Genetic counseling refers to a process of guiding an individual, couple or a family regarding issues related to genetic disorders. Since it is a process, it involves various steps, including gathering information via history and examination, formulating a diagnosis via various genetic and non-genetic investigations, and then advising about the risks involved, specifically in a prenatal scenario, of inheriting a disorder in the fetus. Finally, the various modes of detection of the disorder in pregnancy are discussed, together with their advantages and disadvantages. In the process, there are many challenges that a fetal medicine specialist or geneticist faces, and these are evident in the cases described below.

Case 1

A 26-year-old healthy woman, presented in her second pregnancy. Her first born was a healthy girl. The pregnancy was noted to be 14 weeks, and reason for referral for genetic counseling was a positive first trimester screen. The parameters on a first trimester screen were as follows: PAPP-A 0.86 Multiples of Median (MoM), HCG 3.1 MoM. Nuchal translucency was 2.45 mm at crown rump length (CRL) of 66.5 mm (90th centile; 1.68 MoM). The combined risk for Down syndrome was 1:90, whereas age-related risk for same was 1:1244. She was counseled regarding the increased risk of Down syndrome in the baby as suggested by the screen test, and also about the implications of an increased nuchal translucency, even though the NT was strictly within the normal limits (<95th centile). The couple chose to go for amniocentesis at 16 weeks. At 16 weeks, the ultrasound showed some abnormality of amniotic sac (wavy sac) and hence deferred the procedure. The couple then went ahead with NIPT (non-invasive prenatal test) as an alternate option. The NIPT showed positive result for Trisomy 21. This was then confirmed by amniocentesis and fetal FISH and karyotype. Genetic counseling was performed following which couple chose not to continue with the pregnancy.

Case 2

A 36-year-old pregnant woman presented at 13 weeks of pregnancy. In view of advanced maternal age, and a bad obstetric history, she was referred to clinical geneticist. In the family history, she was married non-consanguineously and had had 5 prior pregnancies. The first pregnancy had been terminated at 19 weeks of gestation, in view of a complex congenital heart disease detected in fetus. This was evaluated with a fetal autopsy and genetic studies that included a fetal karyotype. The autopsy showed fetus to have an isolated hypoplastic left heart, with no associated malformation. Karyotype had been normal, 46,XY. The second and third pregnancies were uneventful, with two healthy girls. Fourth and fifth pregnancies ended in first trimester missed abortions. The couple underwent chromosomal study in view of the repeated miscarriages, and it

was normal. Our proband conceived spontaneously in the current pregnancy, and had a normal NT scan at 13 weeks (Nuchal translucency of 1.3 mm at CRL of 76 mm). Nasal bone was visible. After taking the history, and keeping in mind a normal NT scan, there was the advanced maternal age issue that was still there. After counseling the couple, they took the option of a Non-Invasive Prenatal Test (NIPT) for common aneuploidies.

The results of NIPT revealed a high risk for Trisomy 13 and low risk for aneuploidy of chromosome 18, 21 and sex chromosomes. The next step was to confirm the status using an invasive test. Since the pregnancy was less than 16 weeks, hence couple decided to go for a chorionic villous sampling for FISH and a karyotype. The FISH report stated a mosaicism, with 30% cells out of total of 200 cells counted, showing three signals in chromosome 13 (figure 1). This report was not conclusive and required more investigations. A subsequent karyotype was normal, and an early fetal anomaly scan at 16 weeks was also normal. In view of the normal karyotype and healthy fetus, decision of a termination was deferred and an amniocentesis was performed. The amniotic cells are the true depiction of fetal genome (chromosomes), in contrast to the chorionic villi which may have an abnormality confined to the placenta (confined placental mosaicism). The amniocentesis revealed a normal FISH report – no aneuploidy. A further chromosomal microarray analysis was performed to ensure no submicroscopic copy number variation in any of the chromosomes, which was also normal.

The case is an example of a confined placental mosaicism, and also shows the NIPT results cannot be relied upon completely. NIPT remains a high efficiency screening test.

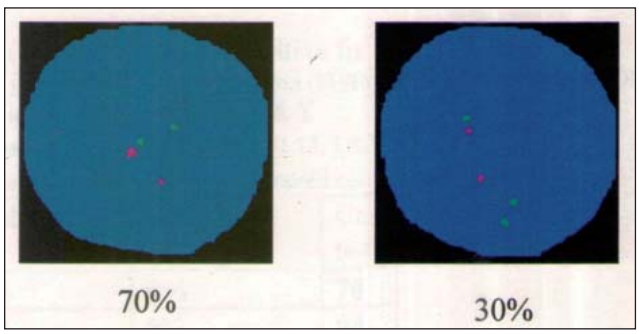


Fig 1: FISH analysis on CVS showing trisomy 13 in 30% and normal disomy 13 in 70% cells

Case 3

A non-consanguineously married couple presented to the genetic centre with foetal malformations detected on the level II ultrasound. The nuchal translucency measured at 12 weeks was 1.1mm (<95th) at a crown rump length (CRL) of 66 mm. The anomalies detected on the level II scan at 21 weeks of gestation included bilateral ventriculomegaly (Right ventricle-18.1mm, Left ventricle-20.1mm) and dangling choroids were seen. Posterior fossa showed direct communication between the fourth ventricle and cisterna magna suggestive of Dandy-Walker malformation (DWM). The right kidney was multi-cystic and enlarged (45mm). The left kidney was normal in shape and size. Right-sided congenital talipes equinovarus was also identified. Their first pregnancy was terminated in view of antenatally detected ventriculomegaly at 18 weeks. Genetic testing was not performed in that pregnancy.

In regard to the current pregnancy, the couple was counselled regarding the grave prognosis and need for genetic testing to identify the likely aetiology. Following this, the pregnancy was terminated at 25 weeks following the disappearance of cardiac activity. The couple opted for a post-natal evaluation and autopsy.

At autopsy, there was no facial dysmorphism on gross examination (Figure 2A). Fetal parameters were corresponding to 25-26 weeks of gestation. Contractures at elbow joint and right-sided congenital talipes equinovarus were noted, along with a rocker bottom foot on the left side (Figure 2 A & B).

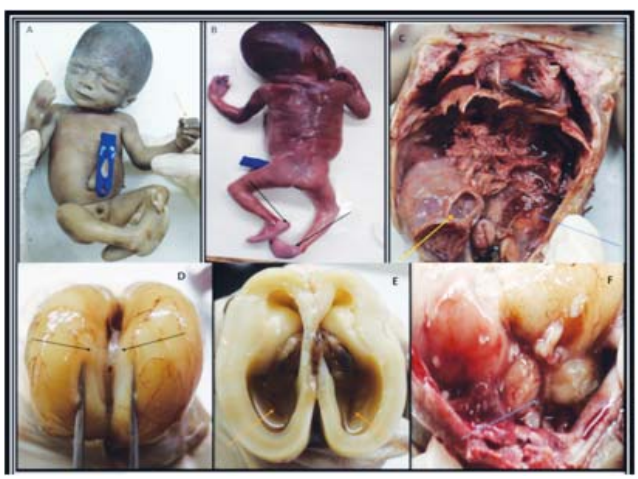


Fig 2: Autopsy pictures of fetus showing external features (A,B) and internal abnormalities (C: enlarged cystic kidneys, D:ageneis of corpus callosum, E: ventricular dilation, and F:enlarged cisterna magna and Dandy-walker malformation).

In the internal examination, the right kidney was noted to be grossly enlarged, occupying almost half of the abdominal cavity (Figure 2C). The left kidney, both ureters and bladder as well as the genitalia were normal. The cranial cavity was examined internally. Agenesis of the posterior part of the corpus callosum was noted along with a severe bilateral ventricular dilatation (Figure 2D &E). In the posterior fossa, enlarged cisterna magna and a DWM was observed (Figure 2F). Rest of the systemic examination was unremarkable.

In view of recurrence and a strong suspicion of a genetic disorder, a trio whole exome sequencing (fetus and parents) (Trio-WES) was performed after written informed consent from the couple. The trio–WES revealed a homozygous variant, c.411C>A; (p.Cys137Ter) (ENST00000223528.2) (chr9:108366537C>A) in the *FKTN* gene. It is classified as 'likely pathogenic' according to the 2015 ACMG criteria. This provided a genetic diagnosis of a congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies (type A). This is an autosomal recessive single gene disorder, with a 25% recurrence in each pregnancy for the couple. The couple was counseled accordingly for their next pregnancy[2].

Case 4

A 3rd degree consanguineous couple presented in their fourth pregnancy first, with an abnormal ultrasound showing multiple fetal malformations. Their first child was alive & healthy. One pregnancy ended in a blighted ovum. Third pregnancy had to be terminated due to multiple fetal malformations (figure 3). The fourth pregnancy ultrasound at 18 weeks had revealed atrioventricular septal defect, occipital encephalocele, bilateral polycystic and enlarged kidneys, post-axial polydactyly in all 4 limbs, with bilateral foot deformity. The decision of termination of pregnancy with subsequent fetal autopsy was taken after genetic counseling of the couple. The fetal autopsy confirmed the findings of the ultrasound (figure 4). As there was recurrence of a similar pattern of malformation and in view of the consanguinity in the couple, an autosomal recessive cause was suspected. A clinical diagnosis of Meckel-Gruber syndrome was made, and fetal DNA was subjected to WES as a trio (trio-WES). This revealed a homozygous pathogenic variant, c.1450_1453dup (p.Thr485ArgfsTer107) in MKS1 gene, thus confirming diagnosis of Meckel –Gruber or Meckel syndrome type 1. A prenatal diagnosis for the same was performed in a subsequent pregnancy via CVS at 11 weeks, detecting in a normal unaffected fetus. The couple went on to have a healthy baby at term.

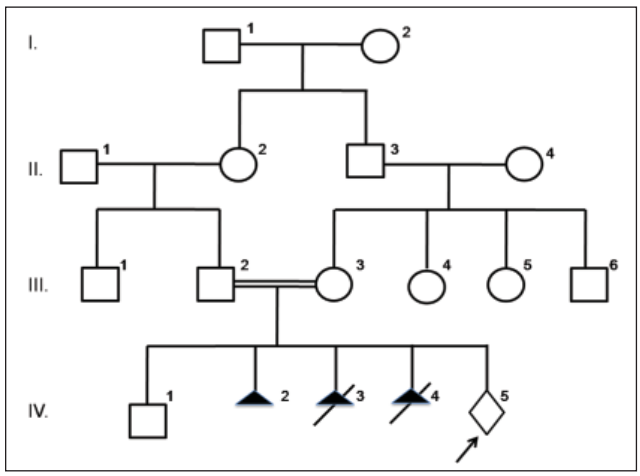


Fig 3: Pedigree of family showing consanguinity (3rd degree) between individuals III2 and III3, three affected pregnancies (IV2-4), and current pregnancy where prenatal diagnosis was performed based on confirmed genetic diagnosis.

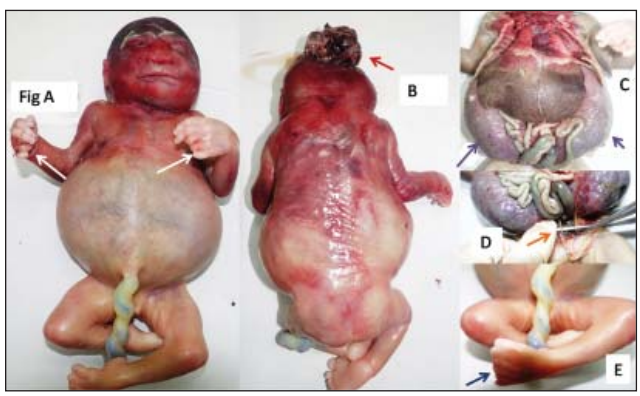


Fig 4: Fetus with Meckel-Gruber Syndrome, showing microcephaly and dysmorphic facies, with bilateral post –axial polydactyly (fig A), posterior encephalocele (B), and enlarged kidneys (C). The ureters are normally seen (D). There is post-polydactylyj in feet as well (E).

Case 5

A healthy young, non-consanguineous couple presented to genetic clinic with history of two first trimester miscarriages. The obstetric work up had not revealed any cause – the uterus was normal in size and shape, there were no medical concerns with the woman such as hypothyroidism or diabetes or auto-immune or any chronic illness. In the family, while the husbands' family had no significant history,

the wife had a similar history in her maternal uncle and aunt, who had multiple miscarriages before a healthy child. Her brother had infertility, and during work up of infertility, a karyotype was performed. His karyotype was abnormal, showing an apparently balanced translocation between chromosome 1 and 9 (46, XY, t(1:9)(q21;q13). Because of the family history, the wife was suspected to be carrier of translocation similar to the brother and was tested. Her karyotype performed at our laboratory, revealed similar translocation as her brother, 46, XX, t(1;9)(q23;q12) (figure 5). She was then provided with one of three reproductive options for planning her next pregnancy.

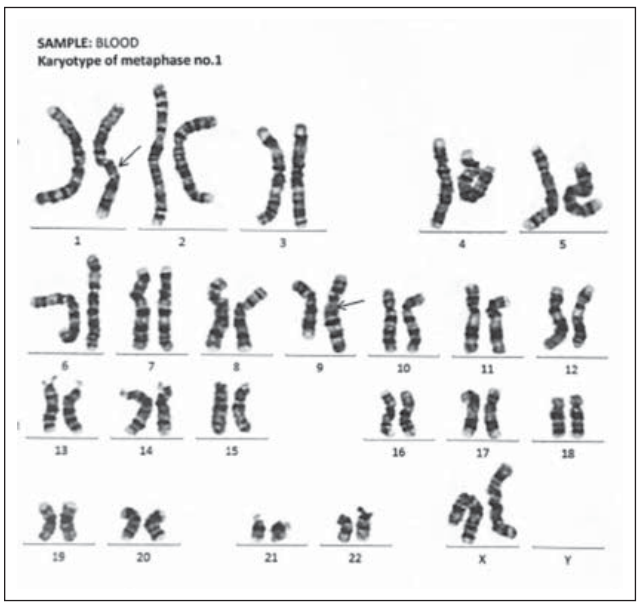


Fig 5: Pedigree of family showing consanguinity (3rd degree) between individuals III2 and III3, three affected pregnancies (IV2-4), and current pregnancy where prenatal diagnosis was performed based on confirmed genetic diagnosis.

i. Option for natural and spontaneous pregnancy. Once successful, then monitor with ultrasounds in first trimester till NT scan. If normal, then proceed with pregnancy and also perform amniocentesis at 16 weeks to look for imbalance in the chromosomes. This is done to prevent abnormality in the baby who will be born, as translocations leading to copy number variations within the genome can lead to not only to miscarriages but also intellectual disabilities and other more serious problems later.
ii. Assisted reproduction – in-vitro fertilization (IVF) using a donor egg. The point to clarify is that the donor should not be a blood relative, and should have a normal karyotype.
iii. Assisted reproduction – IVF with pre-implantation genetic diagnosis (PGD). The embryos created in the laboratory are tested for copy number variations, specific to the couple, along with other aneuploidies, and a healthy unaffected embryo is selected to be implanted. The PGD process is usually fool-proof and gives reasonable assurance of no chromosomal issues with baby, but amniocentesis during pregnancy can also be offered for surety.

In the family, brother of the proband with infertility, was concurrently noted to have hypothyroidism with serum TSH levels above 150 mIU/L. The couple soon presented with pregnancy after initiation of treatment for hypothyroidism. Amniocentesis for fetal karyotype and a microarray – revealed normal results. The couple gave birth to a healthy baby girl.

Case 6

A 29-year-old healthy woman presented in her first pregnancy at 13 weeks. This was a spontaneous pregnancy of non-consanguineously married couple and there was no background or family history suggestive of a genetic disorder. The ultrasound at 12^{+2} weeks showed a raised nuchal translucency (NT). It was 2.7 mm with CRL of 60 mm (above 95^{th} centile, less than 99^{th} centile). The other parameters on ultrasound were normal, such as nasal bone, ductus flow velocity and fetal heart rate. The couple was counseled regarding the increased risk of chromosomal disease in fetus, including Down syndrome, other birth defects such as heart defects, skeletal dysplasia or rare metabolic diseases. This was a condition where NIPT was not suitable as the implications of increased NT extended beyond Down syndrome and other common aneuploidies. The couple underwent amniocentesis at 17 weeks. At this juncture, an early fetal growth restriction was also noted on ultrasound. The karyotype of the fetus was abnormal, showing deletion at chromosome 2q (karyotype 46,__. del(2)(q32q33) (figure 6). This pregnancy was terminated after counseling of the couple. Subsequent parental karyotypes were performed showing normal results.

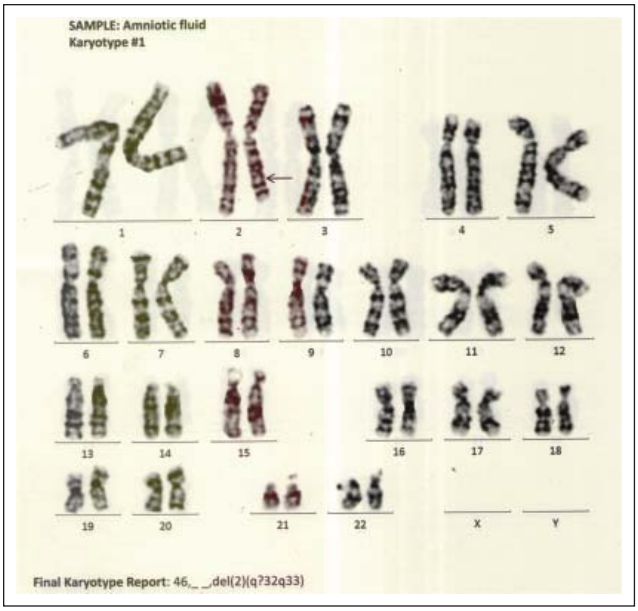


Fig 6: Karyotype on cultured amniocytes showing deletion on chromosome 2q (46,__. del(2)(q32q33)

Case 7

A 3rd degree consanguineous couple presented in their 3rd pregnancy at 16 weeks (figure 7). Their previous child, a 4-year-old boy had history of neuro-regression after 1 year of age, on a background of mild motor developmental delay. He was brought along for a clinical and genetic evaluation. He was noted to have spasticity, visual loss, seizures, and feeding difficulty. Multiple Mongolian spots were noted along with an exaggerated startle response. There was no organomegaly. He was completely bedridden. Eye examination showed bilateral cherry red spot. MRI brain showed evidence of hypomyelination. All the features in the child led to strong suspicion of a gangliosidosis. He was investigated with leucocyte enzyme assay for GM1 and GM2 gangliosidosis. The enzyme assay revealed a deficiency of beta galactosidase enzyme (3.23 nmol/hr/mg; *normal range*: 58-676 nmol/hr/mg , mean : 277.4), thus confirming the diagnosis of GM1 gangliosidosis, another autosomal recessive disorder with a 25% recurrence rate. This was possible in only a few days. In view of the diagnosis, genetic counseling was done and a prenatal diagnosis was performed using a placental biopsy and enzyme assay on chorionic villi. The result was normal. A subsequent gene sequencing was also performed, making the diagnosis more confirmatory.

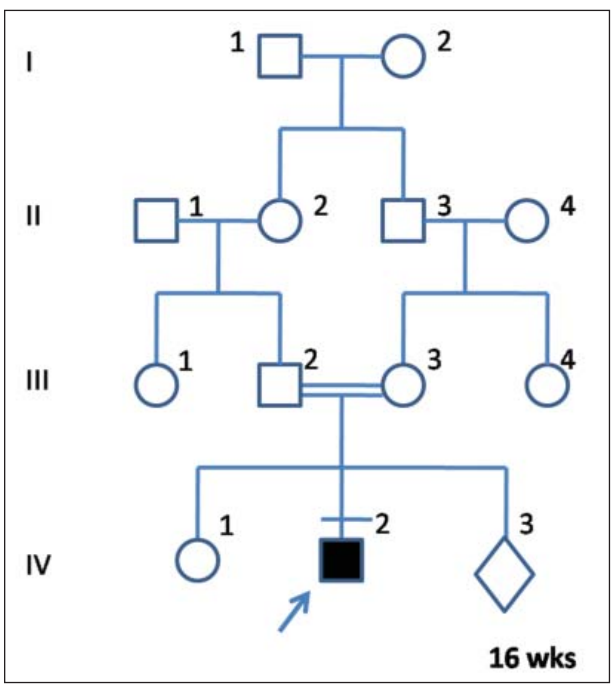


Fig 7: Four generation pedigree showing consanguinity and previous affected child and ongoing pregnancy.

Discussion

In the practice of obstetrics and fetal medicine, the cases provide a helpful learning. As the practice is closely linked with genetic disorders, hence the role of a geneticist seems to be central to the counseling and management. The cases described are real and commonly encountered in our genetic practice and each case provides a few learning points.

The first case was a straight forward case of a young healthy couple who underwent a routine screening, which showed a high risk for Down syndrome. Although the NT was slightly on the higher percentile, but strictly speaking it was less than the 95th centile for gestation. In such a scenario, the options are open for either non-invasive prenatal testing (NIPT) through cell-free fetal DNA in maternal blood or invasive testing. According to the latest guidelines from the American College of Obstetrics and Gynaecology (ACOG), cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies[3]. NIPT is a high efficiency screening test for aneuploidies, especially Down syndrome, where trisomy 21 can be ruled out with > 99% accuracy[4]. It also carries the advantage of non-invasiveness and thus not worrying about chances of miscarriage. The invasive testing, on the other hand provides a foolproof answer, not only for Down syndrome but all the other chromosomes

that may incidentally show abnormality. Incidental other chromosomal aberrations have been noted in 0.7-4.5% of cases[5,6]. The couple had to make the choice for NIPT in view of greater risk of miscarriage associated with amniocentesis in their case, as the amniotic sac look unsteady and wavy. The NIPT result of Trisomy 21 was finally confirmed via amniocentesis.

In case number 2, the choice between non-invasive and invasive stood between the couple. The non-invasive was an attractive option because of no associated abortion risk, but the background history of two miscarriages and a baby with a malformation was raising the possibility of there being a structural chromosomal abnormality, even though karyotypically visible aberrations had been ruled out. In the end, the couple decided upon NIPT. The NIPT result in this case was Trisomy 13 which required further the invasive testing, and finally ruled out by amniocentesis. This was a case of confined placental mosaicism, as the NIPT picked the mosaicism in the placental cellular population, whereas the amniotic fluid cells which were purely fetal showed a clear normal result.

Cases 3 and 4 were that of recurring malformations in fetuses in the families. Chromosomal cause was not found in any of the families, which is more common in any sporadic pregnancy, detected in upto 49% & 17% cases of structural malformations detected in first and second trimesters, respectively[7,8]. However, in case of recurrence and of a similar malformation, whether consanguinity or not, a single gene etiology is to be considered. Both the families were detected to have autosomal recessive disorders, a recurrence for which is 25% in each subsequent pregnancy. For future pregnancies, an early chorionic villous sampling for molecular genetic testing, or a pre-implantation genetic diagnosis becomes an option after a confirmed diagnosis.

Case 5 was that of a chromosomal translocation giving rise to multiple miscarriages in a family. The reproductive counseling for such cases has been provided. Balanced chromosomal translocations account for 2-4% of cases with recurrent spontaneous abortions[9,10].

In case 6, there was increased nuchal translucency due to a structural microdeletion in chromosome 2, which is different from the usual aneuploidies detected. Thus, in such cases, invasive testing (either CVS or amniocentesis) for chromosomal microarray analysis is recommended.

The last case was that of an autosomal recessive disorder, with 25% recurrence risk for the ongoing pregnancy. This is a common scenario where a pregnancy occurs without a proper evaluation of an existing (or deceased) child with a suspected genetic disorder. The prenatal diagnosis was made possible only because of their good fortune of timely detection of specific genetic disorder in the previous child. Bringing along their child for testing proved immensely helpful. This is not always the case. Hence, the take home message here would be to advice couples to get a timely, preferably pre-pregnancy, genetic evaluation of the affected person in the family. If not possible, then at least his or her blood should be sent for DNA storage to a lab for testing anytime in the future, in case of untimely demise of the affected patient.

The cases cover a wide spectrum of fetal and genetic medicine and provides clues for improving our obstetric and genetic practice.

Acknowledgments: The author wishes to acknowledge the mentorship of Prof I C Verma, who has been the guiding light always, Dr Ratna D Puri, and all the doctors and scientific staff at the Institute of Medical Genetics & Genomics, Department of Fetal Medicine, and the Institute of Obstetrics & Gynaecology, which are all closely knitted. Sincere thanks to all the referring doctors and patients.

References

1. Bijarnia-Mahay S, Arora V, Puri RD, Lall M, Saxena R, Verma IC et al. The changing scenario in prenatal diagnosis of genetic disorders: Genetics to genomics. Current Medicine Research and Practice 8 (2018) 201e206.
2. Arora V, Bijarnia-Mahay S, Kulshreshtra S, Singh K, Puri RD, Verma IC. Prenatal presentation of a rare genetic disorder: a clinical, autopsy and molecular correlation. Autops Case Rep. 2019 Sep 30;9(4):e2019124. doi: 10.4322/acr.2019.124.
3. Rose NC, Kaimal AJ, Dugoff L, Norton ME; American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics; Committee on Genetics; Society for Maternal-Fetal Medicine. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. Obstet Gynecol. 2020 Oct;136(4):e48-e69. doi: 10.1097/AOG.0000000000004084. PMID: 32804883.

4. Verma, I.C., Dua-Puri, R. & Bijarnia-Mahay, S. ACMG 2016 Update on Noninvasive Prenatal Testing for Fetal Aneuploidy: Implications for India. J. Fetal Med. 4, 1–6 (2017). https://doi.org/10.1007/s40556-017-0116-4
5. Lall, M., Mahajan, S., Saviour, P. et al. FISH is not Suitable as a Standalone Test for Detecting Fetal Chromosomal Abnormalities. J. Fetal Med. 2, 53–59 (2015). https://doi.org/10.1007/s40556-015-0043-1
6. Neagos D, Cretu R, Sfetea RC, Bohiltea LC. The importance of screening and prenatal diagnosis in the identification of the numerical chromosomal abnormalities. Maedica (Bucur). 2011 Jul;6(3):179-84. PMID: 22368694; PMCID: PMC3282538.
7. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, Maher EJ, Meller CH, Williams D, Wapner RJ, Maher ER, Kilby MD. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2013 Jun;41(6):610-20. doi: 10.1002/uog.12464. Epub 2013 May 7. PMID: 23512800.
8. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018;109(2):201-212. doi:10.1016/j.fertnstert.2018.01.005
9. Fan HT, Zhang M, Zhan P, Yang X, Tian WJ, Li RW. Structural chromosomal abnormalities in couples in cases of recurrent spontaneous abortions in Jilin Province, China. Genet Mol Res. 2016 Jan 22;15(1). doi: 10.4238/gmr.15017443. PMID: 26909916.
10. Tunç E, Tanrıverdi N, Demirhan O, Süleymanova D, Çetinel N. Chromosomal analyses of 1510 couples who have experienced recurrent spontaneous abortions. Reprod Biomed Online. 2016 Apr;32(4):414-9. doi: 10.1016/j.rbmo.2016.01.006. Epub 2016 Jan 28. PMID: 26874988.

Nuchal Translucency and The First Trimester Anomaly Scan

Varun Duggal[1], Anupam Bhalla[2], Jiten Kumar[3]
[1]Senior Consultant, [2]Consultant, [3]Consultant, Dr. Varun Dugal Ultrasound Clinic, Green Park Extention, New Delhi

Introduction

What started as the nuchal translucency (NT) and nasal bone (NB) scan for detection of aneuploidy has evolved to detect structural anomalies and combined with serum biochemistry the risk of preeclampsia, IUGR and preterm delivery.

It leads to early identification of abnormalities and early reassurance of normality for women. It gives access to earlier genetic testing, gives additional time to consider termination of pregnancy and safer termination if required.

Timing

12-13 weeks is the best time as both structural analysis and visualisation of NT & NB are good.

At 13-14 weeks although structural analysis is better, however NT visualisation is not that good and at 11-12 weeks structural analysis is not as good.

If CVS is planned then a scan prior to CVS at 11 weeks may be done with a follow up at 13 weeks.

Transabdominal versus transvaginal (TVS) scan

Transabdominal probe is easier to manoeuvre and is preferred for NT measurement.

TVS gives better resolution especially in mothers with high BMI or a retroverted uterus, it is better when size of the foetus is less than 60 mm, and also when there is an abnormality on the transabdominal scan.

Ideally start with transabdominal and then TVS.

Protocol

Crown rump length (CRL), NT, NB, Intracranial Translucency (IT) measurements on mid sagittal view. Axial section head for calvarium, midline falx, lateral ventricles with choroid plexuses, aqueduct of Sylvius, mid sagittal view for facial profile & brainstem, coronal and axial views for orbits with lenses, lips and nose. Sagittal, coronal and transverse spine. Situs, heart 4chamber view(4CV) ,3vessel trachea view, outflow tracts, symmetric lung fields, stomach, abdominal wall, kidneys, urinary bladder, cord insertion, three vessel cord,12 long bones, hands and feet. Ductus venosus flow, tricuspid flow & uterine artery Doppler. Placenta size and appearance. Cervix preliminary evaluation (cervix is ideally evaluated at 16 weeks) (Fig 1-9)

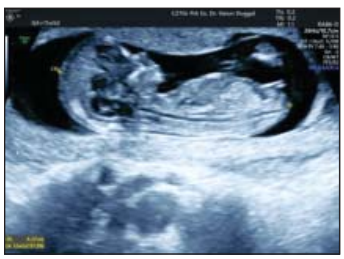


Fig 1: CRL

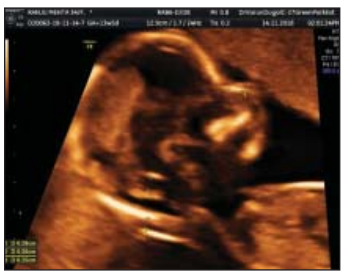


Fig 2: NT, NB, IT

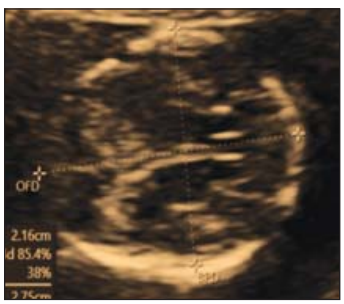


Fig 3: BPD

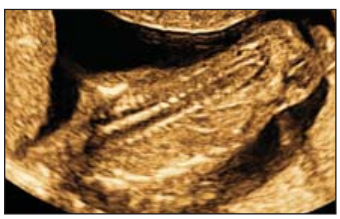


Fig 4: Spine

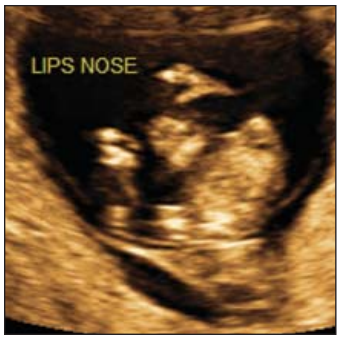


Fig 5a: Lips & Nose

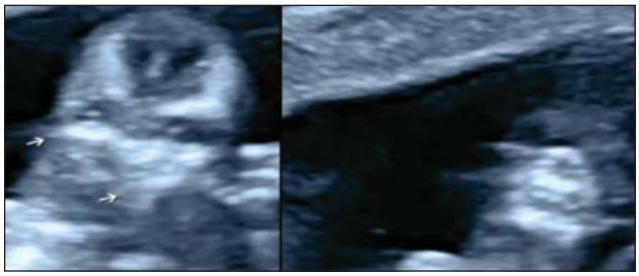


Fig 5b: Orbits **Fig 5c:** Retronasal Triangle

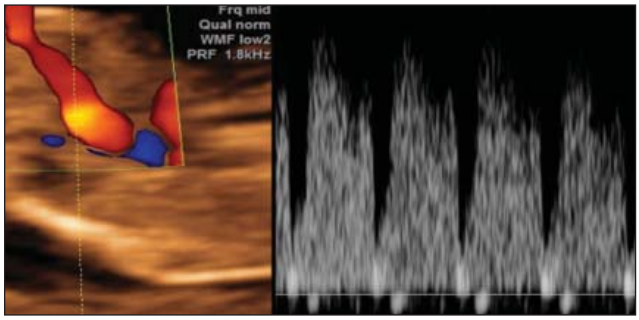


Fig 6a: Ductus Venosus Flow

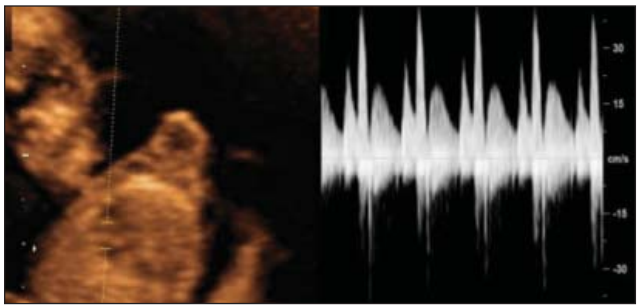


Fig 6b: Tricuspid Flow

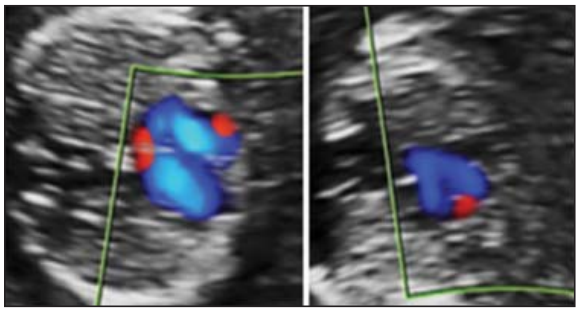


Fig 6c: Heart 4 Chamber View and 3 Vessel View

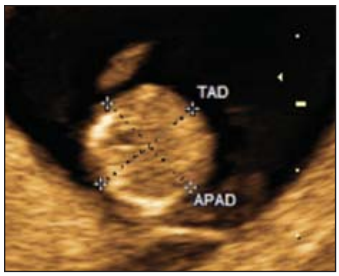


Fig 7a: Abdomen

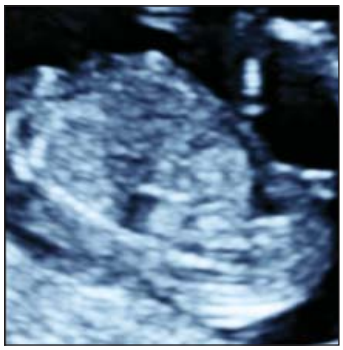


Fig 7b: Stomach & Urinary Bladder

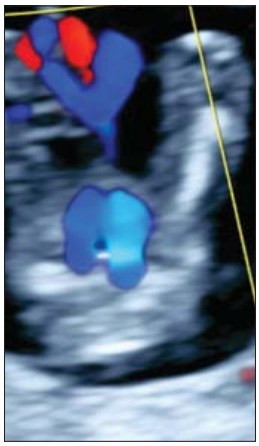


Fig 7c: Vessel Cord

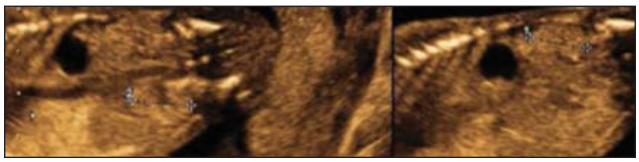


Fig 7d: Kidneys

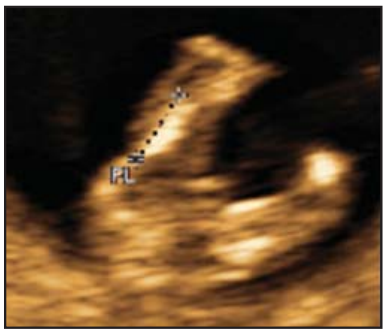


Fig 8a: Femur

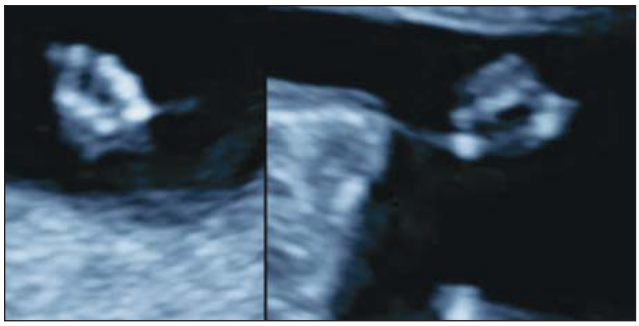


Fig 8b: Hands

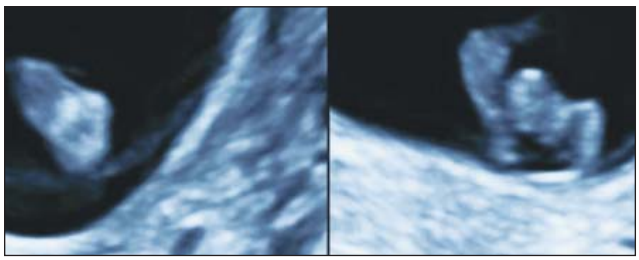


Fig 8c: Feet

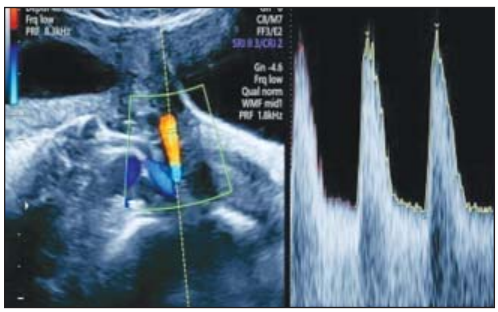


Fig 9: Uterine Artery Doppler

Biometry

CRL is used for dating and so is the biparietal diameter (BPD). Head circumference (HC), abdominal circumference (AC) and femur length (FL) are measured.

Level of measurement of BPD is on an axial view,in a plane showing midline falx and both thalami with symmetric appearance of cerebral hemispheres. The cavum septum pellucidum which is the level at which BPD is measured in the second trimester is not visualised in the first trimester

The Aqueduct of Sylvius can also be seen on the axial plane of the fetal head to make a subjective assessment of its distance from the occipital bone. (explained later with open neural tube defect)

A small BPD may be seen with holoprosencephaly, encephalocele, and open spina bifida.

Trisomy 18 and triploidy are associated with moderately severe growth restriction. Discrepancy in dimensions between the head and abdomen is seen in digynic Triploidy.

EARLY IUGR- a growth lag of greater than one week as based on dating scan. It can be a predictor for subsequent miscarriage. It can occur in both chromosomally normal and abnormal foetus and can be due to abnormal placentation also.

Markers for Aneuploidy

NT, absent/hypoplastic nasal bone, Ductus venosus, Tricuspid regurgitation.

Nuchal Translucency (NT)

Strict criteria for measurement

Valid only for CRL of 45-84 mm, at 11 to 13+6 Weeks

Magnification-- only the upper thorax and foetal head should be included in the image, each movement of the calliper produces a 0.1 mm change in measurement.

True mid sagittal view—tip of the NB should be seen, rectangular shape of the palate, non-visualisation of zygomatic process. (FIG10)

Neutral position- gap between chin and chest should be present

Calliper placement – inner border of the horizontal crossbar of the calliper should be superimposed on the line that defines the nuchal translucency thickness.

Widest part of lucency is measured.

Nuchal membrane should be seen clearly and differentiated from the amnion.

More than one measurement should be taken and the maximum one should be recorded

If nuchal cord is present use the mean of NT above and below the cord.[3]

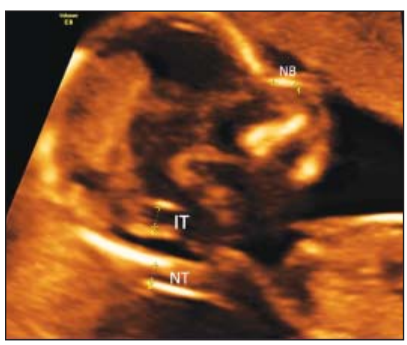


Fig 10: Nuchal Translucency (NT)

Thick NT — Common Associations & Pathophysiology

- Aneuploidy-the prevalence of chromosomal defects increases exponentially with NT, from 0.2 %to those with NT between 5th to 95th centile to 65% for NT of 6.5 mm or more.[2]

  In the chromosomally abnormal group about 50 % have trisomy 21, 25% have trisomy 18 or 13, 10% have Turners syndrome, 5% have triploidy and 10% have other chromosomal defects.
- Cardiac defects
- Skeletal dysplasia's
- Diaphragmatic hernia
- Exomphalos and body stalk anomaly
- Fetal infections –particularly Parvovirus B19.
- Single gene disorders, genetic syndromes.

Pathophysiology

Cardiac failure, venous congestion in the head and neck in diaphragmatic hernia and skeletal dysplasia, altered composition of the extracellular matrix, failure of lymphatic drainage, fetal anaemia and hypoproteinaemia, fetal infection.[1] (Fig 11 & 12)

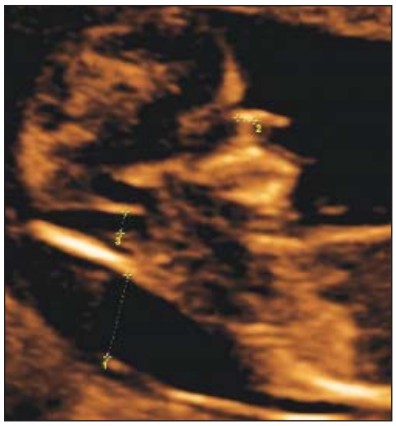


Fig 11: Thick NT Sagittal

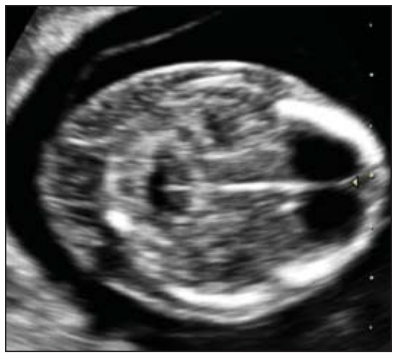


Fig 12: Thick NT Axial

Table 1 Relation between NT and prevalence of chromosomal defects, fetal death and major abnormalities and the prevalence of delivery of a healthy baby with no major abnormalities. (Souka et al)[3]

The chances of delivering a baby with no major abnormality is about 97% for NT below the 95th centile and 93 % for NT between 95th and 99th centile (table 1).

The percentile value of NT varies with CRL and various reference charts are available. It can be calculated from specialised software available online.

An increased NT leads to patient counselling with options for additional screening work up or invasive diagnostic testing.

Persistence of Increased NT

If karyotype and microarray are normal and NT is still thick at 14 weeks then further evaluation with infection screen and Noonan/exome testing should be done.

Maternal blood for toxoplasmosis, CMV, Parvovirus and a Rasopathy panel to test for Noonan's syndrome. **Rasopathy testing** is recommended when a foetus shows isolated NT ≥5 mm after chromosomal abnormalities have been excluded;

Table 1:

NT	Chromosomal Abnormalities	Fetal death	Major fetal Abnormalities	Alive with no major abnormality
<95th centile	0.2%	1.3%	1.6%	97%
95th-99th centile	3.7%	1.3%	2.5%	93%
3.5-4.4 mm	21.1%	2.7%	10%	70%
4.5-5.4mm	33.3%	3.4%	18.5%	50%
5.5-6.4mm	50.5%	10.1%	24.2%	30%
≥6.5mm	64.5%	19%	46.2%	15%

or when NT ≥3.5 mm with associated distended jugular lymphatic sacs, hydrops, polyhydramnios, cardiac defects or renal anomalies.[4]

Nasal Bone

Nasal bone (NB) length increases linearly with gestation

Criteria for assessment-Gestational age 11-13+6 weeks, CRL 45-84 mm, Magnification so that only head and upper thorax are seen

Mid sagittal view of the fetal profile

Echogenicity of nasal bone should be more than the skin overlying it.

3 distinct lines should be considered --top line skin and bottom line which is more echogenic than skin is NB (forming an equal sign). Third line in front of the bone at a level higher than skin is tip of the nose (fig 13,14).

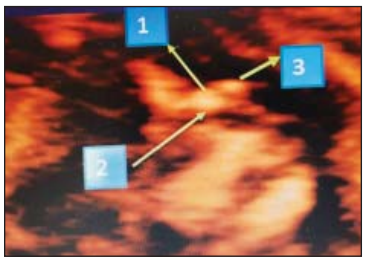


Fig 13: Skin (1), Nasal Bone (2), Tip of Nose (3)

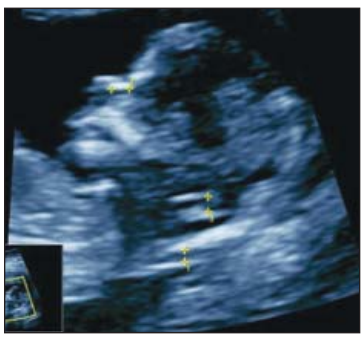


Fig 14: NB

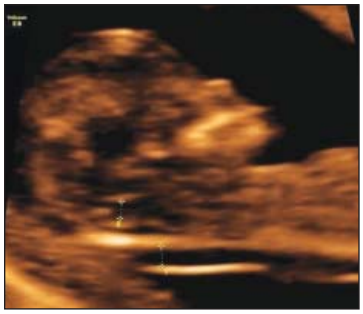


Fig 15: Absent NB

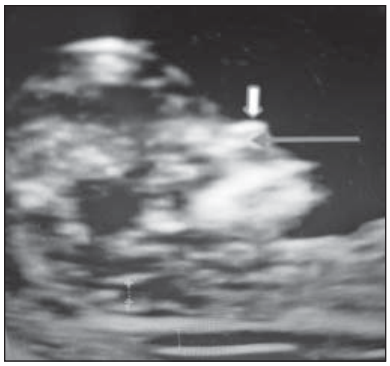


Fig 16: Arrow – Absent NB, Chevron--Skin

Non ossified NB is a marker for aneuploidy, most notably trisomy 21.

May be non-ossified in many craniofacial anomalies such as midface hypoplasia's and syndromic conditions

Absent nasal bone is found in 1-3% euploid fetuses and in 60% of trisomy 21, 50% of trisomy 18 and 40 % of trisomy 13 fetuses. (1) (FIG 15, 16)

Maternal ethnic origin may play a role in evaluation of the nasal bone was suggested by Prefumo et al. A mother of African origin is associated with an increased likelihood of absent nasal bone compared to Caucasians[5]. Cicero et al have mentioned the incidence of absent nasal bone in the chromosomally normal group as 2.2% for Caucasians, 9% for AfroCaribbeans and **5% for Asians**[6]

Incorporation of the nasal bone improves the performance of the combined screening test.

Ductus Venosus

Sagittal magnified section of abdomen, area of maximum aliasing on colour.

Reversed of a wave is seen in 3 % euploid foetuses. 65 % of trisomy 21 have reversed a wave and 55 % of trisomy 18 and 13.[3]

Reversed "a" wave implies **an increased risk for cardiac defects**, chromosomal defects and foetal death.

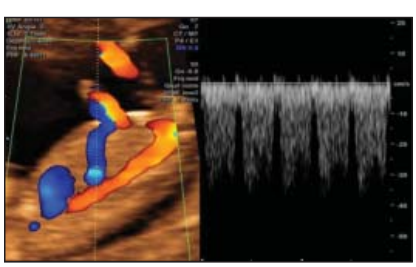


Fig 17: Normal Ductus Venosus

80% of foetuses with reversed a wave will have normal outcome. (FIG 17 & 18)

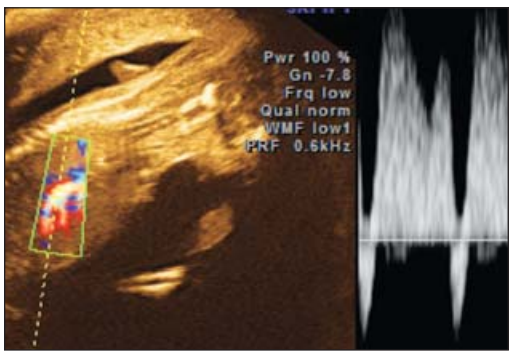


Fig 18: Reversed a wave DV

Contamination of waveform by hepatic vein or umbilical veins can occur. If pulsed Doppler sample is large, the cursor can fall partly into the hepatic vein and a false positive for reversed a wave may occur as hepatic veins normally have a backward flow during atrial contraction. (FIG 19 & 20)

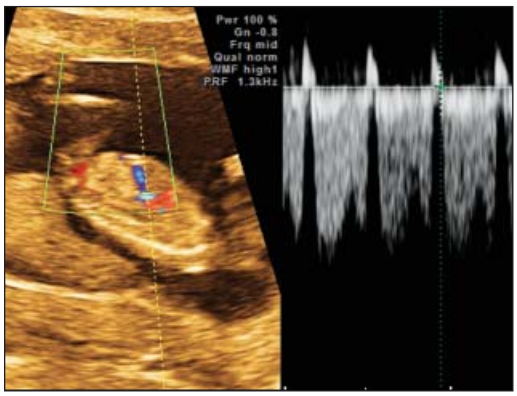


Fig 19: Contaminants in DV - Hepatic Vein- False Positive for reversed a wave

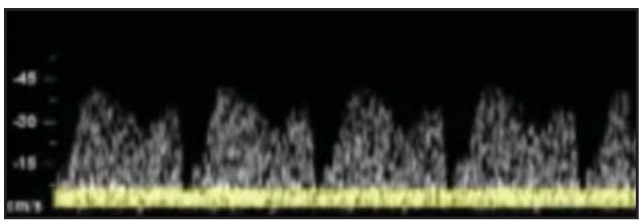


Fig 20: Contaminants in DV – Umbilical vein-continuous forward flow in the umbilical vein overlaps the a wave of DV.

Tricuspid Flow

Magnified 4 chamber view of foetal heart. Regurgitation is when peak systolic velocity (PSV)> 60cm/sec (differentiates regurgitation from contamination due to backward flow from aortic or pulmonary valve which have velocity of less than 60 cm/sec) lasting for more than half of systole (differentiates it from small jets corresponding to closure of the tricuspid valve) (FIG 21)

Association with cardiac defects and chromosomal aneuploidies.

It is **a marker for congenital heart disease**. The likelihood ratio of tricuspid regurgitation (TR) for cardiac defects is 8.4.

It is found in 1% of euploid fetuses, in 55% of fetuses with trisomy 21 & 30 % of fetuses with trisomy 18 and trisomy 13. (1, 3 AND 7)

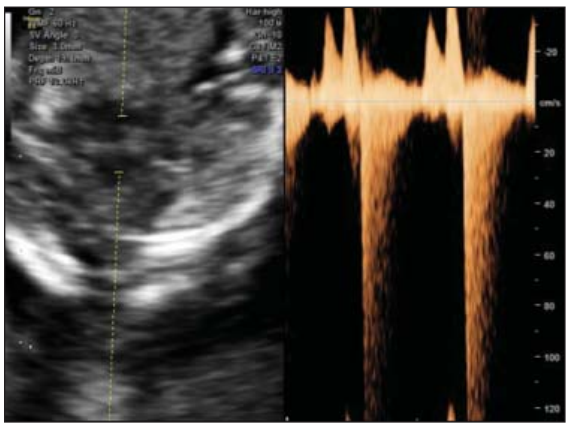


Fig 21: Tricuspid Regurgitation PSV >60cm/sec

Tricuspid regurgitation and reversal of a wave in DV can be transient and reverse to normal, (it has been postulated that as the foetal heart at 11-13 weeks is not compliant, has stiffer myocardium and more cartilage, with increasing gestation, myocardium becomes more resilient with more muscle and also the umbilical artery impedance falls as placenta implants better into uterine wall.

Only a small impairment of cardiac diastolic function is enough to cause tricuspid regurgitation.

DV a wave reversal and Tricuspid Regurgitation– both are associated with cardiac anomalies and early fetal echo is a must. They improve the performance of the combined screening test.

Screening by a combination of maternal age, fetal NT and serum free beta HCG and *PAPP-A* identifies 90% of trisomy 21 with a false positive rate of 3 %.By adding the additional ultrasound markers(NB,DV, Tricuspid flow) to this, the detection rate of trisomy 21 increases to 95% with a false positive rate of 2.5%.[1]

New Marker –
Aberrant Right Subclavian Artery (ARSA)

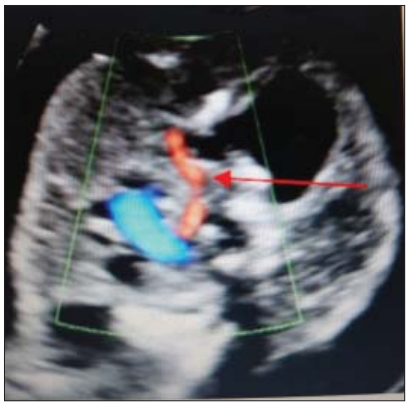


Fig 22: ARSA

An aberrant right subclavian artery (ARSA) is found in 1.5% 0f the normal euploid population and therefore when isolated is considered a normal variant.

It is considered an ultrasound marker for chromosomal and cardiac abnormalities and is also associated with genetic defects (22q11. 2 microdeletion).

ARSA is seen behind the trachea, as a straight vessel arising from the aortic arch and lies in front of the spine. (FIG 22)

Fetal Anomalies

- **ALMOST ALWAYS DETECTABLE (90-100%)**

  Acrania –Anencephaly

  Cephalocele

  Alobar holoprosencephaly

  Iniencephaly

  Ectopia cordis

  Omphalocele

  Gastroschisis

  Body stalk anomaly

  Megacystis

- **Potentially Detectable (2-90%)**

  Cerebral ventriculomegaly, posterior fossa abnormalities, open neural tube defects, micrognathia, cleft lip & palate, congenital heart defects, diaphragmatic hernia, bilateral renal agenesis, lower urinary tract obstruction, bladder exstrophy, limb abnormalities like transverse reduction defects and aplasia radii, polydactyly, lethal skeletal dysplasia's, hand & foot absence.

- **Anomalies Undetectable (<2%)**

  Agenesis of corpus callosum— non visualisation of the cavum septum pellucidum is a normal feature before 18 weeks.

  Cerebellar vermian hypoplasia

  Microcephaly

  Ventricular septal defects, Pulmonary Stenosis, Aortic Stenosis, Arrhythmias

  Congenital pulmonary airway malformation and sequestration

  Bowel obstruction, duodenal atresia, anal atresia

  Renal abnormalities

  Talipes

  Foetal tumours.

  Ovarian cysts

Reasons for Being Undetectable

Embryologic- some structures are not developed at this time

Some anomalies do not develop till later in gestation such as foetal tumours, ovarian cysts, microcephaly and ventriculomegaly.

Some anomalies evolve over time- hypoplastic left heart syndrome, coarctation of aorta, short limbs in achondroplasia, fractured limbs in osteogenesis imperfecta.

Some anomalies in which the phenotypic expression becomes apparent later in pregnancy. -increased urinary production unmasking urinary tract obstruction or reflux; increased production of lung fluid unmasking congenital cystic pulmonary airway malformation.

Overall Prenatal Detection Rate of Anomalies

Syngelaki et al (2019) studied 100997 pregnancies, overall incidence of nonchromosomal abnormality was 1.7% including 27.6% detected in first trimester, 53.8% detected in second trimester and 18.6 % detected in third trimester or postnatally. Some of these are listed below.[8]

Acrania 100%

Alobar holoprosencephaly 100%

Encephalocele 100%

Open spina bifida 59.3%

Hypoplastic cerebellum/vermis 13%

Agenesis of corpus callosum 0%

Schizencephaly 0%

Septo -optic dysplasia 0%
Microcephaly 0%
Severe ventriculomegaly 0%
Anophthalmia/microphthalmia 0%
Cataract 0%
Cleft lip and palate 34.6%
Cleft lip only 0%
Cleft palate only 0%
Micrognathia 14.3%

Congenital diaphragmatic hernia 29.2%
Congenital pulmonary airway malformation 0%
Pleural effusion 0%

Tricuspid atresia 100%
Pulmonary atresia 100%
Polyvalvular dysplasia 100%
Hypoplastic left heart 92.5%
Atrioventricular septal defect 90.9%
Complex heart defect 60%s
Left atrial isomerism 57.1%
Tetralogy of Fallot 39.3%
Arch abnormalities 31.6%
Tricuspid valve abnormalities 25%
Transposition of great arteries13.3%
Double arch/right aortic arch 15.6%
Aortic stenosis, pulmonary stenosis, common arterial trunk 0%
Arrhythmias 0%
Rhabdomyomas0%
Ventricular septal defects 0%

Cloacal abnormality 100%
Meconium peritonitis 100%
Right sided stomach 100%
Oesophageal atresia, duodenal atresia, imperforate anus, small bowel obstruction 0%

Exomphalos with bowel or liver 100%
Gastroschisis 100%
Bladder exstrophy 0%

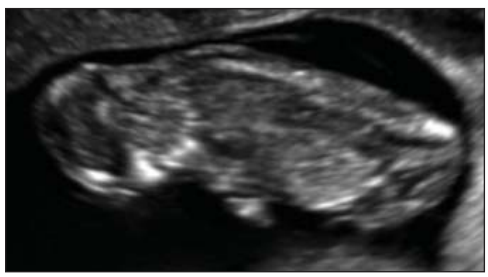
Lower urinary tract obstruction 71.2%
Bilateral renal agenesis 15.4%
Bilateral polycystic kidneys 7.1%
Unilateral pelvic kidney/agenesis 2.4%
Multicystic kidney unilateral/bilateral 0%
Severe hydronephrosis 0%
Duplex kidney/horseshoe kidney 0%
Ovarian cyst 0%
Ambiguous genitalia 0%
Hematocolpos 0%
Absent leg, arm, hand or foot 75%
Fetal akinesia deformation sequence 72.7%
Lethal skeletal dysplasia 71.4%
Non-lethal skeletal dysplasia 0%
Abnormal digits 42.4%
Hemivertebra /scoliosis 33.3%
Talipes 2.2%

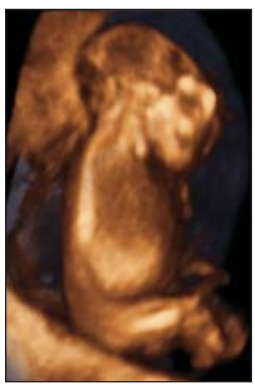
Sacrococcygeal teratoma 50%

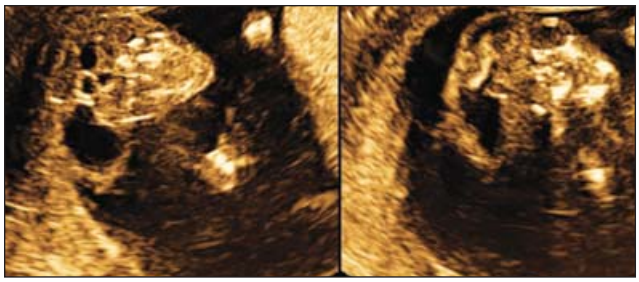
Body stalk anomaly 100%
Pentalogy of Cantrell 100%
Ectopia cordis only 100%
Hydrops fetalis 0%

Head and Spine

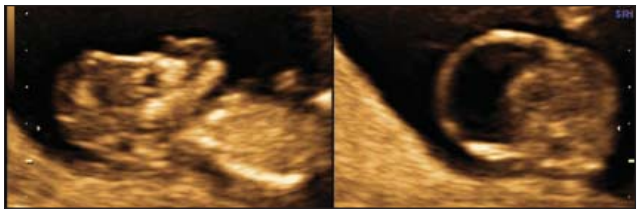
ACRANIA-EXENCEPHALY-ANENCEPHALY—absence of the ossified cranial vault-acrania, abnormal foetal head shape with exposed foetal brain tissue-exencephaly ,as gestation progresses the exposed tissue gets absorbed –anencephaly . Normal cranial bone ossification is seen by 11 weeks. (FIG 23 A-E).


A

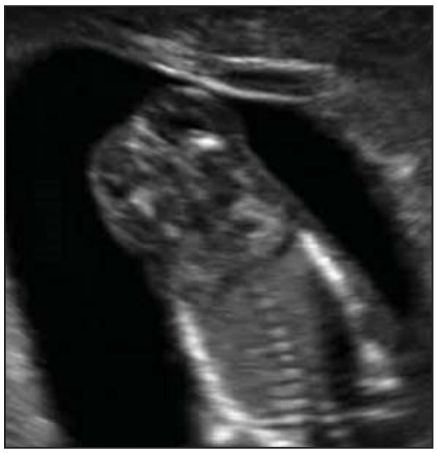

B



C



D



E

Fig 23: A, B Acrania, C, D Exencephaly, E Anencephaly

Cephalocele - protrusion of intracranial structures through a cranial bone defect. The herniated structures can consist of meninges only (meningocele) or meninges plus cerebral tissue (meningoencephalocele). Commonly occipital however frontal or parietal location may be there. (FIG 24)

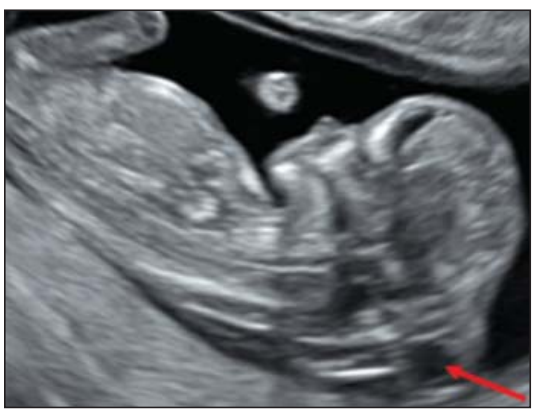


Fig 24: A Small Cephalocele

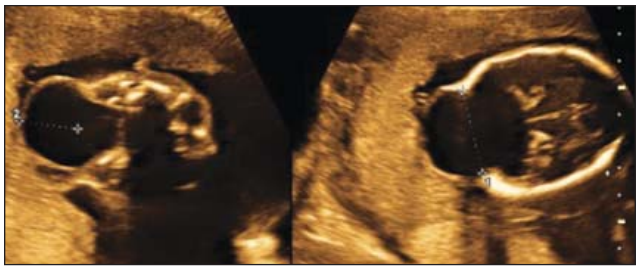
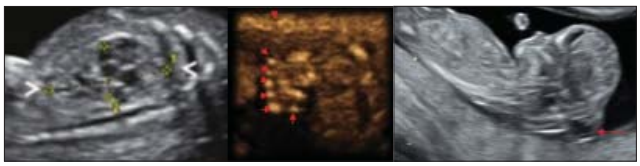


Figure 24 B: Cephalocele at 19 weeks, this is a follow up of small cephalocele at 12 weeks who initially refused termination

Associated abnormalities should prompt the diagnosis of a syndrome such as Meckel Gruber, Walker Warburg and Joubert.

Meckel Gruber Syndrome

Characterised by a triad of large echogenic kidneys with cystic dysplasia, posterior encephalocele, polydactyly. Autosomal recessive inheritance pattern. Important in families at risk with a positive history. FIG 25 A, B, C.



A B C

Figure 25 A, B, C: Meckel gruber syndrome-. Cephalocele, cystic kidneys, polydactyly

Holoprosencephaly- Failure of cleavage of forebrain, single midline ventricle replacing the two lateral ventricles. Complete or partial absence of the falx. Butterfly sign is absent. Fusion of midline structures, thalami and lateral ventricles. Midline facial anomalies including cyclops, absent nose, hypotelorism, proboscis and median cleft lip. Small BPD. Strong correlation with trisomy 13. (FIG 26)

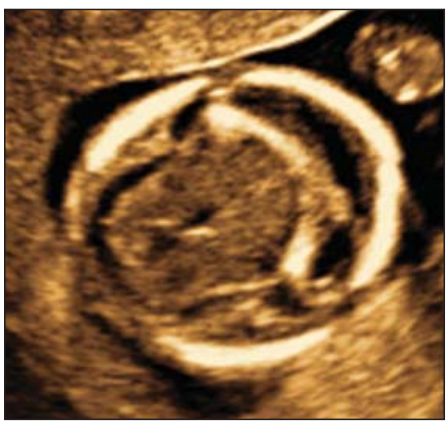


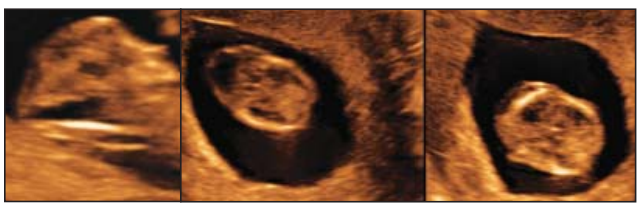
Figure 26: Holoprosencephaly-fusion of lateral ventricles, absent falx

Posterior Fossa Abnormalities

A cystic posterior fossa should raise suspicion of a posterior fossa malformation, which needs confirmation after 18 weeks. It is a strong marker for chromosomal abnormalities. Other causes include Dandy Walker malformation and Blake's pouch cyst. It could be a transient finding too. Predictors are—increased brainstem occipital distance, large fourth ventricle and nonvisualisation of the choroid plexus of fourth ventricle. (FIG 27 A, B,C AND FIG 28)

Some pointers for Joubert's syndrome are enlarged IT and absence of future cisterna magna.

Newer signs to differentiate Blake's pouch cyst from Dandy Walker Malformation have been described and include the position of the choroid plexus with respect to the cystic lesion and compression of the aqueduct of Sylvius in Dandy Walker Malformation.[9]



A B C

Fig 27 A,B,C: Posterior fossa cyst-sagittal and axial views

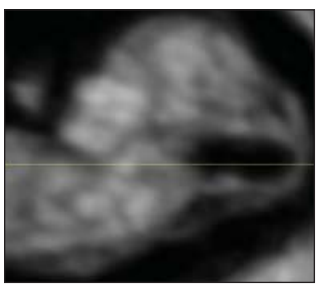


Figure 28: Posterior fossa cyst 3D rendering

First Trimester Open Spina Bifida Detection, Sonographic Signs to be Looked for

Intracranial Translucency (IT)

There are three spaces in the posterior fossa between the sphenoid and occipital bones, these are the brainstem, the fourth ventricle (defined as intracranial translucency.)and the cistern magna.

There are 4 lines in the normal posterior brain—anterior border of brainstem, posterior border of brainstem, choroid plexus, and occipital bone. (FIG 29)

Between the posterior border of brainstem and choroid plexus is the IT, behind it is the future CM

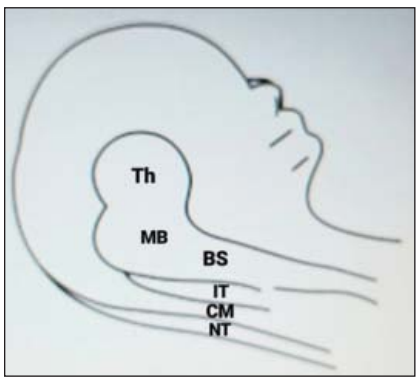


Fig 29: Diagram showing brainstem, it and cisterna magna

Table 2: IT according to CRL-

CRL	MEAN	SD
45-54mm	1.46	0.22
55-64mm	1.65	O.19
65-74mm	1.84	O.24
75-84mm	2.03	0.25
	1.7	0.27

Chiari II malformation is characterised by caudal displacement of the brainstem and compression of the fourth ventricle and cistern magna causing the obliteration /disappearance of the intracranial translucency, enlargement of the anechoic/ hypoechoic space representing the brainstem and nonvisualisation of cisterna magna. (FIG 3O)

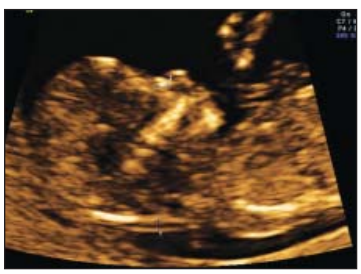


Fig 30: obliterated it and cisterna magna with posterior displacement of brainstem in open spinal defects.

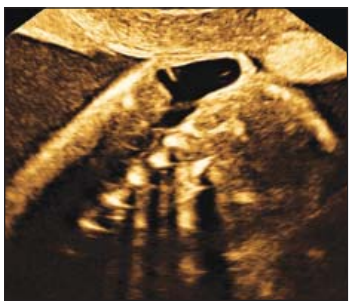


Fig 31: meningomyelocele at lower end of spine at 16 weeks of gestation. This was not identified at 12 weeks and only reduced intracranial translucency was seen at 12 weeks.

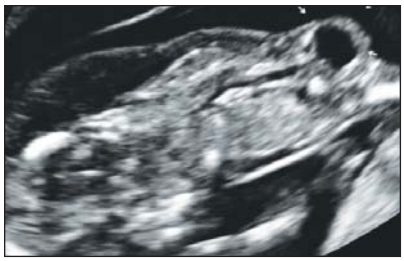


Fig 32: obliterated intracranial translucency with cystic space occupying lesion lower end spine-open spina bifida.

Signs for Open Spinal Defect

Absent /reduced IT, obliterated cistern magna (FIG 30)

Enlarged brainstem (FIG 30)

Increased brainstem /brainstem occipital bone diameter ratio

Reduced brainstem occipital bone distance

Reduced Aqueduct occipital distance

Parallel cerebral peduncles

"Crash" sign –displacement of mesencephalon posteriorly due to Chiari II malformation

Obliterated cistern magna

Dry brain- fluid in ventricle reduced so choroid plexus fills up the ventricle

Reduced BPD

Direct demonstration of the defect. (FIG 31 & 32)

TVS and 3D sagittal images are recommended

BS /BSOB Diameter Ratio On the mid sagittal plane the brainstem (BS) thickness is normally shorter compared to brainstem occipital distance (BSOB). Posterior and downward shift of the brainstem in fetuses with open spina bifida, results in thickening of the brainstem and shortening of the brainstem occipital distance. The normal ratio is < 0.9. The thickened brainstem and the reduced brain stem occipital distance due to obliteration of IT and cisterna magna in open spina bifida increases the ratio to > 1. (FIG 30, 33, 34 A, B)

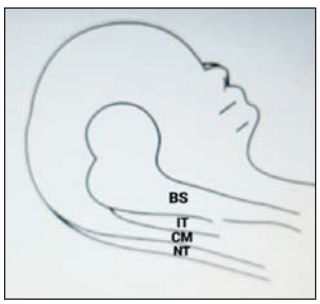
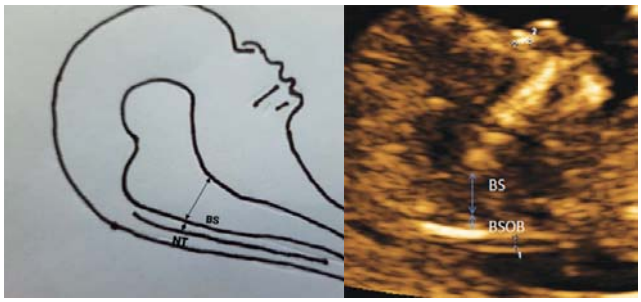


Fig 33: Normal

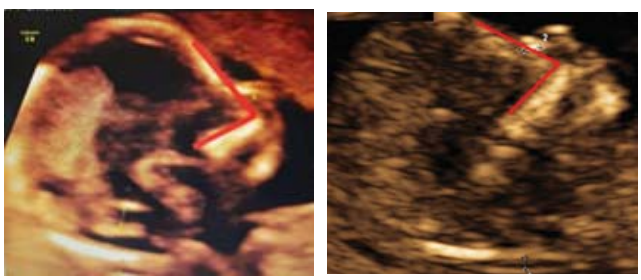


A B

Fig 34 A, B: Thickened brainstem and reduced braintem occipital distance

Newer signs have been described for detection of open spina bifida, the potential of which are yet to be evaluated

FMF ANGLE- In foetuses with open spina bifida the fronto- maxillary facial angle is decreased. (FIG 36 A, B) Normally the frontomaxillary facial angle decreases with CRL from a mean of 84 degrees at CRL of 45mm to 76.5 degrees at a CRL OF 84 mm .In the spina bifida group the mean angle corrected for CRL was 9.9 degrees lower than in the controls. Caudal displacement of the foetal brain due to Chiari malformation results in impaired development of the frontal bones.[10]



A B

Fig 36 A, B: Frontomaxillary Facial Angle A- Normal; B-Reduced Angle in Open Spina Bifida

Maxillo Occipital Line

J Ramakrishna et al have described a line drawn along the superior border of the maxilla touching the occipital bone posteriorly on the mid sagittal view of the foetal head as a marker for open spinal

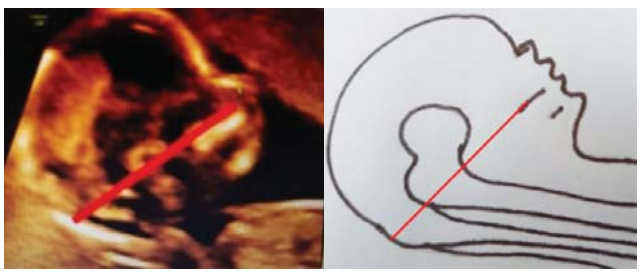


Fig 37 A & B: Normal Position of Maxillo Occipital Line.

defects based on the caudal displacement of the brainstem in open defects.[11] In open spinal defects the junction between midbrain and brainstem is below the line. (FIG 37 A, B, C, D)

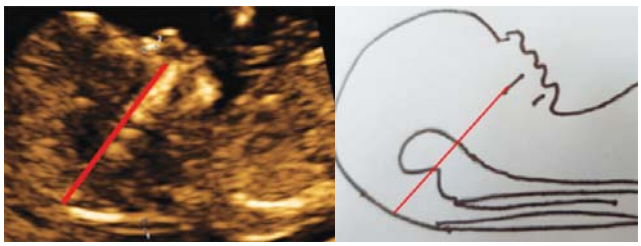


Fig 37 C & D: The junction between midbrain and brainstem is below the line in open spina bifida due to caudad displacement of the brainstem in open spina bifida.

Aqueduct of Sylvius to occipital bone distance (AOD)-juxta position of the midbrain to the occiput seen on the axial view. Distance between posterior border of aqueduct to anterior border of the occiput is measured. It is reduced in open spina bifida. (FIG 35)

Table 3: Aqueduct of Sylvius to occiput distance(mm)

CRL (mm)	Mean -2SD	Mean	Mean + 2SD
45-49	1.7	2.3	2.6
50-54	2.0	2.8	3.6
55-59	2.1	3.5	4.9
60-64	2.5	3.9	5.3
65-69	2.6	4.2	5.8
70-74	3.1	4.7	6.3
75-79	3.6	5.2	6.8
80-84	3.7	5.7	7.7

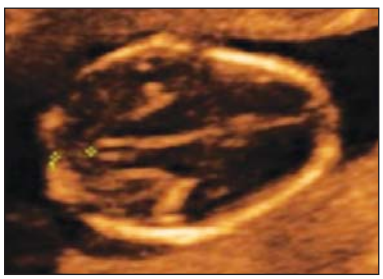


Fig 35: Aqueduct Occipital Distance

Ventriculomegaly in the First Trimester

Normally the choroid plexus should fill up the lateral ventricle, if it fills up half or less than half of the lateral ventricle it could suggest ventriculomegaly. Ventriculomegaly cannot be diagnosed in first trimester, however if it is suspected it is necessary to review, mostly it is a second or third trimester diagnosis (FIG 38)

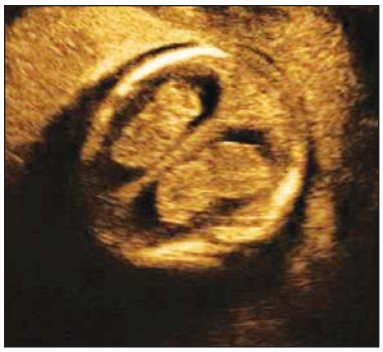


Fig 38: Normal Choroid Plexus Filling Up The Lateral Ventricles.

Face

Sagittal, coronal, axial views of the face and 3D are required. (FIG 39-44)

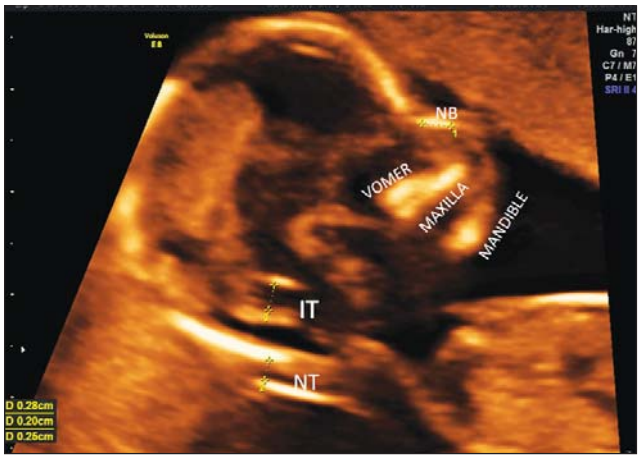


Fig 39: Profile view face

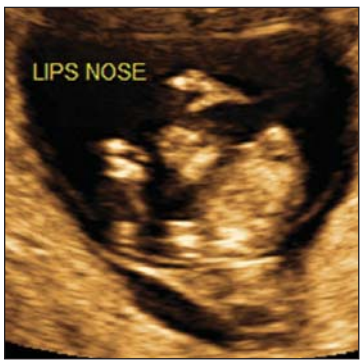


Fig 40: Lips and nose coronal view

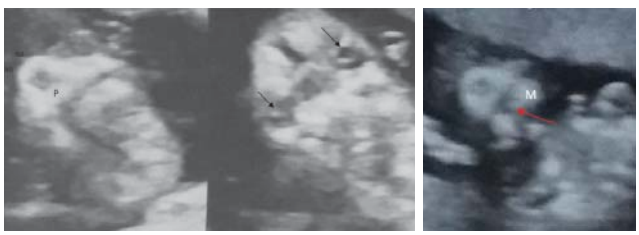


Fig 41: Normal coronal viewsa-retronasal triangle NB nasal bone, p palate b-orbits with lens, c-mandibular gap

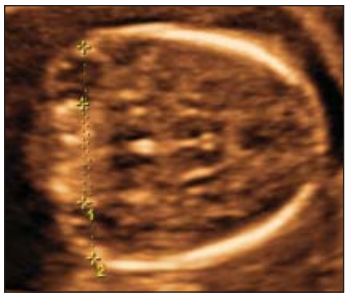


Fig 42: Hypertelorism

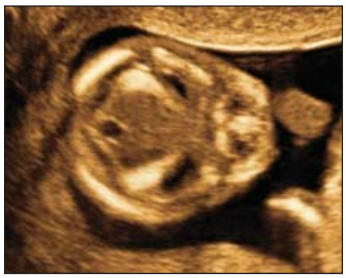


Fig 43: Hypotelorism

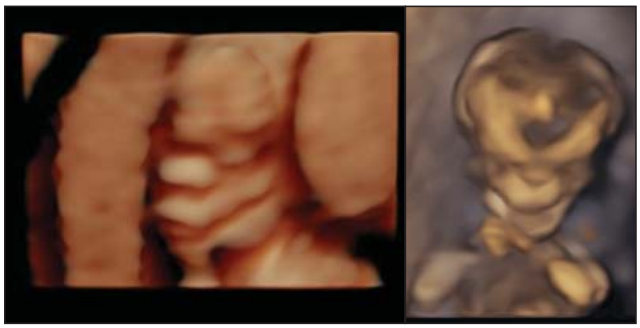


Fig 44: 3D Face lips and nose

Retrognathia/Micrognathia - A subjective finding of prominent upper lip and receding chin in the mid-sagittal view of the face. This may be due to micrognathia (short mandible) or retrognathia (backward displacement of mandible) it is difficult to assess in first trimester. Severe micrognathia may be detectable. In the normal foetus the tip of the mandible reaches under the anterior aspect of the maxilla. In retrognathia the mandibular tip does not reach the anterior aspect of maxilla (FIG 45). Association with chromosomal abnormalities mainly trisomy 18 and many genetic syndromes is found. On the coronal view the normal mandibular gap seen on the retronasal triangle view may be absent as the chin is displaced posteriorly and visualised as an echogenic structure filling the mandibular gap.

Caudal extension of a line drawn between nasal bone and palate will show the chin anterior to the line. However in retrognathia this line will be at level of chin. if suspected then it should be reviewed later .

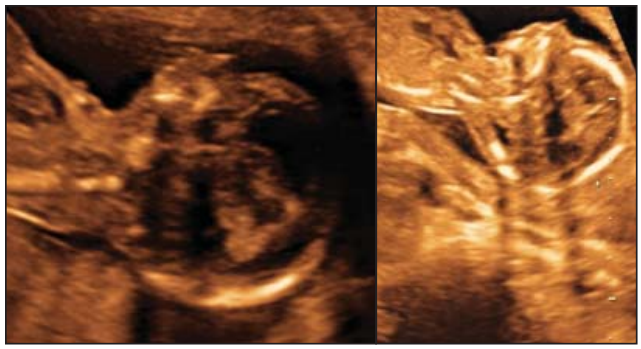


Fig 45: Retrognathia/micrognathia-receding chin

FACIAL ANGLES are available but not used routinely, may be of potential use

Frontomaxillary facial angle (FMF) objective evaluation of the maxilla with respect to forehead. A smaller maxilla gives a wider angle. In Trisomy 21 the angle measures more. A smaller maxilla gives a wider angle. In Trisomy 21 the angle was more than 85 degrees in 69 % of trisomy 21 and 5% of euploid fetuses

Mandibulomaxillary facial (MMF) angle-- objective method of evaluating the location of the mandible with respect to maxilla, for early diagnosis of micrognathia and retrognathia. (FIG 46) **(**normally measures 114.5 at 45mm CRL and 103.1 degrees at CRL of 84 mm.)

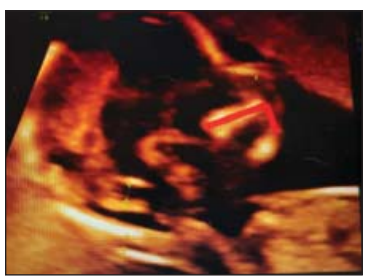


Fig 46: Mandibulo Maxillary Facial Angle

The normal FMF/MMF ratio remains constant at 0.74. In Trisomy 18 the mean FMF angle to MMF angle ratio was 0.86. The FMF angle increased and MMF angle was smaller in trisomy 18.

Cleft Lip and Palate–

Cleft lip may be unilateral, bilateral or median. (FIG 47, 48)

Isolated cleft palate is very difficult to diagnose in first trimester and may be seen when associated with cleft lip and can be attempted at best in late second trimester Approximately 30 % of oral clefts are syndromic.

Cleft palate is seen as a gap in the alveolar ridge on axial view. (FIG 49)

A gap in the maxilla on the mid sagital plane and in the retronasal triangle view known as the maxillary gap is a hint for cleft palate.

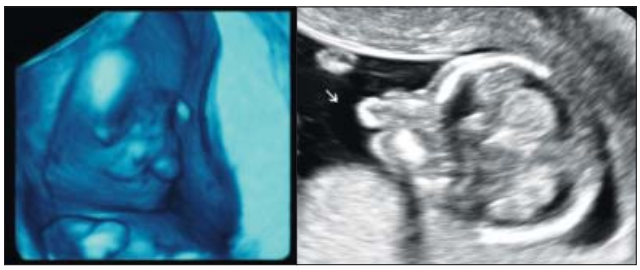


Fig 47: Bilateral cleft lip with median protuberance

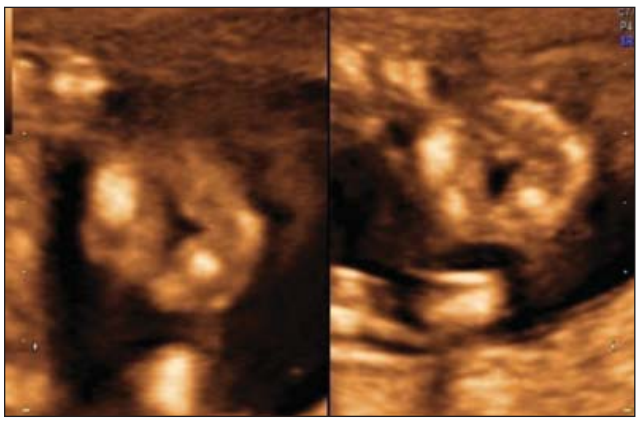


Fig 48: unilateral cleft lip

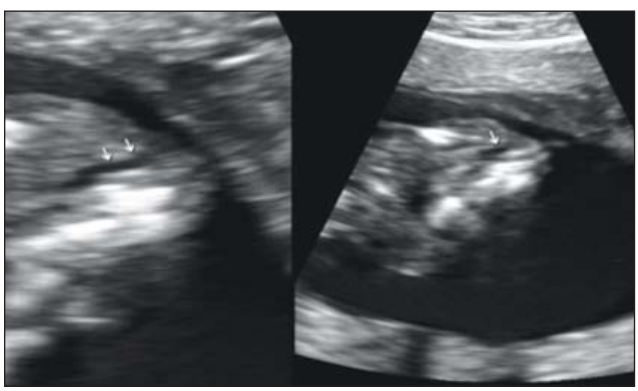


Fig 49: Cleft palate involving alveolar ridge and secondary palate

Lakshmy et al have described the superimposed line sign for early diagnosis of cleft palate using 2D imaging ,in the mid sagittal view normally the vomer appears as a superimposed line merging with the secondary palate. (FIG 39) In midline cleft of the secondary palate the line formed by the palate is absent and only the vomer is visualised creating a single line instead of the normal superimposed double line.[12]

Frontal space distance not used routinely, but may be of potential use in cleft lip, palate and retrognathia. It is the distance between the forehead and an extended line joining the most anterior portions of mandible and maxilla.(FIG 50)[13]

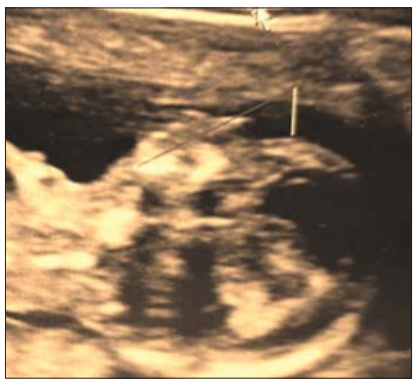


Fig 5O: Frontal Space Distance – Increased Distance in Cleft Palate and Retrognathia

Ears

A new sign which may have potential is the appearance and position of foetal ears. Low set and small malrotated ears may be a clue for Treacher Collins syndrome and other syndromes with facial dysmorphology.

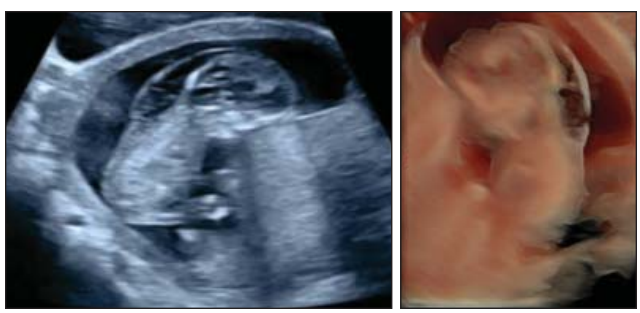
Neck

Iniencephaly

Neck not seen, persistent retroflexion of head, short lordotic spine.

Cystic Hygroma

Bilateral cystic structures of the posterior neck separated by the nuchal ligament. It can progress towards hydrops, resolve or persist in part as nuchal oedema. Association with other malformations in 60 % cases. About 35-50 % have an abnormal karyotype. The most common nonchromosomal syndrome is Noonan syndrome. (FIG 51, 52)



A B

Fig 51 A & B: Cystic Hygroma, 3D Image

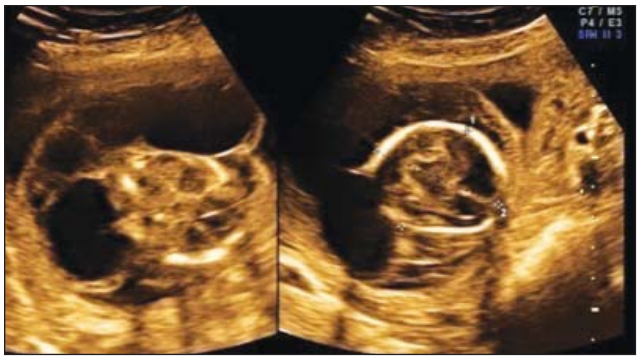


Fig 52: Cystic Hygroma - Axial

Thorax

Cardiac Defects

Foetal situs can be ascertained, heart and stomach on same side. (FIG 53)

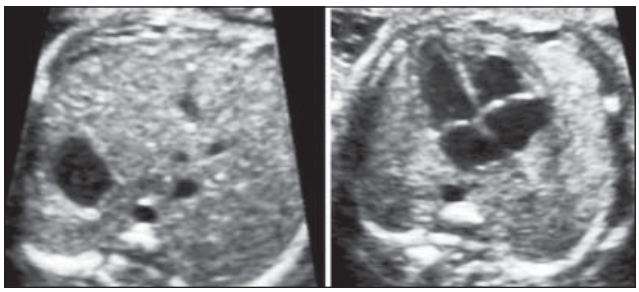


Fig 53: Situs Both Stomach and Heart on Same Side

A practical way of determining situs is-

If foetus with cephalic presentation then the spine, stomach and portal vein in this sequence have a clockwise arrangement. (FIG 54)

Foetus in breech presentation spine, stomach and portal vein in this sequence are in anticlockwise arrangement. (FIG 55 A & B)

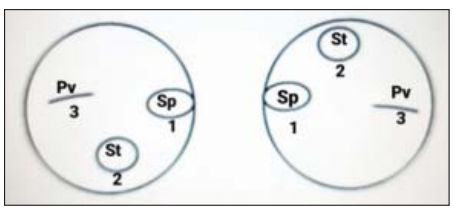


Fig 54: Cephalic presentation clockwise arrangement of fetal spine, stomach and portal vein

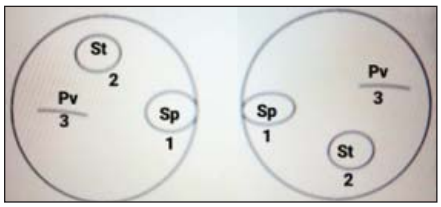


Fig 55 A: Breech presentation anticlockwise arrangement of fetal spine, stomach and portal vein

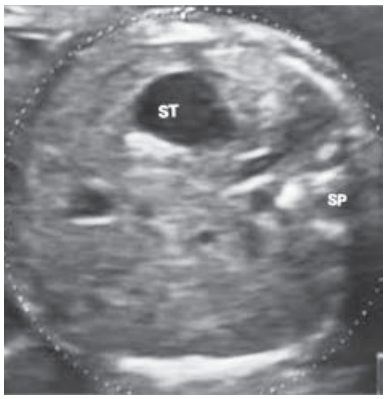


Fig 55 B: Breech presentation anticlockwise arrangement of fetal spine, stomach and portal vein

Four chamber view-two atrioventricular connections on 2D and with colour flow showing equal filling of ventricles and forward flow. Colour flow enhances the detection of anomalies especially in first trimester. (FIG 56 & 57)

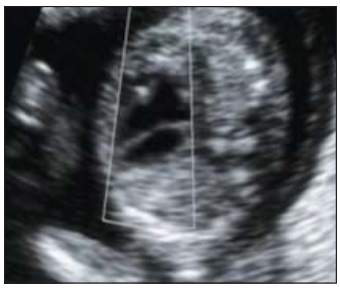


Fig 56: 4 Chamber View

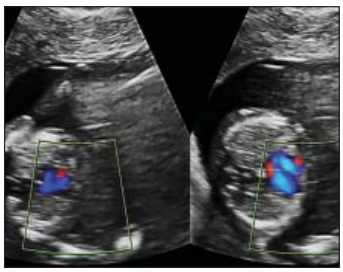


Fig 57: Crossover view showing outflow tracts and 4cv showing inflow into the ventricles-on color flow

Three vessel view –posterior v sign of ductal and aortic arches, demonstrating equal size and forward flow in outflow tracts towards left of spine. (FIG 57 & FIG 58)

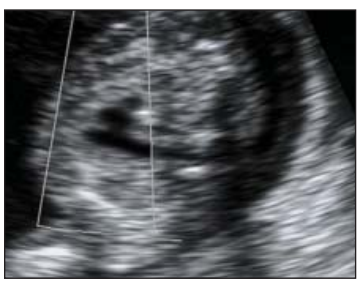


Fig 58: Three Vessel View-Pulmonary Artery, Aorta and SVC

Findings suggestive of foetal cardiac anomalies-

- Cardiac axis deviation. It performs better than NT, TR and reversed a wave in detecting major foetal cardiac defects.

  Left axis deviation is associated with conotruncal anomalies.[14]
- Increased NT –Associated with major cardiac anomalies, almost 10 times higher, an early foetal echo at 16 weeks is proposed for NT more than 99th centile(3.5mm)
- Cardiomegaly-- CT diameter ratio >0.55.
- Chamber disproportion
- Abnormal DV-Reversed a wave in the DV.
- Tricuspid regurgitation(FIG 62)
- Aberrant right subclavian artery.
- Tachycardia

Cardiac defects that should be detected-Hypoplastic left heart (FIG 59)

Tricuspid atresia

Pulmonary atresia

Transposition of great arteries

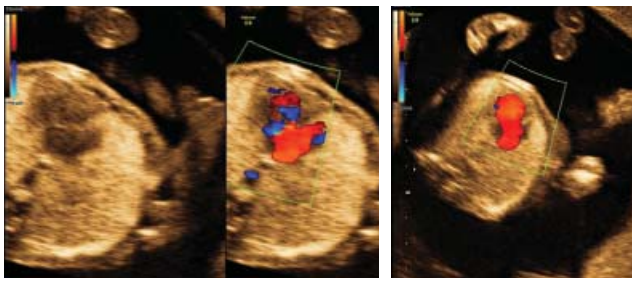


Fig 59: Hypoplastic Left Heart-- Small Left Atrium And Left Ventricle, No Color Flow Across Mitral Valve

Cardiac defects that might be detected

- Double outlet right ventricle
- Tetrology of Fallot
- Truncus arteriosus
- Atrioventricular septal defects (AVSD) (FIG 60)

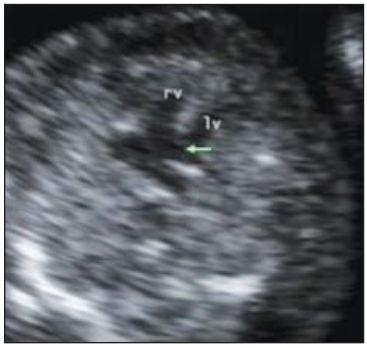


Fig 60: AVSD

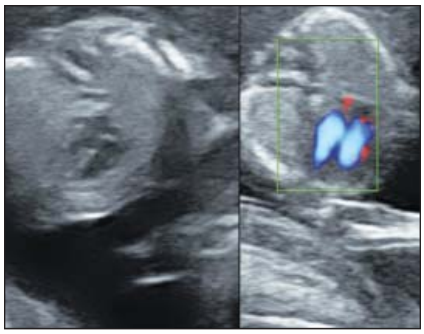


Fig 61 A: Truncus Arteriosus - 4CV Normal

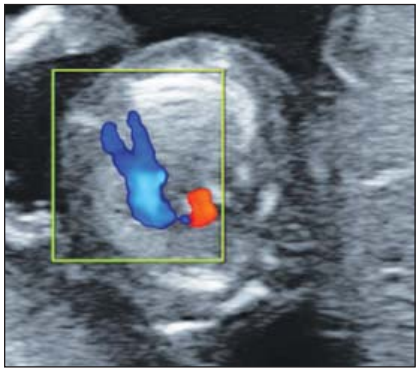


Fig 61 B: Outflow Tact View Showing Single Outflow

Cardiac defects unlikely to be detected—

Ventricular septal defect

Ebsteins anomaly

Aortic and pulmonary stenosis

Cardiac tumours

Myocardial hypertrophy, cardiomyopathies

Abnormal pulmonary venous return

Complete heart block

Absent pulmonary valve

Coarctation aorta (FIG 61),

Aortic arch and branching anomalies

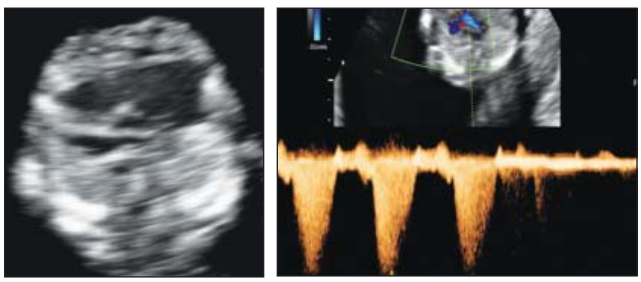


Fig 62: Dilated Right Atrium with TR

Lungs

Congenital Pulmonary Airway Malformation

Usually not a first trimester diagnosis, however the cystic variant with larger cysts may be visualised. (FIG 63)

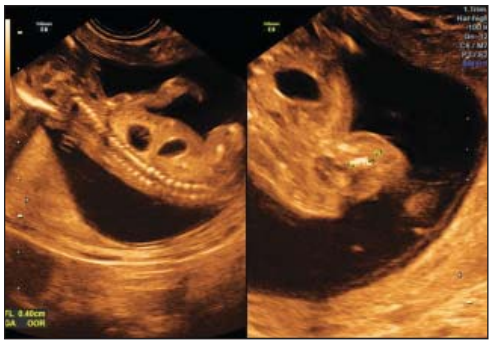


Fig 63: Congenital Pulmonary Airway Malformation, Cystic Variant

Congenital Diaphragmatic Hernia

May present with abnormal cardiac axis and mediastinal shift. Herniation of stomach and intraabdominal contents into the chest confirms the diagnosis. The herniation of intraabdominal contents into the chest can be delayed to the second trimester or beyond, it may not manifest till third trimester. A normal ultrasound examination of the chest in the first trimester does not rule out the presence of a CDH. (FIG 64)

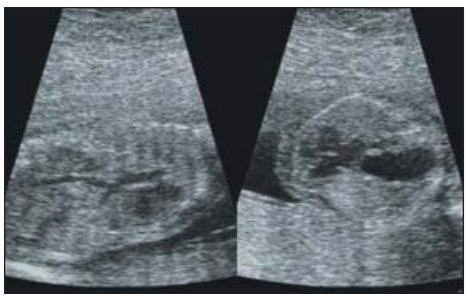
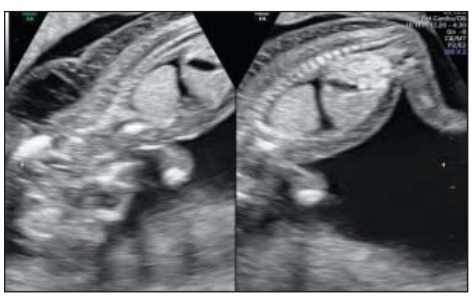


Fig 64: Congenital Diaphragmatic Hernia -Stomach in Chest Adjacent to Heart

Hydrothorax/Pleural Effusion

May be seen in cardiovascular malformations, chromosomal abnormalities including Trisomy 21 and Turners syndrome, infections or as a part of hydrops. Bilateral hydrothorax has a poor prognosis. Isolated unilateral hydrothorax could be a transient finding. (FIG 65 & 66)



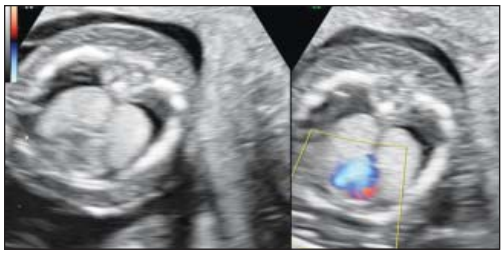
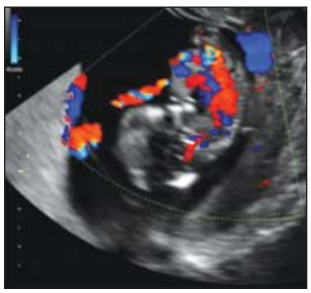


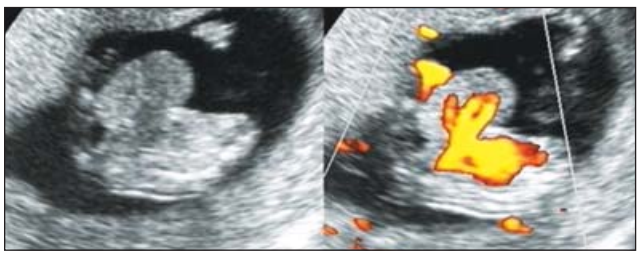
Fig 65 & 66: Hydrothorax

Abdomen

OMPHALOCELE- Midline abdominal wall defect with herniation of bowel loops and sometimes liver. The cord insertion is on the convexity of the mass, contents are wrapped in a sac . Differentiation from a physiologic mid gut herniation is important which occurs at 10 weeks, by 12weeks the bowel moves into the abdomen. To differentiate physiologic midgut herniation from omphalocele a gestational age more than 12 weeks, size of the mass is more than 7 mm, and when it contains liver or stomach are helpful. High association with chromosomal anomalies. (FIG 67 A, B & C)



A



B

Fig 67 A, B: Omphalocele Containing Small Bowel and Liver

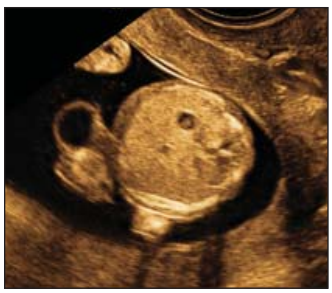


Fig 67 C: Omphalocele

GASTROSCHISIS– Right paraumbilical defect of the abdominal wall through which bowel loops herniate to float freely in the amniotic fluid. There is no membrane wrapping the herniated viscera. The cord inserts normally. May be associated with other major defects.(FIG 68)

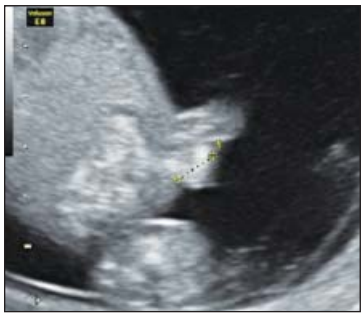


Fig 68: Gastroschisis

Pentalogy of Cantrell- Rare defect. It consists of an anterior abdominal wall defect, diaphragmatic defect, cleft sternum, cardiac defect, communication between pericardium and omphalocele.

Limb Body Wall Complex

Foetus is distorted and adherent to placenta, a large abdominal wall defect with herniation of liver and bowel, severe scoliosis and a short umbilical cord. Diagnosis is based on -cephalocele/exencephaly, facial clefts, abdominal wall defect/ thoracoschisis, limb defect. Some organs are located in the coelomic cavity. (FIG 69)

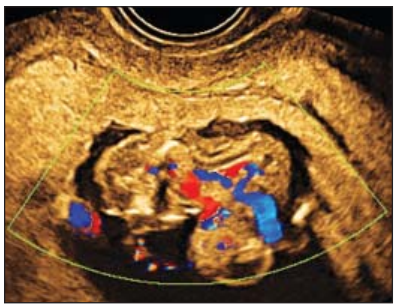


Fig 69: Limb body wall complex- abnormal curvature and shortening of spine, abdominal wall defect, short cord, adherent to placenta

kidneys and Urinary Bladder

Kidneys are assessed on sagittal and coronal views. (fig 70)

Foetal urine production begins at 10 weeks of gestation, when the bladder is seen as an anechoic structure in the pelvis surrounded by two umbilical arteries. (FIG 71)

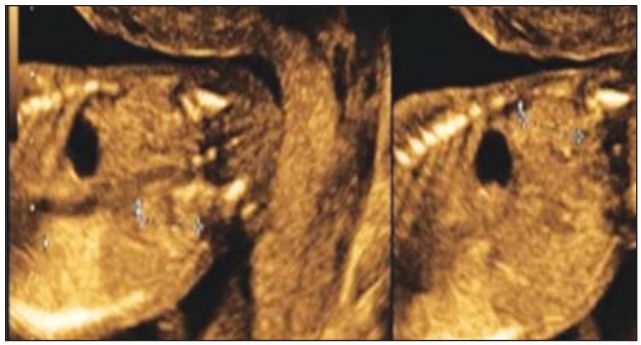


Fig 70: Normal Kidneys

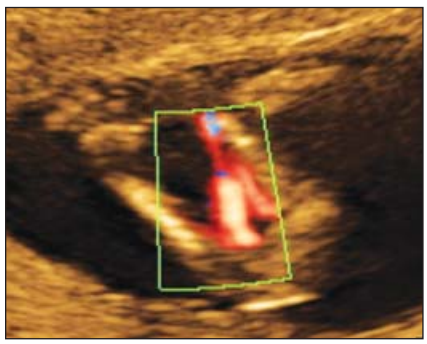


Fig 71: Urinary Bladder With Umbilical Arteries

Bilateral Renal Agenesis - difficult to diagnose early in gestation, if kidneys are not visualised even on transvaginal scan, bilateral renal agenesis should be suspected and absence of both renal arteries looked for, in case of doubt follow up at 16 weeks. Amniotic fluid in the first trimester remains normal.

Megacystis

Longitudinal bladder length≥ 7 mm. (fig 72 &73)

The main cause is bladder outlet obstruction. Differential diagnosis includes chromosomal abnormalities, developmental anomalies and genetic syndromes

Longitudinal bladder length of 7-15 mm-23 % associated with aneuploidies, mainly Trisomy 13 and 18. CVS needs to be done. If no chromosomal abnormality detected then in 90% there is spontaneous resolution by 20 weeks and in 10% uropathy.

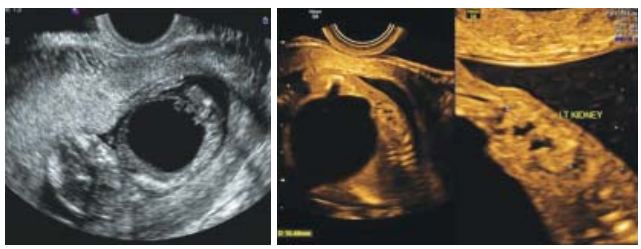


Fig 72: Posterior urethral valve-large bladder with keyhole sign and early renal pelvic dilation.

If length>15 mm there is high likelihood of progressive obstructive uropathy. Spontaneous resolution is unlikely. The risk of chromosomal defects is 10%.

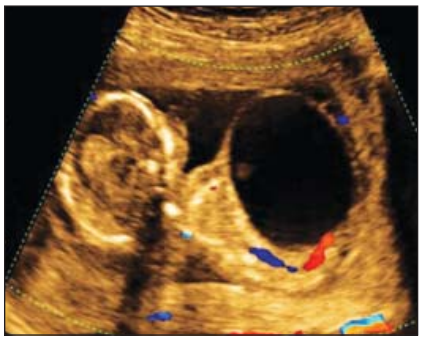


Fig 73: Megacystis

Fontanella et al have also found an association with OEIS complex, VACTERL &fetal overgrowth syndromes like Beckwith Weidman syndrome.[15]

Fontanella et al in their recent study(2019) have mentioned that a cut off of longitudinal bladder diameter of 12mm rather than 15 mm predicts the chance of spontaneous resolution. They found that the presence of an umbilical cord cyst with megacystis is a strong marker for urethral atresia,and a thickened NT rather than the bladder diameter increased the risk of complex megacystis. A normal NT, an longitudinal bladder diameter >12 mm and absence of umbilical cord cysts are best criteria to guide counselling and select isolated posterior urethral valve. They also saw that trisomy 18 and 21 show a more severe degree of bladder distension and suggested that karyotyping should be offered more liberally and the focus should be on the NT.[16]

Bladder Exstrophy

Absence or non-visualisation of the urinary bladder on routine examination, the first step should be to reassess later in the sonogram as a temporarily absent bladder is a normal variant and should be visualised if given time to fill. A bladder should fill within a 30 min time frame. If still not visualised, clues include solid bulging mass in lower abdominal wall with the umbilical arteries running alongside it, low set umbilical cord with normal amniotic fluid volume and kidneys. Splaying of iliac bones. It could be part of the OEIS complex (omphalocele, exstrophy of bladder, imperforate anus and spinal defects), a spectrum of cloacal exstrophy. Omphalocele is absent in isolated bladder exstrophy. Cloacal exstrophy can present with an omphalocele and cystic pelvic mass.

Single Umbilical Artery- Suggest an early follow up ultrasound with foetal echo at 16 weeks as it is associated with cardiac anomalies and IUGR. When in isolation it does not increase the risk of chromosome aberrations. It should initiate a careful search for additional anomalies. (FIG 74)

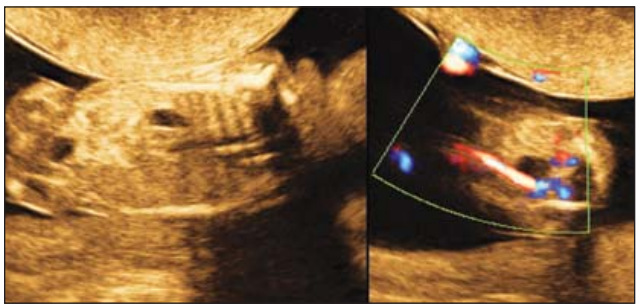


Fig 74: Single Umbilical Artery

Skeletal Dysplasia

With increased NT and a normal karyotype, the prevalence of skeletal dysplasia is increased. A short spine, lack of ossification of the spine, scoliosis, narrow thorax, short ribs ,polydactyly ,short femur, limb contractures are some of the clues seen in first trimester.(fig 79)

Sirenomelia- single lower extremity which can show a wide spectrum depending upon degree of fusion of bony and soft tissue structures. Usually a single umbilical artery is seen with an aberrant origin from the abdominal aorta high up in the abdomen. (FIG 75 & 76)

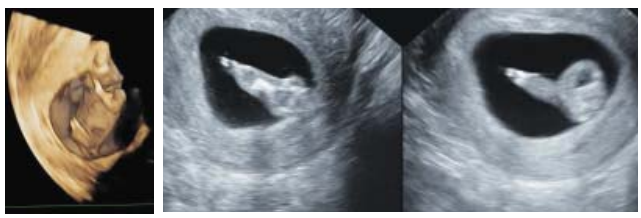


Fig 75 and 76: Sirenomelia Single Small Deformed Lower Limb

Limb Reduction Defects- transverse reduction defects and radial aplasia are potentially detectable, details of hands and feet are not always feasible. (FIG 77 & 78)

It is difficult to differentiate pathologic talipes from positional talipes in the first trimester.

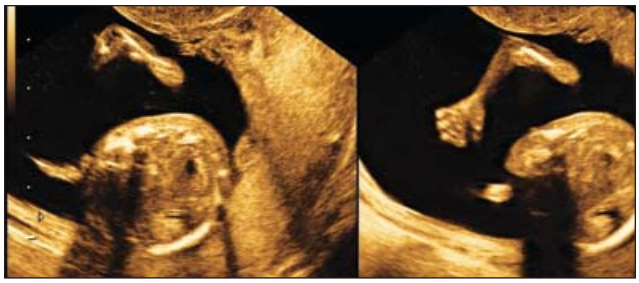


Fig 77: Absent Forearm Bones

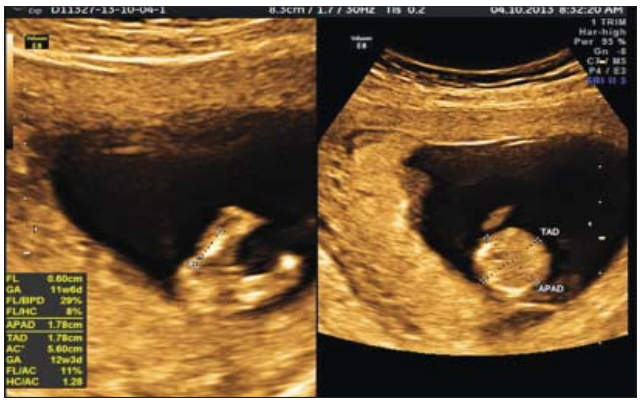


Fig 78: Unilateral Absence of Femur

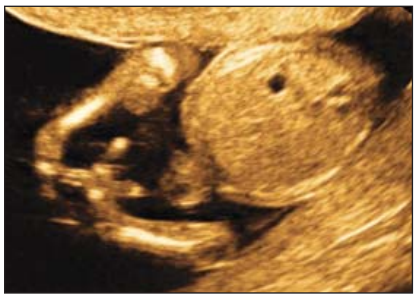


Fig 79: Contractures Both Wrists, Bilateral Clubbed Hands

A sign with future potential has been described in Thanatophoric dysplasia is abnormal gyration of the temporal lobes.

Placenta

The position of the placenta in relation to the internal os should be assessed keeping in mind that, most placenta praevias in the first trimester will resolve in the follow up, however reporting this is of significance so that patient may avoid travel.

The presence and size of any sub chorionic bleeds should also be mentioned

A low anterior implantation of the placenta close to or within the scar of previous LSCS is an early ultrasound sign suggestive of abnormally adherent placenta. Multiple intraplacental lacunae, reduced thickness of retroplacental myometrium, abnormal bladder serosal interface may also be visualised. Best evaluated on a transvaginal scan with a slightly full bladder.

Role of Cell Free DNA (cf DNA) in First Trimester Screening

It can detect more than 99% of fetuses with trisomy 21 and 98 % with trisomy 18 or 13 with a false positive rate of 0.1-0.2%. All abnormal NIPT results require confirmatory studies with invasive testing.

FHR— In euploid fetuses the heart rate is about 170 bpm at 10 weeks decreasing to 150 bpm at 14 weeks. It is mildly increased in Trisomy 21 & mildly decreased in trisomy 18. In Trisomy 13 the FHR is substantially increased and is above the 95th centile in 85 % cases.

Screening for Pre-Eclampsia

Combining maternal characteristics, uterine artery Doppler, mean arterial pressure and biochemistry (PAPPa, PLGF) to estimate the individual post test probability of developing pre-eclampsia.

Mean uterine artery PI is used

PSV greater than 60 cm/sec to ensure arcuate artery is not being sampled.

The detection rate of screening for pre-eclampsia by maternal factors and PlGF at 10% false positive rate was 100% for pre-eclampsia <32 weeks, 75% for pre-eclampsia less than 37 weeks and 43 % for preeclampsia ≥37 weeks.[17]

Studies suggest that administration of low dose aspirin (150 mg) every night, started at ≤ 16 weeks until 36 weeks of gestation in the high-risk group reduces the incidence of preeclampsia.

ASPRE (combined multimarker screening and randomised patient treatment with aspirin for evidence-based preeclampsia prevention) trial found aspirin to reduce the incidence of pre-eclampsia before 37 weeks by 62 %. The beneficial effects of aspirin may not apply in pregnancies with chronic hypertension.[18]

Low dose aspirin is effective in the prevention of preterm rather than term pre-eclampsia.

Screening in Twin Pregnancies

Screening for chromosomal abnormalities is provided by a combination of fetal NT and

first trimester biochemical screening in twin pregnancies.[19]

Dichorionic twins - risk for each foetus is calculated. Individual measurements of NT for each foetus give a foetus specific risk, because of distinct karyotype of each foetus.

Monochorionic twins- risk for the pregnancy is calculated. The false positive of NT screening is higher because increased NT in one of the twins is an early manifestation of twin to twin transfusion as well as a marker for chromosomal abnormalities. For screening for trisomy 21 Vandecruys et al have advocated the use of the average NT measured in the two fetuses.[20]

Cf DNA in twins– Data is limited and additional studies are required however it seems promising. Monni et al[21] have studied that in monozygotic twins NIPT is as efficient as in singleton pregnancy. In dizygotic twins, the two fetuses which are not identical may be discordant for aneuploidy and the test may be inaccurate if the amount of foetal DNA produced by the affected foetus is low.

Vanishing twin – NT alone in combination with maternal age should be used and not serum biochemistry as serum beta HCG AND PAPPa values are biased.

Twin reverse arterial perfusion (TRAP)-A rare condition of monochorionic twins with embryonic demise in one twin. One twin, the pump twin appears structurally normal, the other twin, the acardiac twin is usually partially formed (commonly with missing head and upper thorax and no heart) with reversed flow in umbilical artery. (FIG 80)

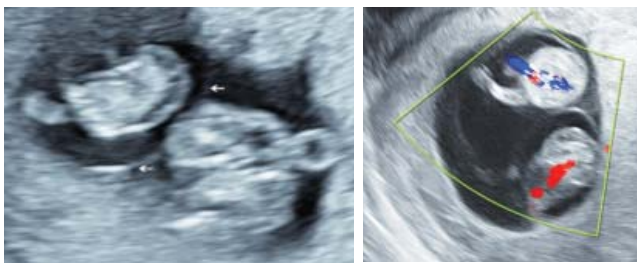


Fig 80: Trap sequence in monochorionic twins. One twin is structurally normal, the other has edema and no cardiac activity with reversed flow in umbilical artery that is towards the foetus.

Practical Tips

Agenesis of corpus callosum cannot be diagnosed in first trimester.

Isolated cleft palate and ventriculomegaly if suspected need to be confirmed on follow up.

A cystic posterior fossa needs to be reviewed at 16 weeks as it could be a transient finding.

Flattening of the nasal bridge when isolated is to be followed up

Renal pelvic dilatation without lower urinary tract obstruction can resolve spontaneously or could be a marker for aneuploidy in second trimester and should be re- evaluated at the time of level 2 scan.

Conclusion

The 11-13+6 weeks scan is the time for assessment of NT and when carried out according to a standardised protocol can identify many abnormalities. It also helps us categorise those patients who need an earlier follow up scan.

References

1. Internet course the 11-13-week scan. Available from www.fetalmedicine.com
2. Souka AP, Von Kaisenberg CS, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005; 192 issue 4,1005-1021
3. Kypros H. Nicolaides. Screening for foetal aneuploidies at 11-13 weeks. Prenat Diagn 2011; 31:5-7
4. Kyra E Stuurman, Marike Joosten, Ineke van der Burgt et al Prenatal ultrasound findings of rasopathies in a cohort of 424 fetuses: update on genetic testing in the NGS era. Journal of Medical Genetics Volume 56, Issue 10 2019; 654-661.
5. F. Prefumo, Shanthi Sairam et al Maternal ethnic origin and foetal nasal bones at 11-14 weeks of gestation. BJOG 2004 volume111, issue 2.
6. S. Cicero, KH Nicolaides et al Likelihood ratio for trisomy 21 in fetuses with absent nasal bone at the 11-14 week scan. Ultrasound Obstet Gynecol 2004; 23, issue3.
7. S Faiola, K H Nicolaides et al Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13 +6 –week scan. Ultrasound Obstet Gynecol 2005; 26, issue1
8. A. Syngelaki Hammmi, S Bower, R Akolekar, KH Nicolaides. Diagnosis of foetal nonchromosomal abnormalities on routine ultrasound examination at 11-13 weeksUltrasound in obstetrics and gynaecology/volume 54, issue 4.13. August 2019
9. D. Paladini, R. Chaoui et al Differentiating features of posterior fossa at 12-13weeks gestation in fetuses with Dandy –Walker malformation and Blake's pouch cyst. Ultrasound in Obstetrics & Gynecologyvolume 53, issue 6.
10. R Lachmann, Picciarelli, J Moratella et al Frontomaxillary facial angle in foetuses with spina bifida at 11-13 weeks' gestation Ultrasound Obstet Gynecol

2010;36,issue3:268-271.

11. Jayshree Ramakrishna, Simon Meagher et al Maxillo-occipital Line: A Sonographic Marker of Open Spina Bifida in the First Trimester of Pregnancy. J Maternal Fetal& Neonatal Medicine. Volume 32,2019-issue 24
12. SR Lakshmy, NityRose, Praveenkumar Masilamani," Absent superimposed line sign": a novel marker in early diagnosis of secondary cleft palate, Ultrasound in obstetrics and gynecologydoi:10.1002/uog21931.
13. M Hoopmann, K O Kagan et al Frontal space distance in facial clefts and retrognathia at 11-13 weeks gestation. Ultrasound in Obstetrics & Gynecology 2015/Volume 48, Issue 2.
14. Edgar Hernandez-Andrade, Manasi Patwardhan, Monica Cruz-Lemini et al Early Evaluation of the Foetal Heart Fetal Diagn Ther 2017;42:161-173
15. F.Fontanella, L.Maggio et al Fetal megacystis a lot more than LUTO Ultrasound in Obstetrics & Gynecology/Volume 53, Issue 6.
16. F Fontanella, L. Duin et al Antenatal Workup of Early Megacystis and Selection of Candidates for Fetal Therapy Fetal Diagn Ther 2019;45:155-161
17. N.Gorman, DWright, Poon LC et al. Accuracy of competing risks model in screening for pre eclampsia by maternal factors and biomarkers at 11-13 weeks gestation. Ultrasound Obstet Gynecol 2017 volume 49, issue6
18. DL Rolnik,N Gorman et al Early screening and prevention of preterm preeclampsia with aspirin; time for clinical implementation Ultrasound Obstet Gynecol 2017 volume 50, issue 5
19. Whitney Bender, Lorraine Dugoff. Screening for aneuploidy in multiple gestations. The Challenges and options. Obstet Gynecol Clin N Am 45 (2018)41-53
20. Vandecruys H, Faiola S et al. Screening for trisomy 21 in monochorionic twins by measurement of fetal nuchal translucency thickness Ultrasound Obstet Gynecol 2005:25:551-3
21. G Monni, A Lucolano et al Screening and Invasive Testing in Twins J Clin Med 2014Sep;3(3) 865-882

Cervical Length: Predicting Preterm Birth and Beyond

Ashok Khurana[1], Karthik Senthilvel[2]
[1]The Ultrasound Lab, New Delhi, India, [2]MS Hospital, Trichy, Tamilnadu, India

Globally, preterm birth (PTB) is the single largest cause of neonatal deaths. In India, among the total 2.7 crore babies born annually, 35 lakh are born preterm. Over 3 lakh of these die each year because of complications associated with PTB, the leading cause of death in children less than 5 years old.

India contributes 25% of the overall global preterm related deaths. In more than 10 lakh babies the adverse effects of PTB extend beyond early infancy with substantial long-term adverse consequences in late childhood and adult life.

India has the highest number of preterm births in the world; 3.5 times more than China, which is second on the list.

All births before 37 weeks of gestation are defined as preterm.

The overall prevalence of spontaneous delivery before 34 weeks is about 1%. In one-third of preterm births the delivery is carried out for medical indications, mainly preeclampsia and fetal growth restriction, and in two-thirds it is spontaneous preterm birth (sPTB), because of premature onset of labor or preterm pre-labor rupture of membranes.

Currently, as a prognostic timeline, preterm birth is defined as:

a. Extreme (<28 weeks), which occurs in about 0.25% of pregnancies,
b. Early (28-30 weeks), which occurs in about 0.25% of pregnancies,
c. Moderate (31-33 weeks), which occurs in about 0.6% of pregnancies, and,
d. Mild (34-36 weeks), which occurs in about 3.0% of pregnancies.

The risk of fetal death and handicap is mainly increased in the extreme, early and moderate subgroups.

In 2015, a WHO Technical Consultation publication[1] led to the adoption of 10 main recommendations and 17 additional sub-recommendations. These cover antenatal corticosteroids, tocolysis, magnesium sulfate, antibiotic prophylaxis, mode of preterm birth (for the mother) and Kangaroo mother care, plastic wraps, continuous positive airway pressure therapy, surfactant and oxygen therapy (for the newborn). Adoption of these recommendations has undoubtedly improved outcomes. However, the most brilliant impact on preterm birth is elucidated in an editorial by Stuart Campbell[2] in the American Journal of Obstetrics and Gynecology 2018 entitled

What is described in this article is a perfect marriage of technology and pharmacy:

a. An effective screening test which is a simple ultrasound measurement of the cervix in the midtrimester (a biomarker)
b. piggybacked onto an already existing screening test (the anomalies scan at 18-22 weeks) and,
c. Treatment with vaginal progesterone for women at risk, which is safe, efficacious, and cost effective.

Understandably, this strategy was expected to make significant inroads into the greatest problem for mothers and babies worldwide and it has not failed its promise.

Several guidelines are available for the management of preterm labor and delivery and these are excellent references for clinicians. These are evidence based guidelines from the United States (ACOG[3]), the United Kingdom (RCOG/NICE[4]) and Canada (SOGC[5]). The largest evidence base, however, comes from the Fetal Medicine Foundation (FMF) and is summarized in an online course[6]. This review draws extensively from the publications that led to the formulation of the content of this course.

Background for Prediction

There are essentially two groups of pregnant women contributing to spontaneous deliveries before 34 weeks.

15% of such deliveries come from the group of women who had a previous late miscarriage or

spontaneous PTB. This group constitutes about 3% of the pregnant population. In these women the risk of recurrence is inversely related to the gestational age at the previous PTB.

85% of such deliveries come from the 97% of women who are either in their first pregnancy or their previous pregnancies resulted in deliveries at term. Consequently, any strategy at reducing the rate of PTB that is focused on the subgroup of women with a previous PTB would have a very small impact on the overall rate of PTB.

These statistics underline the significance of the need for universal screening of pregnancies for preterm birth and the consequent treatment of those at high risk with vaginal progesterone to prevent this catastrophe.

Fetal Fibronectin

Early efforts in predicting preterm birth focused on fetal fibronectin that is an extracellular matrix glycoprotein produced by amniocytes and by cytotrophoblast. It is localized between chorion and decidua where it acts as 'glue' between the pregnancy and the uterus. Fetal fibronectin can be detected in cervicovaginal secretions in all pregnancies. Levels are high before 22 weeks but low (less than 50 ng/mL) at 22-34 weeks. Measurement of fetal fibronectin at 22-24 weeks is useful in predicting pregnancies at increased risk of spontaneous preterm birth. At 22-24 weeks the test is positive in about 5% of the population and this group contains 25% of pregnancies with spontaneous preterm birth (sPTB) at < 34 weeks. It is not, therefore, a very sensitive screening test.

Cervical Length: Diagnostic Considerations

The risk of spontaneous preterm birth (sPTB) is increased in women with a history of previous sPTB and the risk is related to the gestational age at which the PTB occurred. Additionally, the risk of PTB is inversely related to cervical length at 20-24 weeks of gestation in the current pregnancy.

Cervical length at 20-24 weeks in pregnancies that deliver at term is normally distributed with a mean of 34 mm.

In pregnancies with sPTB at < 34 weeks there is a bimodal distribution in cervical length. Cervical length is < 15 mm in 1% of the population and this group contains 20% of cases of sPTB at < 34 weeks. Cervical length is < 25 mm in 10% of the population and this group contains 40% of cases of sPTB at < 34 weeks.

A model combining cervical length and obstetric history provides a better prediction of spontaneous preterm birth than either factor alone and the sensitivity of screening improves for increasing degrees of prematurity. A free of cost calculator is available for this calculation at the FMF website, www.fetalmedicine.org.

For a screen positive rate of 10%, the detection rate is about 80% for birth at < 28 weeks of gestation, 60% for birth at 28-30 weeks and 50% for birth at 31-33 weeks.

Ultrasound measurement of cervical length is clinically useful in the prediction of PTB in the following situations:

a. In asymptomatic women with previous history of PTB and in those with uterine abnormalities, such as unicornuate uterus, the cervical length should be measured every two weeks at between 14 and 24 weeks of gestation
b. In asymptomatic women with no previous history of PTB, measurement of cervical length should be carried out routinely at the time of the second trimester scan at 18-24 weeks.

The technique is simple. The urinary bladder should be emptied. The patient is then put in a dorsal lithotomy position. Strapping the legs into stirrups is neither necessary nor recommended. The vaginal transducer is introduced after routine covering with a condom and a blob of standard ultrasound gel. The transducer is then directed towards the anterior fornix. Attention is paid to avoid exerting undue pressure on the cervix because this may artificially increase the length.

A sagittal view of the cervix is obtained and the endocervical mucosa is used as a guide to the true position of the internal os, thereby avoiding confusion with the lower segment of the uterus (Fig. 1). The endocervical mucosa may be of increased (Fig. 2) or reduced echogenicity (Fig. 3) compared to the cervix.

Each examination should be performed over a period of 2-3 minutes. In about 1% of cases the cervical

length may change due to uterine contractions and in such cases the shortest measurement should be recorded.

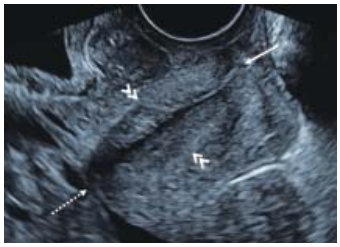


Fig 1: Transvaginal Sonoanatomy of the cervix: solid arrow: external os, dashed arrow: internal os, arrowheads: endocervical mucosa.

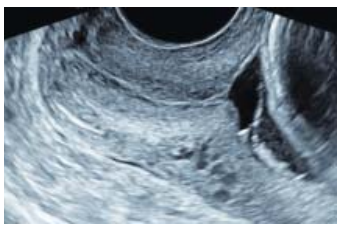


Fig 2: Echogenic cervical canal.

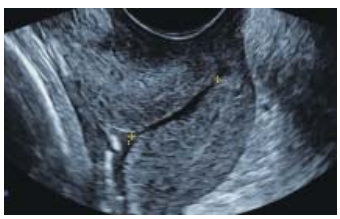


Fig 3: Hypoechoic cervical canal. Calipers are used to measure the linear distance between the triangular area at the external os and the V-shaped notch at the internal os.

Attempts have been made to assess cervical length with transabdominal scanning[7]. Even with newer high-resolution ultrasound equipment it is not possible to adequately image the cervix in about half the cases. Although a transabdominal cut-off value of 35-36 mm is appropriate for detection of short cervix, the technique does not yield very reliable landmarks for measurement. Practitioners must validate the technique in their own practice before adopting or accepting this or similar prescreening protocols.

The cervix can be examined with a transperineal approach by placing a curvilinear probe in a sagittal plane between the labia majora. Using this technique, the cervix can be visualised adequately in about 80% of patients and the measurements of cervical length obtained by this approach are very similar to those obtained by transvaginal ultrasound. In about 20% of cases the cervix either not be visualised, or, the external or internal os is obscured by a translucent artifact that cannot be abolished despite vertical movement of the transducer or lateral angulation and rotation. The technique is time consuming and offers no advantage. The method bears root in the mistaken beliefs that a transvaginal ultrasound is risky or may introduce infection or is unacceptable to the patient. Transvaginal scans are safe, do not introduce infection and are acceptable to almost all patients.

The cervix is often curved and in these cases the measurement of cervical length taken as a straight line between the internal and external os is inevitably shorter than the measurement taken along the endocervical canal. Curved cervices should be measured using a trace or a polyline. From the clinical point of view the method of measurement is not important because when the cervix is short it is always straight (Fig. 4). Quantification of a cervico-uterine angle has been suggested as a feature for improving the prediction of preterm delivery but offers no sizable advantage for the effort involved.

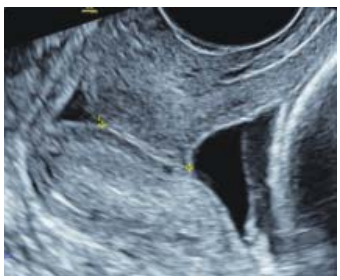


Fig 4: Short cervices are almost always straight. The normal curve gets obliterated with shortening.

Dilatation of the internal os, observed as funneling, is no more than a simple reflection of the process of producing cervical shortening that will eventually result in preterm birth. Many women with a short cervix have funneling of the internal os (Fig. 5). Women with a long cervix and funneling are not at increased risk of preterm delivery.

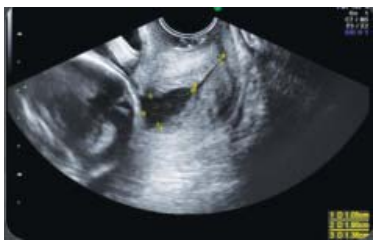


Fig 5: Funneling in association with a short cervix. Funneling, in association with a long cervix, is not associated with preterm birth.

Echogenic sludge (Fig. 6) can sometimes be seen within the amniotic cavity near the internal os. This is associated with microbial invasion of the amniotic cavity resulting in an increased risk for preterm rupture of membranes or preterm birth[8].

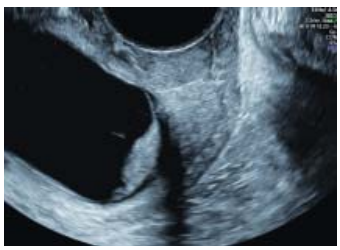


Fig 6: Many researchers believe that amnionitis is the primary cause of preterm birth. Sludge is not infrequent in association with a short cervix. The association of sludge with preterm birth is not robust.

3D Ultrasound, Elastography and MRI have been explored as advanced techniques to enhance detection rates. Although some studies suggest enhanced results, the methods currently do not meet the criteria necessary for being put to use in a perspective of routine universal screening.

Artificial Intelligence based evaluation of the cervix is now commercially available[9]. Quantitative analysis of tissue texture on ultrasound (Fig. 7) has been used to extract robust features from the ultrasound image to detect subtle changes in its microstructure[10]. A result of "low risk" on this test reduces the risk of sPTB < 34 weeks to 1.1% and < 37 weeks to 4.1%. A "high risk" result increases the risk to 25.8% for sPTB < 34 weeks and 46.7% for < 37 weeks.

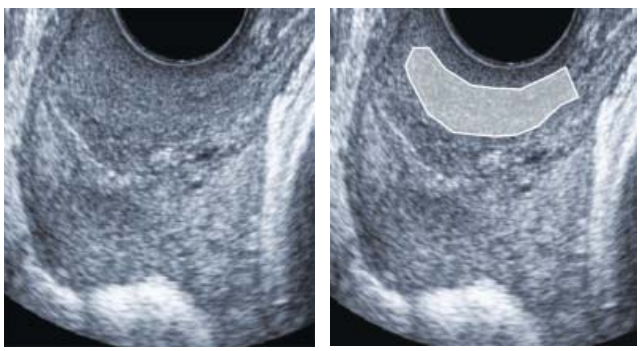


Fig 7: a. Transvaginal image of a cervix b. An automated algorithm, developed using Artificial Intelligence, identifies an island for assessment of texture c. Computer generated report of the evaluation

Cervical Length: Management Considerations

In low risk singleton pregnancies with a short cervix, cervical cerclage reduces the risk of birth before 34 weeks by about 25%. Progesterone given from 20 to 34 weeks reduces the risk of birth before 34 weeks by about 25%. Progesterone can be given as a vaginal pessary of the natural product (200 mg) every night or as an intramuscular injection of the synthetic 17 alpha-hydroxyprogesterone caproate (250 mg). Natural progesterone is preferable because of lack of undesirable side effects, such as sleepiness, fatigue and headaches. Additionally, there is some concern that injections of 17-OHP-C may increase the risk of fetal death. The Arabin cervical pessary has been examined in randomized trials in singleton pregnancies with a short cervix. Reported results concerning benefit in reducing sPTB are contradictory.

There is no benefit in reducing the risk of recurrence with bed rest, betamimetics, decrease in manual labor, increase in visits to antenatal clinics, psychological support, or diet supplementation with iron, folate, calcium, zinc, magnesium, vitamins or fish oil. Bed rest may have some adverse effects including increased likelihood of venous thrombosis, muscle atrophy and stress.

For the management of patients with previous preterm births no benefit in reducing the risk of recurrence has been shown for bed rest, betamimetics, decrease in manual labor, increase in visits to antenatal clinics, psychological support, or diet supplementation with iron, folate, calcium, zinc, magnesium, vitamins or fish oil. Bed rest may have some adverse effects including increased likelihood of venous thrombosis, muscle atrophy and stress. There are two options for managing women with a previous sPTB. The first option is an elective cerclage in all such women, soon after the 11-13 weeks scan. The second option is measurement of cervical length every two weeks and placement of a suture only if cervical length becomes less than 25 mm. The overall rate of preterm birth is similar with the two approaches but the second approach is preferable because it reduces the need for cerclage by about 50%.

In twin pregnancies with a short cervix (< 25 mm) diagnosed by routine transvaginal ultrasound at 20-24 weeks three strategies have been attempted to reduce the risk of preterm birth (PTB). Vaginal progesterone from 20-34 weeks may reduce the rate of spontaneous PTB at < 34 weeks by up to 30%. Cervical cerclage may increase the rate of

spontaneous PTB at < 34 weeks. Cervical pessary in unselected twin pregnancies does not reduce the rate of spontaneous PTB at < 34 weeks.

The risk of sPTB is doubled in women with bacterial vaginosis and may be increased fivefold if the infection is present before 16 weeks. Antibiotic treatment can eradicate bacterial vaginosis in pregnancy but does not reduce the risk of PTB after 16 weeks of pregnancy.

The majority of women (about 90%) presenting with painful and regular uterine contractions at 24–36 weeks of gestation are not in true labor and do not deliver within the subsequent 7 days. Hospitalisation and the administration of tocolytics and steroids should be reserved for women that are truly in labor and such women can be identified by ultrasund measurement of cervical length and / or a positive fetal fibronectin test in cervicovaginal secretions at presentation.

In women with threatened preterm labor the rate of delivery within the subsequent 7 days is inversely related to cervical length at presentation. The cervical length is < 20 mm in about 20% of women with threatened preterm labor and this group contains 75% of those that deliver within 7 days. In patients with cervical length > 20 mm the risk of birth within 7 days is about 3%.

The fetal fibronectin test is positive in about 20% of women with threatened preterm labor and this group contains 75% of those that deliver within 7 days. In patients with fetal fibronectin test negative the risk of birth within 7 days is about 3%.

A combination of cervical length and fetal fibronectin could potentially reduce the screen positive rate to about 5% (rather than 20% with each test alone) and also reduce the risk of birth within 7 days in those with a negative combined test to about 1%.

Randomised studies on the use of tocolytics in threatened preterm labor have demonstrated a significant prolongation of pregnancy by about seven days but no significant reduction in the incidence of preterm birth. Consequently, the primary aim of tocolysis is to achieve prolongation of pregnancy for a couple of days for effective treatment with steroids for fetal lung maturity, rather than prevention of preterm birth.

Traditionally, cervical assessment for induction of labor has been done by the Bishop scoring system. This can be replaced by transvaginal cervical assessment. Pre-induction transvaginal cervical length predicts the risk for cesarean section better than the Bishop score[11]. Cervical length is a better predictor than the bishop score in predicting the success of induction of labor[12]. Women with a pre-induction cervical length of less than 19 mm, are more likely to deliver within 24 hours after the start of induction of labor and women with a pre-induction cervical length of more than 31 mm have an 85 % chance of remaining undelivered[12].

In nulliparous women undergoing induction of labor for prolonged pregnancy with a pre-induction cervical length of less than 20 mm, there is an 80% chance that they might deliver within the next 24 hours and those with a cervix measuring more than 30 mm have a 90% chance of remaining undelivered[13]. Multiparous women with a pre-induction cervical length of less than 20 mm, have a 90% chance of delivering within the next 24 hours and those with a cervix measuring more than 30 mm have a 60% chance of remaining undelivered[13].

With each completed week of gestation between 37 and 41 weeks, neonatal respiratory morbidity is halved[14]. However, waiting can convert an elective cesarean into an emergency cesarean section and this increases maternal mortality and morbidity. Instead of planning elective cesarean section for all women (with indications of cesarean section) at 38 weeks, cervical length measurement can help decide timing of elective cesarean section[15]. If cervical length is less than 20 mm at 37 weeks, then 96 % of these cases will deliver within the next 7 days. Logically, these women can be planned for elective cesarean section between 37 to 38weeks. On the other hand, women with a cervical length of more than 30 mm at 37 weeks can have their elective cesarean planned at a later period of gestation because only 10 % of these cases are likely to progress into spontaneous onset of labor[15].

Concluding Comments

A simple ultrasound measurement of the cervix in the anomalies scan at 18-22 weeks can identify low risk women at risk for spontaneous preterm birth. Treatment with vaginal progesterone from

20-34 weeks for women identified high risk is safe, efficacious, and cost effective.

In women presenting with preterm regular uterine contractions, it is useful to distinguish between true and false labor using cervical length and cervicovaginal fetal fibronectin. Those with true labor need hospitalization at a hospital with appropriate nursery facilities and tocolytics to delay delivery until steroids to mature fetal lungs have been administered.

Cervical length measurements can also replace Bishops score for induction of labor, decide success rates for induction of labor in prolonged pregnancy, and, optimise timing of cesarean sections for indicated elective cesarean sections at 37-41 weeks.

References

1. WHO recommendations on interventions to improve preterm birth outcomes: evidence base 2015 www.who.int/reproductivehealth/publications/maternal_perinatal_health/ preterm-birth-guideline.
2. Campbell S. Prevention of spontaneous preterm birth: universal cervical length assessment and vaginal progesterone in women with a short cervix: time for action! Am J Obstet Gynecol. 2018 Feb;218(2):151-15
3. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins-Obstetrics. Practice Bulletin No. 171: Management of Preterm Labor. Obstet Gynecol. 2016 Oct;128(4):e155-64
4. Preterm labour and birth NICE guideline [NG25] Published 20 November 2015 Updated: 02 August 2019 https://www.nice.org.uk/guidance/ng25
5. Jain V, McDonald SD, Mundle WR, Farine D. Guideline No. 398: Progesterone for Prevention of Spontaneous Preterm Birth. J Obstet Gynaecol Can. 2020 Jun;42(6):806-812
6. Internet Based Course https://www.fetalmedicine.org/education
7. Pandipati S, Combs CA, Fishman A, Lee SY, Mallory K, Ianovich F. Prospective evaluation of a protocol for using transabdominal ultrasound to screen for short cervix. Am J Obstet Gynecol. 2015 Jul (1);213:99
8. Kagan, K.O. and Sonek, J. How to measure cervical length. Ultrasound Obstet Gynecol. 2015;45: 358-362
9. Baños N, Perez-Moreno A, Julià C, Murillo-Bravo C, Coronado D, Gratacós E, Deprest J, Palacio M. Quantitative analysis of cervical texture by ultrasound in mid-pregnancy and association with spontaneous preterm birth. Ultrasound Obstet Gynecol. 2018 May;51(5):637-643
10. Banos N, Perez-Moreno A, Migliorelli F, Triginer L, Cobo T, Bonet-Carne E, Gratacos E, Palacio M. Quantitative analysis of the cervical texture by ultrasound and correlation with gestational age. Fetal Diagn Ther 2017; 41: 265–272
11. Gabriel R, Darnaud T, Chalot F, Gonzalez N, Leymarie F, Quereux C. Transvaginal sonography of the uterine cervix prior to labor induction. Ultrasound Obstet Gynecol. 2002;19(3):254-257 43.
12. Pandis GK, Papageorghiou AT, Ramanathan VG, Thompson MO, Nicolaides KH. Preinduction sonographic measurement of cervical length in the prediction of successful induction of labor. Ultrasound Obstet Gynecol 2001; 18: 623– 628
13. Rane SM, Pandis GK, Guirgis RR, Higgins B, Nicolaides KH. Pre-induction sonographic measurement of cervical length in prolonged pregnancy: the effect of parity in the prediction of induction-to-delivery interval. Ultrasound Obstet Gynecol. 2003;22(1):40-44. 37.
14. Cohen M, Carson BS. Respiratory morbidity benefit of awaiting onset of labor after elective cesarean section. Obstet Gynecol 1985; 65: 818–824.
15. Ramanathan G, Yu C, Osei E, Nicolaides KH. Ultrasound examination at 37 weeks gestation in the prediction of pregnancy outcome: the value of cervical assessment. Ultrasound Obstet Gynecol. 2003; 22(6):598-603.

Twin to Twin Transfusion Syndrome (TTTS)

Anita Kaul
MS, FRCOG, FICOG, Dip Fetal Med, Apollo Centre for Fetal Medicine

Scientific Background

TTTS is a complication seen in 10-15% of monochorionic multiple pregnancies where there is a vascular anastomosis between the placentae. Clinically it is first recognised by ultrasound by a severe discrepancy in amniotic fluid between the twins ("oly-poly sequence")

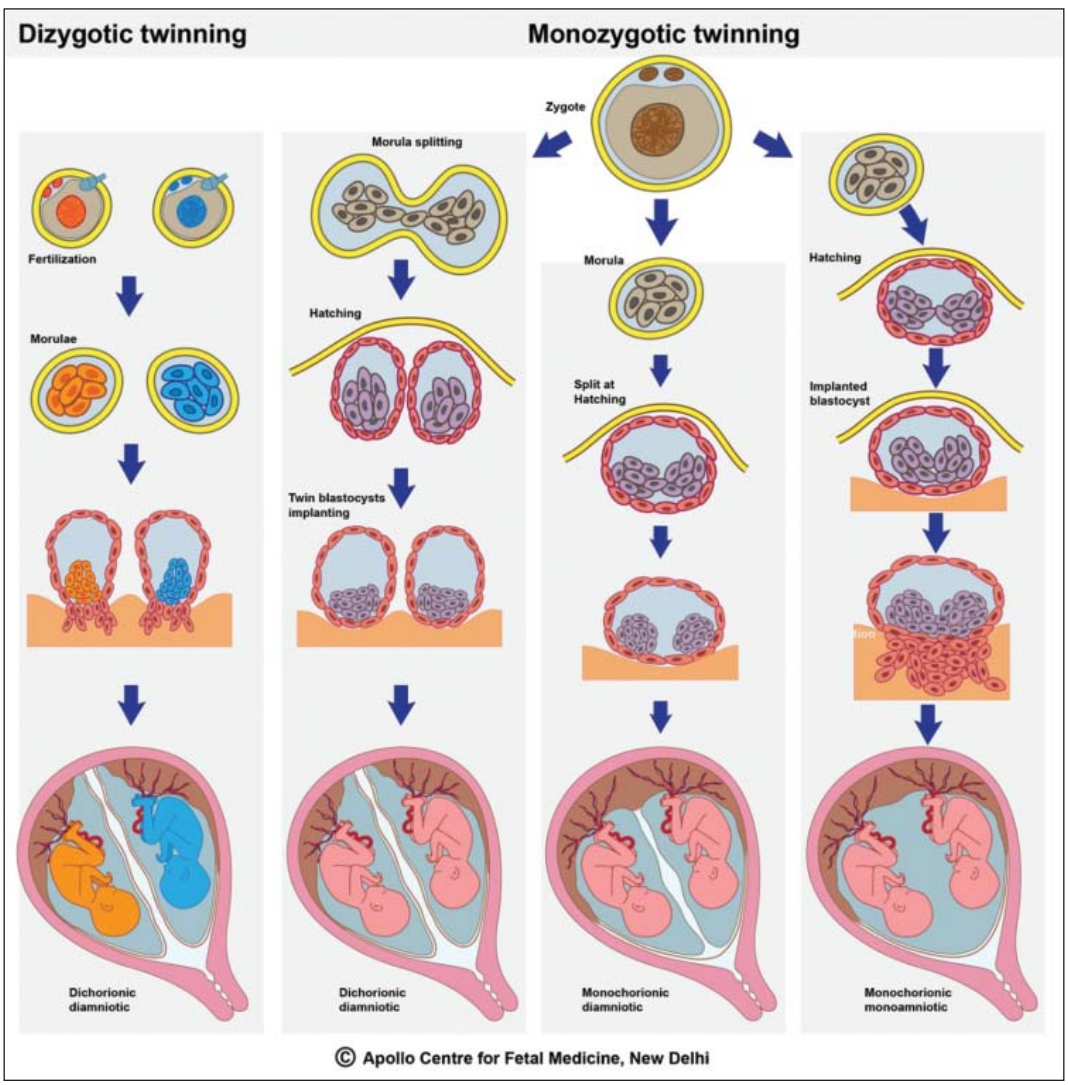
It was first described by a German obstetrician Friedrich Schatz in 1875 (fried)when on his work on twin pregnancies he discovered and described the inter-placental connections.[1] For many decades subsequently, scientists recognised that this condition was not amenable to treatment.

We now know that if untreated, there would be at least 90% perinatal loss rates with >50% neuro-morbidity in any surviving babies [2 3]

Angio-architecture

The most common theory put forward for its development is the timing of the zygote split, post fertilisation,of a single ovum and sperm. This determines the type of placentation and the subsequent angioarchitecture that will develop.

If splitting occurs after 72 hours then a single placental disc develops, with two amnions. It is these types of placentation, that most commonly develop TTTS. **(Fig1)**



The anastomoses between placental circulations is formed randomly, at the time of embryogenesis when both the embryonic and the extra embryonic circulations are established.

These anastomoses are either direct connections -arterial to arterial(AA)veno to veno(VV) or indirect via a shared cotyledon where an artery from one twin drains into the vein of the other twin (AV connection)[4] **Fig 2**

TTTS is shown to occur when the anastomoses pattern facilitates an overall unidirectional flow from donor to recipient.[5] AA anastomoses are considered protective and found to be in roughly 95% of uncomplicated pregnancies versus 25% in TTTS pregnancies.

Another type of placental angioarchitecture results in TAPS (Twin-Anaemia Polycythemia syndrome) rather than TTTS. This is a form of chronic feto-fetal transfusion (rather than the acute seen in TTTS) which results in a large intertwin Hb difference, without signs of oligoamnios-hydramnios as seen in TTTS. The pathogenesis of TAPS is because of the presence of few minute AV anastomoses (<1mm diameter) allowing a slow transfusion of blood from the donor to the recipient. Protective AA anastomoses are again very few in number in TAPS at around 20%[6]

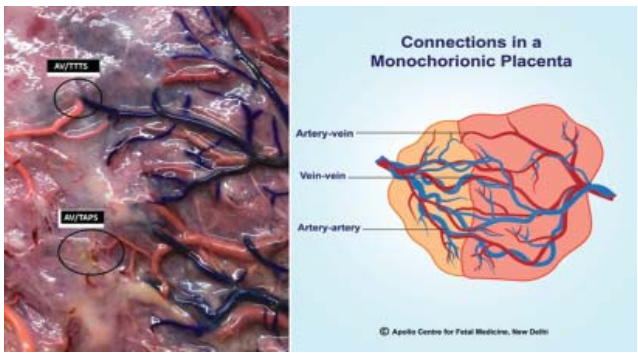


Fig 2

Fetal Renal, Vascular Regulators and Cardiac Effects of TTTS

The clinical features of TTTS involve hypovolemia and oligoamnios in the donor twin whilst there is polyhydramnios and cardiac failure in the recipient twin. It is however too simplistic to attribute the pathophysiology merely to volume changes because of the transfusion from donor to recipient. It is often accompanied by cardiac dysfunction in the recipient. This is thought to be is mainly due to fetal cardiac hypertrophy and systemic raised blood pressure (BP) which underlies TTTS.[7] The blood pressure control and fluid balance in the fetus, much like in the adult is controlled by the Renin-Angiotensin system(RAS)[8]

The RAS responds to low blood pressure, caused by decreased blood flow secondary to the anastomoses, low urine production by causing the juxtaglomerular apparatus in the kidneys to secrete renin.[9]

This renin stimulates angiotensinogen which converts to angiotensin I and then angiotensin II (AT11) under the influence of the angiotensinogen converting enzyme (ACE).

This causes the blood vessels to constrict, in an attempt to increase the BP. It also causes the secretion of aldosterone from the adrenal cortex, which causes an increase in re-absorbtion of sodium and water, with an increase in circulatory volume and thus again an increase in the BP.paul [10]

It is known that the placenta has local RAS from the first trimester itself and it may be possible that upregulation of RAS may account for the high levels of renin and ATII seen in both the donor and recipient.

The other regulators of blood pressure and fluid and electrolyte balance are the natriuretic peptides which comprise of the atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP)and C-type natriuretic(CNP)[11] These peptides exert diuretic as well as natriuretic properties and have an additional role in vasorelaxation. It is seen these peptides are increased in the recipient twin. They are released from the cardiac myocytes following freewall stretch omland, ventricular dilatation or increased pressure from volume overload. Fetal BNP release may be to counteract the sodium and water retention which ensues, and ANP may try to ameliorate the hypertension in the recipient by its direct action on the fetal kidney by increasing urine production, thus worsening the polyhydramnios seen in the recepients.

Endothelins

Endothelin (ET-1) is shown to be increased in amniotic fluid in fetal blood of the recipient as compared to the donor and non TTTS controls.

It is thought to contribute to recipient cardiac failure by inducing cardiac hypertrophy through vasoconstriction -mediated increased peripheral resistance.[12]

Clinical Features

The severity of TTTS in terms of staging and prognosis was first introduced by Dr Rueben Quintero based on ultrasound stages seen in monochorionic pregnancies.[13]

Clinical follow up, once monochorionic twins is diagnosed, is based on strictly 2 weekly ultrasounds between 16-36 weeks of pregnancy. The purpose of the scans is to diagnose emerging monochorionic complications such as TTTS, TAPS and selective fetal growth.

TTTS is staged when the following features are seen and treatment is offered when TTTS is Stage 2 or Stage 1 with fetal cardiac changes.

Quintero Staging Classification (Figure 3)

Stage 1: Oligoamnios < 2cm -Polyhydramnios> 8cm. Donor bladder seen

Stage 2: Donor bladder not seen

Stage 3: Critically abnormal fetal Dopplers noted

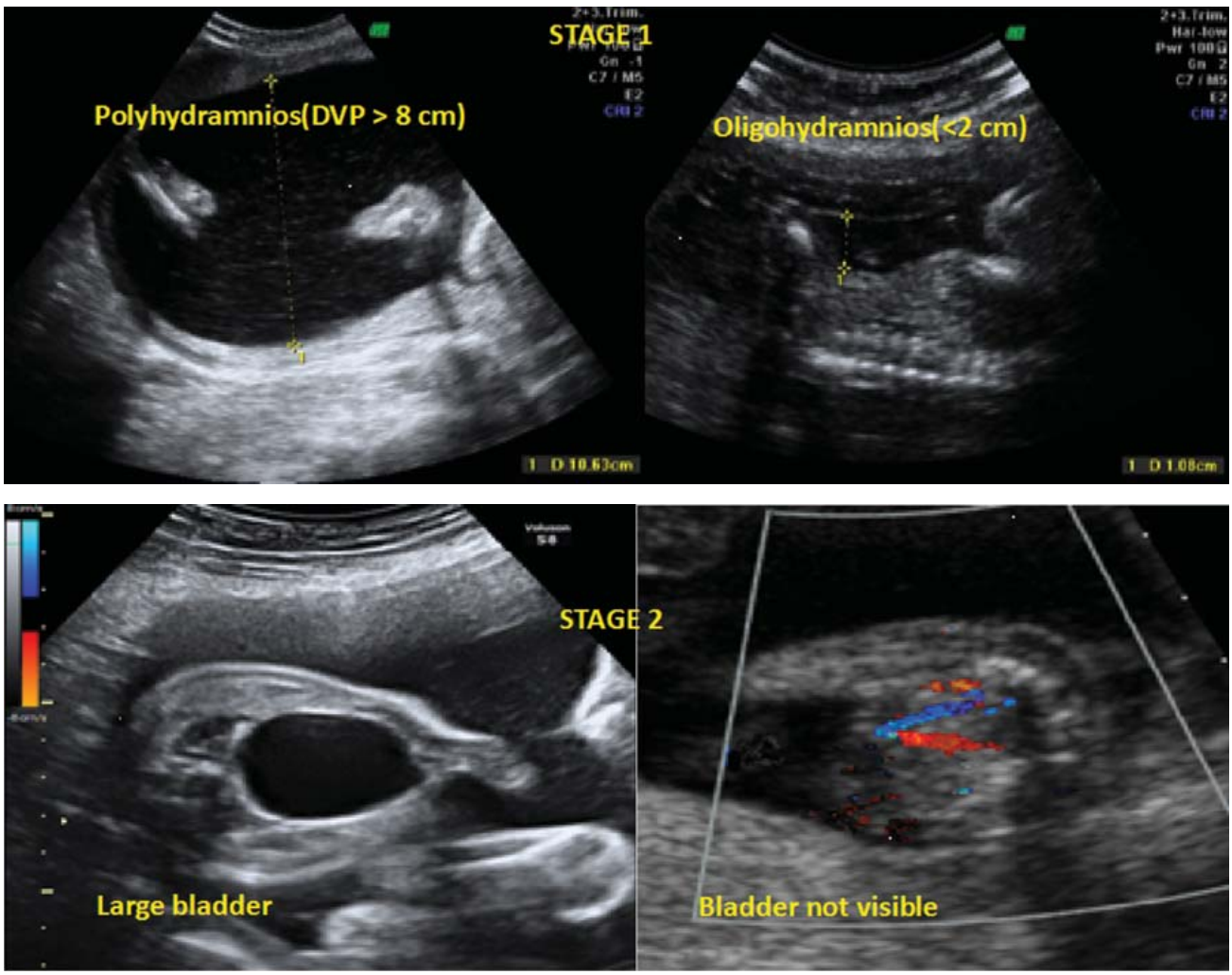
Stage 4: Fetal hydrops of either twin

Stage 5: Fetal demise of either twin

It was seen however that the cardiovascular changes seen primarily in the recipient, affected survival and prognosis and therefore needed to be incorporated in the TTTS workup of the pregnancy once the oligo-poly sequence started.

The cardiac deterioration in TTTS is largely manifested in right ventricle deterioration which is the dominant ventricle responsible for fetal systemic circulation, in utero.

The cardiac manifestation is progressive[14,15] through ventricular dilatation, right ventricular hypertrophy, decreased RV compliance, impaired



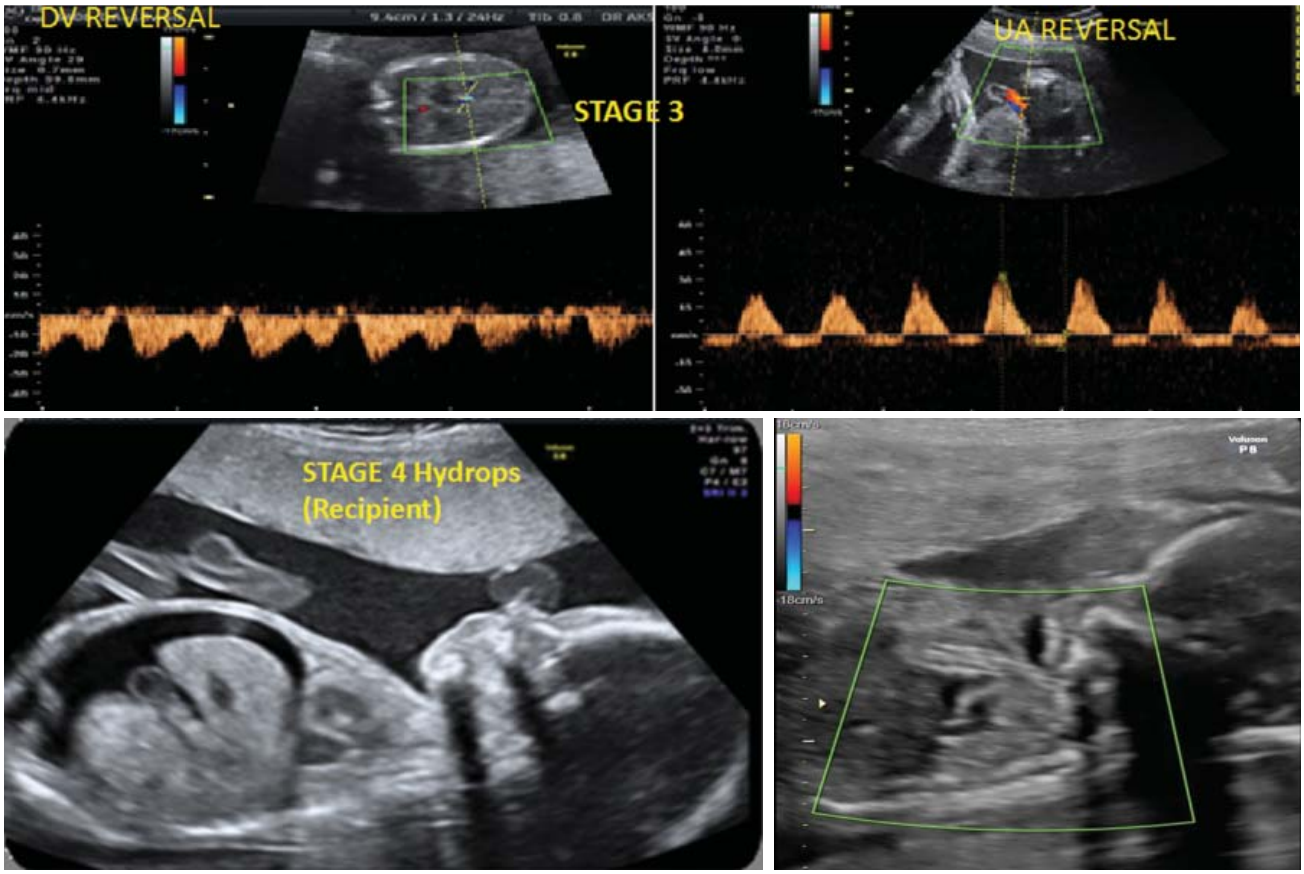


Fig 3: Quintero Staging Classification

diastolic function, abnormal inflow across the atrio-ventricular valves, worsening of umbilical vein and ductus venosus pulsation as diastolic dysfunction progresses. The filling of the RV is compromised and in 10-15% of recipient twins there is reduced antegrade flow through the right outflow track (pulmonary vessel) with pulmonary valve obstruction/hypoplasia/stenosis/ or atresia with further worsening of the ventricular hypertrophy after with the systolic function starts deteriorating with reduced perfusion across the ductus arteriosus.

As the Quinetro staging did not account for the cardiovascular changes in TTTS, the Childrens Hospital of Philadelpia developed a CHOP score[16] specific for the cardiac changes seen in TTTS. This score incorporates features unique to TTTS that quantify the cardiovascular burden of disease.(diagram)

If cardiac changes are present ,we generally upstage the Quintero staging by 1.

As already discussed the natural history of untreated TTTS leads to intra or perinatal death in as many as 90% of cases or impaired neurological development in upto 50% of survivors either due to prematurity or IUFD[17,18] thus it is important to diagnose and treat TTTS as early as possible.

Treatment Options

TTTS management has encompassed non-specific, or symptomatic therapeutic options such as amnioreduction,septostomy or even expectant management.

Selective feticide is not indicated in TTTS unless one twin is on the brink of death (including hydrops) which reverses following successful laser coagulation.

Fetoscopic laser coagulation of placental vessels is the only treatment which addresses the pathophysiology of the syndrome as proven through randomised controlled trial against amnioreduction.[19]

A Cochrane review has also indicated that laser ablation of placental anastomoses is the preferred treatment.[20]

A meta-analysis reviewed literature between 1997-2007 on the controversy of best first line treatment for TTTS. This suggested that the fetus treated

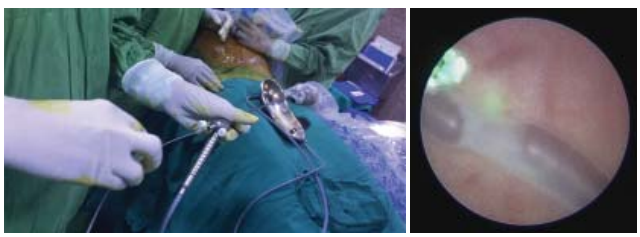
with severe amnioreduction has a higher risk of intrauterine and neonatal death. [21]

Laser Treatment

In 1973 Benirschke and Kim[22] were the first to suggest a surgical intervention for TTTS. The credit for the development of the original technique of laser occlusion of placental vessels goes to De Lia al[23] who used a mini-laparatomy to gain access to the uterus, but was subsequently simplified by Ville[24] to become a minimally invasive percutaneous approach under local anaesthesia.

The technique consists of a small skin incision performed under local anaesthesia, through which a trocar is inserted into the recipient sac. The site of trocar access is the most important step in the procedure as it has to be directed at right angles to the presumed vascular equator where the anastomoses lie for subsequent lasering. Care has also to be taken so as not to cause an inadvertent septostomy, as the intertwin membrane is generally wrapped around the donor, thus disguising its true insertion site. A 3mm (Cooks cannula Cook Ireland Ltd, Limerick Ireland) is inserted percutaneously using the Seldinger technique under ultrasound guidance in a placenta free area. A curved anterior fetoscope or an integrated straight fetoscope is inserted depending on the position of the placenta to visualise the anastomoses and map these to identify the A-V anastomoses, the AA and the rarely seen VV anastomoses.

Using a diode laser fibre 600 microns and a Diode laser (Biolitic) using energy of between 25-40 watts (depending on gestation), the AV anastomoses are lasered over a 1-2 cm section of the vessel.



The discussions about subtle differences between laser techniques have not been elaborated as being of not much interest to an obstetrician.

The procedure is done under IV antibiotics (cefuroxime) and tocolysis (Indomethacin 100mg one hour before the procedure) as both infection and preterm labour are the Achilles heel of this procedure. Subsequent follow up is to identify these complications or the development of TAPS and early signs of recurrence of TTTS[25]

Ultrasound is done 12, 24 hours after the procedure and the patient discharged 24-48 hours after the surgery.

Follow Up

Maternal Complications

The rate of maternal complications is generally low and about 5.4%.[26] These range from severe complications in about 1.8% such as pulmonary embolism and placental abruption to intermediate complications such as chorioamnionitis, bleeding, amniotic fluid leakage into the maternal peritoneal cavity.

Fetal Complications

Laser coagulation is still associated with distinct complications. Early complications occur in the first 6 days post-surgery robyrand include single or double intra-uterine deaths in 13-33% and 3-22% respectively[27,28] ville. It also can cause TAPS in about 9% of the cases.

Late complications comprise recurrent TTTS, late TAPS, IUD of one or both twins, late neurological sequalae.

It is also associated with PROM in 12-28% and preterm labour in 30.5% delivering before 32 weeks.[29,30,31]

Iatrogenic PROM after complex fetal surgery is a grave threat.

Limb ischaemia occurs in 1-2%of cases as a result of pseudoamniotic syndrome following membrane laceration or septostomy during or after laser surgery.[32,33]

Conclusion

Laser photocoagulation is the only treatment option which addresses the underlying pathology. The technique has improved overall survival as well as the rate of neurologically intact survival.

Although awareness about the TTTS condition has improved, there are still many sonologists who do not identify the condition in time to allow early intervention.

Cardiovascular parameter score	Description
Recipient	
Ventricular hypertrophy	Interventricular septum
0	< 2 SD
1	> 2 SD
Cardiomegaly	Cardiothoracic ratio
0	< 1/3
1	> 1/3 and < 0.5
2	> 0.5
Ventricular systolic function	Shortening fraction
0	≥ 30%
1	< 30% and > 20%
2	≤ 20%
MV regurgitation	Color flow area of regurgitant jet
0	Absent
1	< 25% atria
2	> 25% atria
TV regurgitation	Color flow area of regurgitant jet
0	Absent
1	< 25% atria
2	> 25% atria
MV EA	Merging of E- and A-waves
0	Absent
1	Present
TV EA	Merging of E- and A-waves
0	Absent
1	Present
Ductus venosus	End-diastolic A-wave
0	Positive
1	Absent
2	Negative
Pulsatile umbilical vein	
0	No
1	Yes
Pulmonary regurgitation	
0	No
1	Yes
Right ventricular outflow tract (RVOT)	
0	PA > Ao
1	PA = Ao
2	Ao > PA
3	RVOT obstruction
Donor	
Umbilical artery	End-diastolic flow
0	Positive
1	Absent
2	Negative

Ao, aorta; EA, E- and A-waves on pulsed Doppler interrogation; MV, mitral valve; PA, pulmonary artery; TV, tricuspid valve.

In a survey conducted by us on the knowledge and attitude of obstetrician towards fetal therapy including TTTS, we found Irrespective of the health service tier, most respondents were unaware about the availability of fetal therapy in India.

Even obstetricians serving in the two main health service sectors (government teaching hospitals and corporate hospitals), where these procedures are usually performed/could be offered, showed poor awareness.

One-third of the obstetricians working at tertiary care hospitals (both corporate and governmental institutions including teaching hospitals) were unaware of the indications for fetal interventions.

The most common reasons for not seeking treatment for such procedures among nursing homeowners, which comprises the highest referral group (approximately 40%), was the lack of awareness of the institutions where these procedures are performed and because they would prefer to terminate the pregnancy in such cases.

Half of all doctors working at government hospitals felt that such procedures have uncertain outcomes, are not easily available or are not cost effective (manuscript submitted for publication)

The other problem is in the learning curve. As the experience of the centre and operators increase, the perinatal survival rate for one or both twins increase.[34]

Since overall indications for fetoscopy are limited, a low case load will effect the efficiency and outcome of new centres starting to offer this service. Although the technical challenges and quest for progress is tempting for smaller units, professional ethics should be more of a guide than external regulation. We agree with Bebbington et al[35] that this procedure remains confined to large and limited referral units to optimise technical expertise and so outcomes.

References

1. F S. Klinische Beiträge zur Physiologie des Fötus. 1900.
2. Berghella V, Kaufmann M. Natural history of twin-twin transfusion syndrome. J Reprod Med. J Reprod Med; 2001 May;46(5):480–4.
3. Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, et al. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. American Journal of Obstetrics and Gynecology. 2008 Nov;199(5):514.e1–8.
4. Bajoria R, Wigglesworth J, Fisk NM. Angioarchitecture of monochorionic placentas in relation to the twin-twin transfusion syndrome. YMOB. 1995 Mar;172(3):856–63.
5. Umur A, van Gemert MJC, Nikkels PGJ, Ross MG. MonochorionicTwinsandTwin–TwinTransfusionSyndrome: The Protective Role of Arterio-arterial Anastomoses. Placenta [Internet]. W.B. Saunders; 2002 Feb 1;23(2-3): 201–9. Available from: (http://www.sciencedirect.com/science/article/pii/S0143400401907581)
6. de Villiers S, Slaghekke F, Middeldorp JM, Klumper FJ, Walther FJ, Oepkes D, et al. Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia-polycythemia sequence. Placenta. 2012 Mar;33(3):227–9.
7. Umur A, van Gemert MJC, Ross MG. Amniotic fluid and hemodynamic model in monochorionic twin pregnancies and twin-twin transfusion syndrome. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology [Internet]. 2001 May 1;280(5):R1499–509. Available from: https://journals.physiology.org/doi/full/10.1152/ajpregu.2001.280.5.r1499
8. Taylor GM, Peart WS, Porter KA, Zondek LH, Zondek T. Concentration and Molecular Forms of Active and Inactive Renin in Human Fetal Kidney, Amniotic Fluid and Adrenal Gland: Evidence for Renin-Angiotensin System Hyperactivity in 2nd Trimester of Pregnancy. Journal of hypertension [Internet]. 1986 Feb 1;4(1):121. Available from: https://journals.lww.com/jhypertension/Abstract/1986/02000/Concentration_and_Molecular_Forms_of_Active_and.19.aspx
9. Paul M, Poyan Mehr A, Kreutz R. Physiology of Local Renin-Angiotensin Systems. Physiological Reviews [Internet]. 2006 Jul;86(3):747–803. Available from: https://journals.physiology.org/doi/full/10.1152/physrev.00036.2005
10. Kilby MD, Platt C, Whittle MJ, Oxley J, Lindop GBM. Renin Gene Expression in Fetal Kidneys of Pregnancies Complicated by Twin-Twin Transfusion Syndrome. Pediatr Dev Pathol [Internet]. 2019 Sep 10;4(2):175–9. Available from: https://journals.sagepub.com/doi/abs/10.1007/s100240010124
11. Wilkins MR, Redondo J, Brown LA. The natriuretic-peptide family. The Lancet. Elsevier; 1997 May 3;349(9061):1307–10.
12. Bajoria R, Sullivan M, Fisk NM. Endothelin concentrations in monochorionic twins with severe twin-twin transfusion syndrome. Human Reproduction. 1999 Jun;14(6):1614–8.
13. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of Twin-Twin Transfusion Syndrome. J Perinatol. Nature Publishing Group; 1999 Dec 1;19(8):550–5.
14. Simpson LL, MARX GR, ELKADRY EA, D'ALTON ME. Cardiac Dysfunction in Twin–Twin Transfusion Syndrome. Obstetrics & Gynecology [Internet]. 1998 Oct;92(4, Part 1):557–62. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0029784498002208
15. Zosmer N, Bajoria R, Weiner E, Rigby M, Vaughan J, Fisk NM. Clinical and echographic features of in utero cardiac

dysfunction in the recipient twin in twin-twin transfusion syndrome. Heart [Internet]. BMJ Publishing Group Ltd; 1994 Jul 1;72(1):74–9. Available from: https://heart.bmj.com/content/72/1/74.short

16. Rychik J, Tian Z, Bebbington M, Xu F, McCann M, Mann S, et al. The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease. American Journal of Obstetrics and Gynecology [Internet]. 2007 Oct;197(4):392.e1–392.e8. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0002937807008319
17. Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard J-P, Deprest J, et al. Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. YMOB [Internet]. Mosby; 2006 Mar 1;194(3):796–803. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0002937805013943
18. Weir PE, Ratten GJ, Beischer NA. Acute polyhydramnios--a complication of monozygous twin pregnancy. Br J Obstet Gynaecol. John Wiley & Sons, Ltd; 1979 Nov;86(11):849–53.
19. Senat M-V, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic Laser Surgery versus Serial Amnioreduction for Severe Twin-to-Twin Transfusion Syndrome. N Engl J Med [Internet]. 2004 Jul 8;351(2):136–44. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa032597
20. Roberts D, Neilson JP, Kilby MD, Gates S. Interventions for the treatment of twin-twin transfusion syndrome. Cochrane Pregnancy and Childbirth Group, editor. Cochrane Database of Systematic Reviews [Internet]. 2014 Jan 30;199(6 Suppl 1):S118. Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD002073.pub3/epdf/full
21. Salomon LJ, Örtqvist L, Aegerter P, Bussieres L, Staracci S, Stirnemann JJ, et al. Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. American Journal of Obstetrics and Gynecology [Internet]. 2010 Nov;203(5):444.e1–444.e7. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0002937810011099
22. Benirschke K, Kim CK. Multiple Pregnancy. N Engl J Med [Internet]. 1973 Jun 14;288(24):1276–84. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJM197306142882406
23. De Lia JE, Cruikshank DP, Keye WR. FETOSCOPIC NEODYMIUM:YAG LASER OCCLUSION OF PLACENTAL VESSELS IN SEVERE TWIN-TWIN TRANSFUSION SYNDROME. Obstetrics & Gynecology [Internet]. 1990 Jun 1;75(6):1046. Available from: https://journals.lww.com/greenjournal/abstract/1990/06000/fetoscopic_neodymium_yag_laser_occlusion_of.33.aspx
24. Ville Y, Hyett J, Hecher K, Nicolaides K. Preliminary Experience with Endoscopic Laser Surgery for Severe Twin–Twin Transfusion Syndrome. N Engl J Med [Internet]. 1995 Jan 26;332(4):224–7. Available from: https://www.nejm.org/doi/full/10.1056/NEJM199501263320404
25. Permanent Feto-Fetal Transfusion From The Recipient To The Donor Twin. 576 A Complication of Laser Surgery In Twin-To-Twin Transfusion Syndrome. Acta Obstetricia et Gynecologica Scandinavica. 2004;191(42).
26. Merz W, Tchatcheva K, Gembruch U, Kohl T. Maternal complications of fetoscopic laser photocoagulation (FLP) for treatment of twin-twin transfusion syndrome (TTTS). Journal of Perinatal Medicine [Internet]. De Gruyter; 2010 Jul 1;38(4):439–43. Available from: https://www.degruyter.com/view/journals/jpme/38/4/article-p439.xml
27. Hecher K, Diehl W, Zikulnig L, Vetter M, Hackelöer BJ. Endoscopic laser coagulation of placental anastomoses in 200 pregnancies with severe mid-trimester twin-to-twin transfusion syndrome. European Journal of Obstetrics & Gynecology and Reproductive Biology [Internet]. 2000 Sep;92(1):135–9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0301211500004371
28. De Lia JD, Kuhlmann RS, Harstad TW, Cruikshank DP. Fetoscopic laser ablation of placental vessels in severe previable twin-twin transfusion syndrome. American Journal of Obstetrics and Gynecology [Internet]. 1995 Apr;172(4):1202–11. Available from: https://www.sciencedirect.com/science/article/abs/pii/0002937895914803
29. GILBERT WM, DAVIS SE, KAPLAN C, PRETORIUS D, MERRITT TA, Benirschke K. Morbidity Associated With Prenatal Disruption of the Dividing Membrane in Twin Gestations. Obstetrics & Gynecology [Internet]. 1991 Oct 1;78(4):623. Available from: https://journals.lww.com/greenjournal/abstract/1991/10000/morbidity_associated_with_prenatal_disruption_of.11.aspx
30. Yamamoto M, Murr LE, Robyr R, Leleu F, Takahashi Y, Ville Y. Incidence and impact of perioperative complications in 175 fetoscopy-guided laser coagulations of chorionic plate anastomoses in fetofetal transfusion syndrome before 26 weeks of gestation. American Journal of Obstetrics and Gynecology [Internet]. 2005 Sep;193(3):1110–6. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0002937805010501
31. Habli M, Bombrys A, Lewis D, Lim F-Y, Polzin W, Maxwell R, et al. Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. American Journal of Obstetrics and Gynecology [Internet]. 2009 Oct;201(4):417.e1–417.e7. Available from: https://www.sciencedirect.com/science/article/abs/pii/S000293780900831X
32. Lopriore E, Lewi L, Oepkes D, Debeer A, Vandenbussche FP, Deprest J, et al. In utero acquired limb ischemia in monochorionic twins with and without twin-to-twin transfusion syndrome. Prenat Diagn [Internet]. John Wiley & Sons, Ltd; 2008 Sep 1;28(9):800–4. Available from: https://obgyn.onlinelibrary.wiley.com/doi/abs/10.1002/pd.2000?casa_token = pgWQMvlB4 BEAAAAA: LD logTXtocy2Ryiv30nD6jTxgaNMf_cYupsSeEnqV4DQ4bhl374lStcclf 2znnExzoTZB coimSU9Ogw

33. Winer N, Salomon LJ, Essaoui M, Nasr B, Bernard JP, Ville Y. OP15.04: Pseudo-amniotic band syndrome: a rare complication of monochorionic twins with feto-fetal transfusion syndrome treated by laser coagulation. Ultrasound Obstet Gynecol [Internet]. John Wiley & Sons, Ltd; 2007 Oct 1;30(4):506–7. Available from: https://obgyn.onlinelibrary.wiley.com/doi/full/10.1002/uog.4561
34. Morris RK, Selman TJ, Harbidge A, Martin WL, Kilby MD. Fetoscopic laser coagulation for severe twin-to-twin transfusion syndrome: factors influencing perinatal outcome, learning curve of the procedure and lessons for new centres. BJOG: An International Journal of Obstetrics and Gynaecology [Internet]. John Wiley & Sons, Ltd; 2010 Oct 1;117(11):1350–7. Available from: https://obgyn.onlinelibrary.wiley.com/doi/full/10.1111/j.1471-0528.2010.02680.x
35. Bebbington M. Twin-to-twin transfusion syndrome: current understanding of pathophysiology, in-utero therapy and impact for future development. Seminars in Fetal and Neonatal Medicine [Internet]. 2010 Feb;15(1):15–20. Available from: https://www.sciencedirect.com/science/article/pii/S1744165X09000456

Experience of Intrauterine Transfusion in Rh Isoimmunised Pregnancies

Nandita Dimri Gupta
MD, Department of Fetal Medicine, Sir Gangaram Hospital, New Delhi

Objective

To evaluate the successful perinatal outcome and procedure related complications in fetuses treated for moderate to severe anaemia by intrauterine transfusion at Sir Gangaram Hospital.

Method

Retrospective data of women undergoing IUT for fetal anaemia from Jan 2011-May 2020 were analysed for procedure related complications and perinatal survival.

Results

67 IUTs were performed in 38 fetuses. Fetal and neonatal death were reported in 13.2% cases. There were four intrauterine fetal deaths and all occurred in fetuses with GA less than 22 weeks. Out of these four fetuses, three presented with pre-existing hydrops. There were five neonatal deaths and four of them presented with preexisting hydrops. Hydrops and transfusion in free loop were associated with adverse outcome. Median GA at birth was 32 weeks.

Conclusion

Intrauterine transfusion has a learning curve, a dedicated and specialist team is required to obtain satisfactory results. Pre-existing hydrops brings down the survival rate to 30%.

Transfusion in free loop of cord is best avoided. McA PSV though proven to be a good surveillance tool had its pitfalls in this study probably due to initial learning curve and technical difficulty in measurement.

Introduction

Rh isoimmunisation has been one of the leading causes of fetal mortality in the past leading to foetal anaemia and immune hydrops fetalis. One of the success stories in obstetrics and gynaecology has been introduction of Rh immunoglobulin for prevention of isoimmunisation. Despite adequate immunisation, adequate quantification of antiglobulin dose to combat isoimmunisation was seen lacking leading to effects of antigen- antibody reaction, hemolysis, fetal anaemia and subsequent hydrops fetalis and fetal death (Moise, 2008).

Treatment of fetal anaemia has been a great success story in fetal medicine providing hope to despondent Rh isoimmunised mothers. Treatment timeline started with intraperitoneal transfusion by Liley (1963) to intravascular fetoscopic transfusion by Rodeck (1981) and finally leading to ultrasound guided fetal intravascular transfusions (Bang 1982).

Measurement of MCA peak systolic velocity more than 1.5 MOM for gestational age (16- 36 weeks) is taken as the benchmark for intrauterine transfusion and subsequently to monitor need for further transfusions (Mari et al., 2000).

Ultrasound guided fetal intravascular transfusion is now a standard of fetal care for fetal anaemia with overall survival rates of 80-86% ((Van Kamp et al., 2005; Somerset et al., 2006; Tiblad et al., 2011).

Risk factors for adverse outcome after IUT are multifactorial ranging from relevant obstetric history, presence of red cell antibodies and signs of hydrops fetalis on ultrasound.

In this retrospective study, prenatal and perinatal outcome of all IUTs performed in the department of fetal medicine from January 2011- July 2020 at Sir Gangaram Hospital and risk factors for adverse events will be reported.

Material and Methods

Study Subjects

Data of IUT performed between Jan 2011- July 2020 in the Department of FETAL MEDICINE was collected from electronic prenatal and obstetrical databases of hospital information system of Sir Gangaram Hospital. Details of blood products were obtained from the blood transfusion centre.

Most of the patients were delivered in the hospital. Data of the patients delivering outside the hospital were collected by contacting the referring obstetrician/ patient or neonatologist. Prenatal and perinatal were analyzed retrospectively.

In this study, only IUTs for RBC- alloimmunization were included. Hydrops was defined as mild when there was a distinct rim of ascites, with or without pericardial effusion, and as severe when there was an abundant amount of fluid collection, usually ascites, with skin edema (Van Kamp et al., 2001). Anaemia was predicted by assessment of the MCA PSV, and plotted in a chart specifying range from 1- 1.5 MoM and use of different zones according to severity of anaemia. (Mari et al., 2000). An MCA PSV above 1.5 MoM (zone A) was considered an indication for IUT.

Intrauterine Transfusion Technique

During the study period, one operator performed the IUT with team of obstetricians. Antenatal corticosteroids were given to women carrying fetuses with at least 26 weeks of gestation age before IUT to anticipate the need for an emergency cesarean section. IUT were performed under maternal sedation and local anaesthesia using 2% xylocaine at the proposed site of needling. Fetal pain relief and immobilization was achieved by intravenous fentanyl (10 micrograms/ kg estimated fetal weight). A 12- 14 cms long 22G spinal needle was used for the IUT.

Calculation of Volume of O Negative Packed Cells to be Transfused

The target hematocrit (Ht) was usually 40% (40-45%). The hematocrit of the fetal blood sample was assessed through cell counter Sysmex XP 100. The donor blood was O Rhesus D-negative and compatible with the antibody of the mother. It was leucocyte depleted and obtained from CMV negative donors, collected within 5 days before the procedure. The blood was concentrated to a hematocrit between 75 and 80% and underwent gamma irradiation less than six hours before administration.

IUT was performed into the umbilical vein either at the placental cord root for anterior placenta or into a free loop of cord for posterior placenta in majority of cases. After completion of the IUT, a second blood sample was taken to confirm adequate transfusion in majority of cases. In some cases blood was transfused into the peritoneal cavity as an addition to the IV transfusion. Delivery was usually planned two weeks after the last IUT.

Mild procedure related adverse events were considered e.g. transient contractions requiring tocolysis and transient bleeding from the puncture site. Severe adverse events were defined as rupture of membranes or preterm birth within seven days after transfusion, intrauterine infection, emergency cesarean section for fetal distress within 24 hours after procedure, fetal death and neonatal death (Van Kamp et al., 2005)

Statistical Analysis (SG)

Data was stored and analysed using Excel database SPSS version 17.0.

Results

Population and Hematologic Parameters

Average maternal age at admission: 30 years ranging from 23 years to 38 years(table 1)

Most frequent maternal blood group found in the study was B -ve (50%) followed by O-ve (21%)(38) (Table VI)

67 IUTs were performed in 38 fetuses. Out of 67 IUTS, information of two IUTs missing.

Number of IUTs performed before 2015 (2011-2015) and after (2016- 2020) were equal- 49.3%

Median of one IUT was performed per fetus with range of one to five.

The median gestational age at the time of procedure: 28 weeks ranging from 16- 34 weeks. Most common gestational age at which IUT is performed is 29 weeks.

Only one IUT was performed before 20 weeks. Most of the IUTs were performed between 20 to 34 weeks. (64) (95.5%)

Commonest Fetal blood group found at the time of first IUT were O+ve (28.9%) and A+ve (28.9%) (Table VII)

Mean pre-transfusion hematocrit in this study was 19.4 (Hb 6.2g/ dl) with standard deviation of 8.4 (Table II)

There were twenty six (68.4%) nonhydropic, two

(5.3%) mildly hydropic, and nine (24%) severely hydropic fetuses at first transfusion

At subsequent IUTs, percentage of fetuses with severe hydrops dropped from 24.3% to 12.4% i.e. 50% of fetuses showed improvement in hydrops

The mean haemoglobin level and hematocrit level before transfusion were significantly lower in mildly hydropic and severely hydropic fetuses 3.5 gm/dl (10%) vs 7.5gm/dl (22.3%) in nonhydropic fetuses.

Blood source: All fetuses received O-ve fresh packed cells which were leucodepleted within 6-8 hours of irradiation

Premedication: IV Fentanyl 10 micrograms/kg of estimated fetal weight was given after blood sampling in 60 fetuses (89.6%). Two fetuses (3%) received IV vecuronium (0.1mg/kg body weight) in initial year of IUT.

Technique

The puncture was done at the level of the placental cord insertion in 23 cases (34.3%), transplacental 26, (n = 38.8%) but maximum number of puncture were directly into a free loop of the umbilical cord in 39 cases (58.3%). Intraperitoneal technique was performed in 3 cases (4.5%). Unsuccessful attempt at one intrahepatic route was followed by free loop puncture. Needle insertion in the umbilical cord was done twice in 34 case (50.7%) followed by single prick in the cord in 26 cases (38.8%). Three needle insertions were done in 2 cases (3%) and one attempt was unsuccessful.

Surveillance

In this study, ICT titres have negative correlation with pre-transfusion hematocrit i.e higher the titres, lower the hematocrit (Table IV)

Middle cerebral artery peak systolic velocity and pre-transfusion hematocrit did not show significant correlation. (Table III)

IUTs performed for MCA PSV< 1.5 MOM were n=10 (15.8%) and mean hematocrit at the time of transfusion for those fetuses was 22.5% (10.1-31.4). GA in this group (n=10) ranged from 28- 34 weeks.

Procedure Related Complications and Mortality

Percentage of fetal and neonatal death: 13.2% all observed in the period of 2011-2015 barring one.

Uneventful- 71.6%

Mild adverse events- 4.5%

Severe adverse event- 17.9%

Fetal and neonatal death in this study was strongly related to pre-existing hydrops fetalis. (Table I)

Most common mild event was transient fetal bradycardia resolving on change of position and stopping the procedure.

Influence of Hydrops Gestational Age and Technique

In this study, majority of IUT were intravascular (n=64). intra-peritoneal technique was used for early gestation (16-22 weeks) in three cases (4.4%)

Table I: Relation of percentage adverse events (mild and severe) to hydrops, severity of anaemia (Z-Hb), gestational age at IUT and location of transfusion.

	Uneventful IUT	Adverse events (mild and severe)	p-value	Nr severe adverse events
Hydrops non	43(89.6%)	5(10.4%)	<0.00	
Hydrops mild	3(75%)	1(25%)		
Hydrops severe	2(15.4%)	9(69.2%)		
IUT < 20 weeks	0	1(100%)	p=0.134	0
IUT 20-34 weeks	48(75%)	14(21.9%)		
Cord root transplacental	16(69.6%)	5(21.7%)	p=0.00	0
Free loop	32(82.1%)	7(17.9%)		

Table II: Mean SD and range of hematocrit (Ht) before and after intrauterine transfusion (IUT).

	Before IUT			After IUT		
	n	Mean (SD)	range	n	Mean (SD)	range
Ht (%)	63	19.3	0.7-41	31	40.7	5.4-53

Table III: Correlation of MCAPSV with Pre transfusion hct

		MCAPSV (Ist trans)	Pre transfusion hct
MCAPSV (Ist trans)	Pearson Correlation	1	.056
	Sig. (2-tailed)		.667
	N	65	62
Pre transfusion hct	Pearson Correlation	.056	1
	Sig. (2-tailed)	.667	
	N	62	62

Correlation between MCAPSV (Ist trans) and Pre transfusion hematocrit = **0.56** and its p value is 0.667 > .05 therefore correlation between the two is not significant.

Table IV: Correlation of ICT TIter with Pre transfusion hct

		ICT Titer Denominator	Pre transfusion hct
ICT Titer Denominator	Pearson Correlation	1	-0.275
	Sig. (2-tailed)		.090
	N	41	39
Pre transfusion hct	Pearson Correlation	-0.275	1
	Sig. (2-tailed)	0.090	
	N	39	62

Correlation between ICT Titer and Pre transfusion hematocrit = -0.275 and its p value is 0.09 > .05 therefore correlation between the two is not significant at 5% level of significance. Also as ICT Titer increases Pre Transfusion ICT decreases (negative correlation).

Table V: Pregnancy outcome after intrauterine transfusion (IUT) (GA = gestational age, Ht = hematocrit).

	Birth Weight	**Hematocrit at Delivery**
N Valid	37	30
Missing	30	37
Median	1940	31.90
Minimum	400	12
Maximum	2930	48

Table VI: Maternal Blood Group

		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid		1	2.6	2.6	2.6
	A-ve	4	10.5	10.5	13.2
	AB-ve	6	15.8	15.8	28.9
	B-ve	19	50.0	50.0	78.9
	O-ve	8	21.1	21.1	100.0
	Total	38	100.0	100.0	

Table VII: Fetal Blood Group

		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid		4	10.5	10.5	10.5
	A+ve	11	28.9	28.9	39.5
	AB+ve	4	10.5	10.5	50.0
	B+ve	7	18.4	18.4	68.4
	NA	1	2.6	2.6	71.1
	O+ve	11	28.9	28.9	100.0
	Total	38	100.0	100.0	

Pregnancy Outcome (prenatal and perinatal)

Fetal and neonatal deaths were seen in 13.5% cases with fetal death in 4 fetuses, all less than 21 weeks with three fetuses having pre-existing hydrops fetalis. Five neonatal deaths were reported in which four fetuses started IUTs with hydrops. GA for these neonates ranged from 27-32 (median 29 wks) weeks with weight ranging from 950- 2045 gm.

Median GA at birth is 32 weeks (range) and mean birth weight was 1935 gms.

Spontaneous preterm delivery happened in one fetus. Planned Caesarean section was done in 21 pregnancies and emergency CS in 11 fetuses for bradycardia. Five fetuses with hydrops underwent emergency C section due to cardiac decompensation and bradycardia. One fetus had cord hematoma.

Discussion

In this series of 67 IUT's performed for alloimmune anaemia, overall fetal and neonatal mortality was 13.5% with presence of hydrops fetalis being a major contributor to the poor outcome. (Table I)

Although GA did not show a positive correlation (p=0.134), all fetal deaths were reported in GA less than 22 weeks. (Table I)

Transfusion in free loop had a negative correlation and as part of the technique may be best avoided. (Table I)

In this study ICT had a negative correlation and thereby high titres may be an effective screening tool for such isoimmunised pregnancies. (Table IV)

Middle cerebral artery peak systolic velocity in this study did not show positive correlation wherein possible reason seems to be related to technical alacrity.

Conclusion

Intrauterine transfusion has a learning curve, a dedicated and specialist team is required to obtain satisfactory results. Pre-existing hydrops brings down the survival rate to 30%.

Transfusion in free loop of cord is best avoided. MCA PSV though proven to be a good surveillance tool had its pitfalls in this study probably due to initial learning curve and technical difficulty in measurement.

With increasing number of experience adverse events related to procedure of intrauterine transfusion become lesser and improved perinatal outcome becomes a norm.

References

1. S.A. PASmAn, L. CLAeS, L. Lewi, et al Intrauterine transfusion for fetal anemia due to red blood cell alloimmunization: 14 years experience in Leuven FACtS ViewS ViS obGyn, 2015, 7 (2): 129-136
2. Lindenburg IT, van Kamp IL, van Zwet EW et al. Increased perinatal loss after intrauterine transfusion for alloimmune anaemia before 20 weeks of gestation. BJOG. 2013;120:847- 52.hemolytic disease. Pediatrics. 2010;125:139-44.
3. Mari G, Deter RL, Carpenter RL et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. N Engl J Med. 2000;342:9-14.
4. Tiblad E, Kublickas M, Ajne G et al. Procedure-related complications and perinatal outcome after intrauterine transfusions in red cell alloimmunization in Stockholm. Fetal Diagn Ther. 2011;30:266-73.
5. Van Kamp IL, Klumper FJ, Bakkum RS et al. The severity of immune fetal hydrops is predictive of fetal outcome after intrauterine treatment. Am J Obstet Gynecol. 2001;185:668- 73.
6. Van Kamp IL, Klumper FJ, Oepkes D et al. Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red-cell alloimmunization. Am J Obstet Gynecol. 2005;192:171-7.

Fetal Therapy - An overview of medical management of the unborn

Tina Verma
Division of Fetal Medicine, Dr Doda's Diagnostic and Health Care, Pusa Road, New Delhi

Abstract

Fetal medicine has taken vast strides in the recent past. It revolves around the concept of fetus being the primary patient. Fetal therapy is defined as a therapeutic intervention for the purpose of correcting or treating a fetal anomaly or condition. With advent of new knowledge, growing expertise and refined investigations more and more disorders can be targeted during intrauterine life itself. Current therapeutics in fetus can be classified as Non-invasive pharmacotherapy, minimally invasive and invasive/surgical options. The present manuscript is aimed to provide an overview of various pharmacological options and their therapeutic indications. Specific conditions such as fetal tachyarrhyhmias, congenital heart block, fetal goitre and fetal neonatal alloimmune thrombocytopenia have been discussed.

Keywords: Fetal therapy; fetal tachyarrythmia; fetal goitre; congenital heart block; congenital adrenal hyperplasia; multidisciplinary treatment

Introduction

With rapid advancement in technology, there has been massive expansion in therapeutics across ages. Now fetus is in itself a patient. Directed therapy for various conditions can be planned/ initiated even before birth. Fetal therapy is defined as a therapeutic intervention for the purpose of correcting or treating a fetal anomaly or condition. This branch of medicine is exemplified by its two pillars – Diagnostic imaging and Therapeutic interventions.

Therapeutics in fetus can be largely subdivided under three broad headings: Non-invasive pharmacotherapy, minimally invasive and invasive/ surgical options. Figure 1 provides a summary of currently available therapies grouped under the above headings. This manuscript will be dealing with the current available pharmacological treatment options that can be initiated during intrauterine life. Minimally invasive therapy and surgical procedures are beyond the scope of this article.

At the outset, readers must understand that management of fetal disorders involves a multidisciplinary team with all the stakeholders working closely with the Fetal medicine specialist/ Obstetrician who serves as the nodal person. For Invasive Fetal therapy, allied specialties include pediatric surgery, anesthesiology, neonatology, other pediatric sub-superspecialities and Neurosurgery.[1]

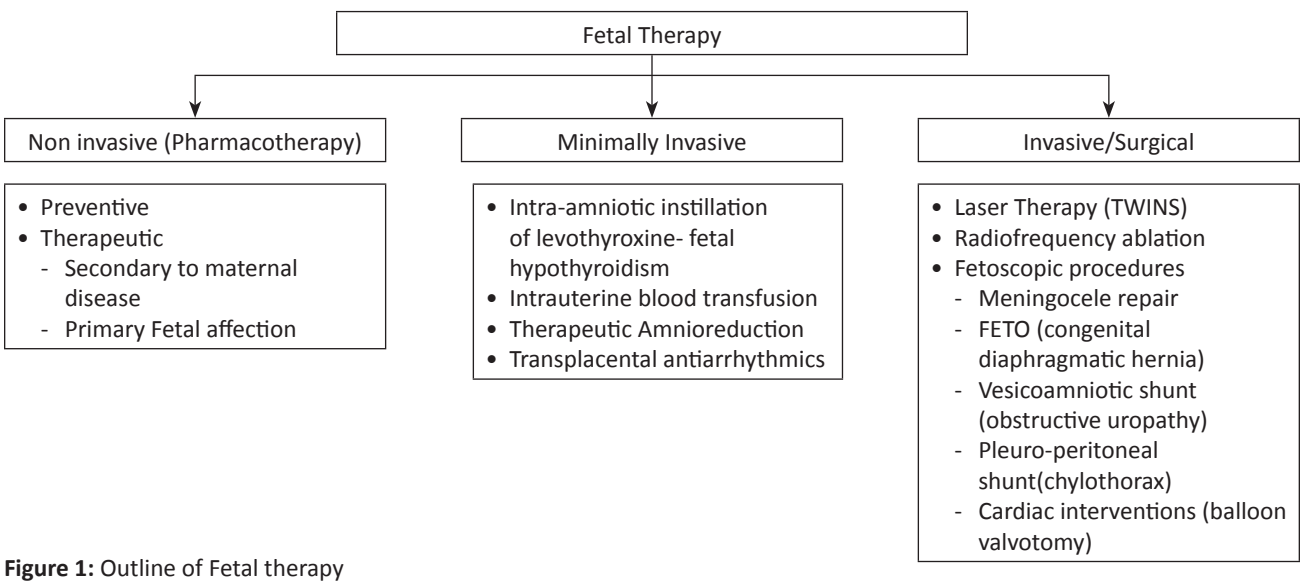


Figure 1: Outline of Fetal therapy

Non-invasive Fetal Pharmacotherapy

Indications

Treatment during fetal life can be most rewarding as interventions can be initiated even before a clinical condition actually develops or any irreversible damage has taken place. The Common Indications of Noninvasive fetal therapy are-

1. **Preventive pharmacotherapy-**
   - Folate for NTD
   - Antenatal corticosteroids for prevention of HMD
   - Magnesium sulphate in preterm for neuroprotection.
   - Anti-D immunoglobulin in rh negative discordant pregnancy.
2. **Maternal disorders impacting fetus**
   - Congenital heart block secondary to maternal connective tissue disorders with Anti-Ro/ SSA or La/SSB positivity
   - Fetal goiter (maternal hyperthyroidism)
   - Platelet alloimmunization-Fetal/ neonatal alloimmune thrombocytopenia.
   - Congenital infection-Toxoplasma.
   - Ambiguous genitalia secondary to maternal congenital adrenal hyperplasia
3. **Primary fetal affection**
   - Fetal tachyarrhythmias

1. **Preventive pharmacotherapy**

   Preventive pharmacotherapy has been the oldest form of therapy aiming at the fetus. The purpose is to prevent the development of fetal diseases. This form of treatment is directed towards an asymptomatic fetus. The most common drugs used are folic acid, antenatal corticosteroids, antenatal anti-D prophylaxis and magnesium sulphate. A tabulated summary of the indications and usage of the same has been provided to the readers as a ready reckoner in table 1.

2. **Maternal disorders impacting fetus**

   Advancement in fetal medicine has not been uniform across all disease types. For certain disorders evidence supports that initiation of early therapy is beneficial, but largely robust class I recommendations are lacking. It is important for the maternal fetal medicine specialists to discuss the pros and cons of various therapeutic

Table 1: Preventive Fetal pharmacotherapy

Preventive pharmacotherapy				
Drug	**Gestational age**	**Indication**	**Dosage**	**Duration**
Folic acid	Ideally 1-3 month Prior to conception	Prevention of Neural tube defects	1-400ugm once daily 2-4mg/day in previous baby with NTD	1-3 moth prior to conception and atleast 12 weeks post delivery
Routine antenatal Anti-D prophylaxis RAADP (Rh discordant couple)	28 weeks-30 weeks	Prevention of Haemolytic disease of new born in subsequent pregnancy	300ugm/1500IU	Stat dose
Anti- D prophylaxis in potentially sensitising events (PSE)	<12 weeks 12-20 weeks 20 weeks to term	Prevention of red cell alloimmunisation (HDN)	50ugm/250IU 100ugm/500IU 100ugm/500IU	Stat dose as and when PSE occur
Antenatal corticosteroids	- 28-34 weeks (single course) - can be given till 36 weeks - rescue dose of steroids can be given after 2 weeks of single course	Prevention of hyaline membrane disease-Prematurity	Betamethasone-12mg 24 hours apart (2 doses) Dexamethasone-6mg/12 hours apart (4 doses)	Single course (2 doses of betamethasone or 4 does of dexamethasone)
Magnesium sulphate for neuroprotection	Less than 32 weeks (anticipated preterm birth12-24 hours)	Neuroprotection	Loading dose-4gm 1st dose (slow iv over 20-30 min) f/b Maintenance dose-1gm/hr infusion for 24 hours	24 hours

options with the concerned obstetrician and the parent. Preconception counselling is done where the maternal fetal medicine specialist aims to identify the fetus at risk as well as availability of treatment and its indications. Some of the fetal conditions may arise de-novo like tachyarrhythmias whereas others such as congenital adrenal hyperplasia, congenital heart block, Intracranial haemorrhage and fetal goitre may reoccur in subsequent pregnancies.

Disease Specific Indications

i. **Congenital complete heart block (CCHB)** is a severe form of fetal bradycardia / bradyarrhythmia which is often fatal unless detected and treated timely. It affects 1 in 15–20,000 pregnancies.[2] The most important risk factor is maternal autoimmune disease with Anti-Ro/ SSA/ or La/SSB autoantibodies. It causes immune mediated damage to Atrioventricular (AV) node resulting in AV dissociation which manifests as fetal bradycardia with heart beat in the range of 40-90 bpm. It has been observed that only a minor proportion (2-5%) of fetuses of autoantibody positive mothers go on to develop CCHB. However, if a previous child is affected the recurrence risk increase to approximately 18%.[3]

Prenatal evaluation of complete heart block involves fetal echocardiography with pulse wave doppler examination across mitral and aortic valve. Weekly measurement of mechanical PR interval is to be done between 16 weeks to 24 weeks of gestation for timely diagnosis and monitoring. The degree of AV block can be categorized as 1st degree, 2nd degree, and 3rd -degree AV block depending upon extent of AV dissociation. The mechanical PR interval in CHB is prolonged in the range of >150 ms (normal<120ms).[4] The disease course of CHB is extremely unpredictable making its treatment challenging and outcome variable. Aim of fetal therapy in CHB is to: -

a. Prevent progression to higher degrees of AV block
b. Reduce risk of hydrops

The available therapeutic options involved are-

1. Fluorinated corticosteroids such as dexamethasone is the most common therapeutic agent used. The initial dose is 4–8 mg/day for 2–4 weeks followed by 2 mg/day throughout pregnancy.
2. β-sympathomimetic drugs are to be added if fetal heart rate falls below 55bpm. An increase of 5-10 bpm can be achieved by oral salbutamol 10mg TDS or terbutaline 2.5-7.5mg TDS (max 30mg/d).[3]
3. Hydroxychloroquine- 400mg /d- decreases the risk of recurrence by 50%.[3]
4. Intravenous immunoglobulin (400 mg/kg every 3 weeks from 12–24 weeks of gestation): potential role.[3]
5. Novel therapies include fetal pacemaker.[3]

ii. **Congenital adrenal hyperplasia (CAH)**

CAH is typically suspected in cases of genital ambiguity in female fetus. The Prevalence varies from 1/5000 to 1/15000 live births. CAH may present as classical CAH with two phenotypic forms namely simple virilising and salt wasting. The non-classic type is late onset with post pubertal hirsutism, menstrual irregularity and better fertility rate compared to classic CAH.

The pathogenesis involves excessive androgen production from fetal adrenal glands, as early as 9 weeks of gestation. The phenotype includes labioscrotal fusion, clitoral enlargement of variable degrees, and a urogenital sinus. Progressive postnatal virilisation includes precocious puberty, menstrual irregularities, reduced fertility rates and salt wasting. In majority (upto 90%) of cases, CAH results from mutations in the CYP21A2 gene encoding the enzyme 21- hydroxylase.[5] Being a recessive genetic disorder, carrier status of the father should be determined however if it cannot be performed then the pregnant mother should be treated with corticosteroids.

Fetal Pharmacotherapy for CAH is currently debated. Earlier, low dose dexamethasone therapy to all pregnant mothers at risk for female genital masculinization from 9 weeks followed by prenatal genetic testing was recommended. In Mutation positive foetuses the therapy is continued throughout pregnancy. The rationale was to suppress fetal androgen production during the period of urogenital organogenesis, which begins by the ninth week of gestation.

The treatment protocol recommended dexamethasone at 20 mg/kg/d of pre-pregnancy weight (maximum dose1.5 mg/d) from 9 weeks of gestation and subsequent tapering after 20weeks to 0.75-1 mg/day. However, a second school of researchers suggest that seven out of 8 fetuses with CAH may not require dexamethasone therapy as it is only beneficial in symptomatic female fetus (one in eight) which may be difficult to identify by 9 weeks.[5]

iii. Fetal Goitre and thyroid disorders

Fetal Goitre is a sonographic clue for fetal thyroid dysfunction, It is one of the important differential to be considered in sonographically detected anterior cervical mass. The lead to the disease is often there in the maternal medical history (autoimmune diseases, medications like antithyroid). A cervical mass should be proactively searched in such cases. Fetal goitre has a worldwide prevalence of 1 in 5000 live births. Untreated fetal goitre is often associated with both perinatal and long-term neurologic sequelae. Goitre is mostly a presentation of hypothyroidism however a subset of cases it may occur with hyperthyroidism as well. Therefore it is critical to determine fetal thyroid status accurately with fetal blood sampling (cordocentesis-gold standard). Associated stigmas of thyroid dysfunction include tachycardia, skeletal maturation, hydrops, fetal growth restriction, and cardiac failure. Pharmacotherapy depends upon the presentation of thyroid function i.e hypothyroidism or hyperthyroidism.

Fetal goitrous hypothyroidism is secondary to transplacental passage of maternal antithyroid drugs (propylthiouracil or methimazole in Grave's disease), or transport of antithyroid antibodies. Once fetal hypothyroidism is confirmed, antithyroid medications are withheld or reduced. In non-responders second line therapy with intra-amniotic instillation of levothyroxine. (100 µg / kg) every 1-2 weeks is done until delivery. As the placenta is relatively impermeable to oral levothyroxine and T3, intraamniotic instillation of levothyroxine is required. The fetus swallowing converts it to triiodothyronine.

Congenital hyperthyroidism though less common is equally serious with major implication on growth and development. Transient neonatal hyperthyroidism is most often caused by transplacental passage of maternal TSH-receptor antibodies (TRAb) .

The aim of therapy is normalisation of Thyroid function. The therapy is empirical with thio-amides (Antithyroid drugs). Propylthiouracil (PTU) has been considered a first-line drug for treatment of fetal hyperthyroidism, and is preferred to methimazole or carbimazole because of fetal complications like esophageal or choanal atresia, aplasia cutis, and embryopathy that includes developmental delay, hearing loss, and dysmorphic facial features.

Fetal hyperthyroidism is treated with the lowest effective dose of Propyl thyrouracil(PTU) -50-100mg three times a day; in order to minimize the risk of fetal hypothyroidism and titrated accordingly.[6]

Serial ultrasound is every 2-4 weeks is advisable to look for aforementioned stigmas of thyroid dysfunction. Delivery should be conducted in tertiary care setup with availability of Ex utero intrapartum treatment (EXIT) procedure.[6]

iv. Fetal and neonatal alloimmune thrombocytopenia (FNAIT)

FNAIT mainly presents as fetal and neonatal intracranial haemorrhage (ICH). The prevalence being1 in 1000 live birth. The fetal human platelet antigen(HPA) is derived from both mother and father. Antenatally, some of the fetal platelets can cross the placenta to cause antibody formation in the mother to paternally derived HPA antigens. These antibodies are called as anti-HPAs which cross the placental barrier and leads to destruction of fetal platelets leading to severe fetal thrombocytopenia. Fetal thrombocytopenia is defined as platelet count less than 25×10^9/l.[7] Fetal haemorrhage most commonly affects brain resulting in intracranial haemorrhage. The presentation varies with the severity of thrombocytopenia. The spectrum involves thrombocytopenia in neonate without ICH to antenatally detected fetal ICH. Serial ultrasound imaging is recommended every 2–4 weeks after 18-20 weeks of gestation to detect ICH.[7] Fetal human platelet antigen (HPA) typing through non-invasive or invasive

techniques may be required in indicated cases for confirmation of FNAIT. Once FNAIT is diagnosed then prompt treatment and close surveillance are required for management. Varying doses of IVIG and/or Corticosteroids may be used alone or in combination. The choice mainly depends upon presence or absence of ICH in previous pregnancy.[7]

a. Previous child with ICH- intravenous immunoglobulin (IVIG) infusions during subsequent affected pregnancies as early as 12 weeks gestation.
   - IVIG 1 g/kg/week at 12–16 weeks increasing to 2 g/kg/week at 20 weeks, OR
   - IVIG 1 g/kg at 12–16 weeks with corticosteroids at 1 mg/kg/day at 20 weeks.

   Mothers should be monitored for IVIG-associated haemolysis.

b. Previous baby with low platelet count but no ICH: Treatment to be initiated not later than 20 weeks of gestation.
   - IVIg 1 g/kg/week with corticosteroids, OR
   - IVIg 2 g/kg/week alone

All mothers are offered elective caesarean section at 37–38 weeks of gestation

Newer approaches found promising for FNAIT are[7]

1. PROFNAIT- anti HPA1a antibody immunoglobulin for prophylaxis
2. Recombinant anti-HPA-1a to treat FNAIT

V. Fetal tachyarrhythmia

Fetal tachycardia is defined as fetal heart rate >210 bpm.[8] Sustained fetal arrhythmias is a threating condition as it leads to occurrence of hydrops fetalis, cardiac dysfunction, or even fetal demise.

The majority of fetal arrhythmias are premature

Table 2: Drugs used in Fetal tachyarrhythmias

Drugs used in Fetal tachyarrhythmias				
Tachyarrhythmia		**First line**	**Second line**	**Third line**
1. Short VA SVT	Without hydrops	Digoxin	Digoxin + Flecainide	Digoxin +Sotalol
	With hydrops	Digoxin +Sotalol	Digoxin+ Flecainide/ Amiodarone	-
2. Long VA SVT		Sotalol	Flecainide	-
3. Atrial flutter	Without hydrops	Digoxin	Digoxin + Flecainide	Digoxin +Sotalol
	With hydrops	Digoxin +Sotalol	Digoxin+ Flecainide / Amiodarone	-
4. Ventricular tachycardia	Without hydrops	Maternal intravenous magnesium	-	-
	With hydrops	intravenous lidocaine, or oral propranolol	-	-

Table 3: Drugs dosage of common drugs used in tachyarrhythmias

Drugs dosage of common drugs used in tachyarrhythmias			
Drugs	**Dosage**	**Therapeutic level**	**Adverse effects**
Digoxin	Loading - 1.2to 1.5 mg/24 h IV in 3 divided doses Maintanence-0.375-750mg/d oral	0.7–2.0 ng/mL	Maternal sinus bradyarrhythmia or AV block
Flecainide	Flecainide 100–300 mg/d in 2-3 divided doses	0.2–1.0 µg/mL,	headache Visual/CNS symptoms, maternal/fetal proarrhythmia
Sotalol	160–480 mg/d in 2-3divided doses	Levels not monitored Bradycardia	Nausea/vomiting, dizzinessmaternal/ fetal proarrhythmia
Amiodarone	Loading: 1600–2400 mg/day 2–4 times per day;		Fetal hypothyroidism maternal thrombocytopenia
Magnesium sulphate	LD: 2–6 g IV over 20 min followed by 1–2 g/h for 48 hrs	<6mEq/L	Watch out for magnesium sulphate toxicity- respiratory depression, renal function. Monitor patellar reflex

contractions which are usually benign and often resolve spontaneously. The most common cause for sustained tachyarrythmia is supraventricular tachycardia (SVT) accounting for 66% to 90% of all cases, other less common forms are atrial fibrillation and ventricular tachycardia.[8] Defining the type of arrhythmia with the help of fetal echocardiography (M-Mode as well as pulse wave doppler evaluation) is prudent as the choice of therapy depends upon its classification based on ventriculoatrial (VA) interval i.e. short or long VA.[9]

The aim of pharmacotherapy is conversion to sinus rhythm. Oral administration of antiarrhythmic drug is recommended (Transplacental therapy) however, maternal intravenous route, intracordal route or direct fetal intramuscular route may be required in certain cases.

The presence or absence of hydrops drives the choice of pharmacotherapy and determines the possible outcome. Pediatric cardiologist plays a key role in initiation of treatment and monitoring of the fetus. Table 2 describes the various pharmacological agents currently approved for fetal tachyarrhythmias.[9] Table 3 provides the recommended dosage schedule.[10]

References

1. Moon-Grady AJ, Baschat A, Cass D, Choolani M, Copel JA, Crombleholme TM, Deprest J, Emery SP, Evans MI, Luks FI, Norton ME, Ryan G, Tsao K, Welch R, Harrison M. Fetal Treatment 2017: The Evolution of Fetal Therapy Centers - A Joint Opinion from the International Fetal Medicine and Surgical Society (IFMSS) and the North American Fetal Therapy Network (NAFTNet). Fetal Diagn Ther. 2017;42(4):241-248.
2. Pruetz JD, Miller JC, Loeb GE, Silka MJ, Bar-Cohen Y, Chmait RH. Prenatal diagnosis and management of congenital complete heart block. Birth Defects Res. 2019 May 1;111(8):380-388.
3. Hansahiranwadee W. Diagnosis and Management of Fetal Autoimmune Atrioventricular Block. Int J Womens Health. 2020 Aug 12;12:633-639.
4. De Carolis S, Garufi C, Garufi E, De Carolis MP, Botta A, Tabacco S, Salvi S. Autoimmune Congenital Heart Block: A Review of Biomarkers and Management of Pregnancy. Front Pediatr. 2020 Dec 22;8:607515.
5. Simpson JL, Rechitsky S. Prenatal genetic testing and treatment for congenital adrenal hyperplasia. Fertil Steril. 2019 Jan;111(1):21-23.
6. Shirin Namouz-Haddad, Gideon Koren, Fetal Pharmacotherapy 4: Fetal Thyroid Disorders, Journal of Obstetrics and Gynaecology Canada. 2014; 36(1):60-63.
7. Regan F, Lees CC, Jones B, Nicolaides KH, Wimalasundera RC, Mijovic A, on behalf of the RoyalCollege of Obstetricians and Gynaecologists. Prenatal Management of Pregnancies at Risk of Fetal Neonatal AlloimmuneThrombocytopenia (FNAIT). Scientific Impact Paper No. 61. BJOG 2019;126:e173–185.
8. Karmegeraj B, Namdeo S, Sudhakar A, Krishnan V, Kunjukutty R, Vaidyanathan B. Clinical presentation, management, and postnatal outcomes of fetal tachyarrhythmias: A 10-year single-center experience. Ann Pediatr Cardiol. 2018 Jan-Apr;11(1):34-39.
9. Yuan, SM., Xu, ZY. Fetal arrhythmias: prenatal evaluation and intrauterine therapeutics. Ital J Pediatr 46, 21 (2020).
10. Donofrio MT, Moon-Grady AJ, Hornberger LK, Copel JA, Sklansky MS, Abuhamad A, et al. Diagnosis and treatment of fetal cardiac disease: A scientific statement from the American Heart Association. Circulation 2014;129:2183-242

Journal Scan

Geeta Mediratta[1] & Veronica Arora[2]
[1]Senior Consultant, [2]Associate Consultant, Sir Ganga Ram Hospital, New Delhi

Fetal Diagnosis and Therapy

An Approach to Integrating Exome Sequencing for Fetal Structural Anomalies into Clinical Practice

Neeta L Vora, Kelly Gilmore, Alica Brandt, Chelsea Gustafson, Natasha Strande, Lori Ramkissoon Emily Hardisty, Ann Katherine M Foreman, Kirk Wilhelmsen, Philips Owen, Karen E Week Jonathan S. Berg, Cynthia M.Powell, and Bradford C. Powell

Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynaecology (N.L.V., K.G., E.H.), Department of Genetics, School of Medicine (A.B., C.G., N.S., L.R., A.K.M.F., K.E.W., J.S.B., C.M.P., B.C.P.), Department of Pathology and Laboratory Medicine (N.S., L.R., K.E.W.) Department of Genetics and Neurology, Renaissance Computing Institute (K.W.), Renaissance Computing Institute (P.O.), and Department of Pediatrics, Division of Genetics and Metabolism (C.M.P.), University of North Carolina at Chapel Hill, Chapel Hill, NC

Genet Med 2020;22(5):954_961

ABSTRACT

Prenatal ultrasound, karyotype, and microarray have become routine tools for the diagnosis of structural anomalies of the fetus. Gene panels are increasingly used as well, although the relatively limited prenatal information that is available regarding fetal phenotypes can make it difficult to choose the appropriate gene panel. Exome sequencing has been shown to improve the diagnostic yield in fetuses with a diagnosis of prenatal abnormalities when testing via microarray or panel is negative. The aim of this study was to describe an approach to exome sequencing for prenatal care practice.

The study cohort was parent-fetus trios, in which the fetus was diagnosed with 1 or more congenital anomalies, but the chromosomal microarray did not identify a genetic cause, The cohort was recruited from various prenatal diagnosis clinics in the United States from July 2014 to February 2019. Consent was obtained before enrollment. Enrolled participants agreed to be informed if there were any findings related to the fetal phenotype, if there were medically actionable findings for a parent, and if both parents were found to be carriers of the same autosomal recessive conditions. Results were reviewed by a multidisciplinary committee of geneticists not involved with the patient's care.

A total of 102 trios were identified, and exome sequencing was performed in 99 of the 102 samples. Of the 102 cases, 30.4% (n = 31) had findings believed to explain the fetal phenotype, 20.6% (n = 21) were positive or positive probable, and 9.8% (n = 10) identified a variant of uncertain significance through to possibly explain the phenotype. In cases with multiple anomalies identified by ultrasound, exome sequencing identified a genetic variant in 33.7% (30/89). In findings related to the parents, exome sequencing reported 2.9% (6/204) had medically actionable autosomal dominant conditions and 2.9% (6/204) were carries for the same recessive condition.

Exome sequencing was found to be a useful diagnostic tool when genetic abnormalities in the fetus are suspected. It can be used not only to diagnosis these prenatal anomalies but also to inform parents of medically actionable genetic conditions.

Editorial Comment

Most women undergo a routine anatomy ultrasound in the midtrimester, and many structural anomalies are diagnosed at this time. When anomalies are identified, counseling involves discussion of the cause, the prognosis for the fetus, and the recurrence risk. Intial investigations typically involve testing for aneuploidy. However, karyotypoing of amately 5% to 10% of cases, depending on the precise abnormally. Chromosomal microarray will detect a cause in an additional 5% to 10% of cases, again depending on

the type and number of anomalies present in the fetus. Single gene variants, either autosomal recessive conditions inherited from carrier parents or autosomal dominant conditions, often due to de novo variants that are not present in the parents, are also responsible for a significant percentage of congenital disorders, including structural birth defects as well as neurocognitive abnormalities. Increasingly, genomic sequencing to screen for such genetic variants is used in children and adults with disorders that are suspected to be genetic. Such testing is beginning to be introduced into prenatal diagnosis and a few large series of exome sequencing for assessment of fetal malformations have been reported (Lancet 2019;393:747-757; Lancet 2019;393:758-767).

In the abstracted article, the authors report on a series of 102 patients who underwent fetal exome sequencing after detection of fetal anomalies. Trio sequencing was performed, in which samples from both parents were also sequenced to determine the inheritance of any identified variants. In 21/102 (20.6%) cases, a pathogenic or likely pathogenic variant was identified that was felt to have caused the detected anomalies. Of those with a positive result, half were autosomal dominant, with most being de novo, and half were autosomal recessive and inherited from carrier parents. There was one case that was x-linked. In another 10(9.8%), there was a variant of uncertain significance (VUS) that was thought to likely be the cause of the abnormalities, but for which data regarding the variant or the gene were not adequate to determine causality with certainty. The authors were aware of at least 2/102(2.0%) in which patient used the information from the study for prenatal diagnosis in a subsequent pregnancy.

The rate of detection of a single gene disorder is somewhat higher than the 8% to 10% reported in prior series (Lancet 2019:393:757; Lancet 2019:393:758-767). This is likely due to selection criteria, in which cases with multiple anomalies or findings highly suggestive of a genetic disorder were prioritized over cases with a single structural anomaly, in which the yield is typically lower. The detection rate was 7.7% in fetuses with one anomaly and 3.7% in fetuses with multiple anomalies.

The field of genomic sequencing is rapidly evolving, and variants are often reclassified overtime. Variants initially thought to be important may later turn out to be unrelated and not pathogenic, and often the nongeneticist does not appreciate the nuances of variant interpretation. Even for geneticists, there is a pressing desire to find the cause, especially once this new, cutting and this can lead to overoptimistic interpretation of a detected VUS.

Another interesting finding in the data includes the highly selected patient population that enrolled in the study. The cohort was 72% white, 87 had a college education or higher degree, and more than 50% had a salary over $90,000.

At present, most insurers do not cover the cost of fetal exome sequencing; therefore, it is primarily available to either patients who are able to pay out of pocket, or to those who enroll in research studies.

Finally and importantly, the turnaround time for these cases was 6 to 12 months, far longer than with clinical sequencing, although even with clinical, nonresearch cases, the turnaround time is an issue, particularly if chromosomal microarray needs to be reported before the sequencing is undertaken. With increasingly stringent gestational age cutoffs for termination access in many parts of the country, this is a real concern. In addition, these tests are very expensive, close to $10,000 for trio sequencing in some commercial laboratories. Clearly, much more data are needed regarding detection rates and appropriate case selection, how to optimize variant interpretation and reporting, and how to manage turnaround time and cost. At present, it is likely optimal if cases are largely managed in experienced, multidisciplinary centers by experts who understand all of these complesities.-MEN)

Prenatal Exome Sequencing Analysis in Foetal Structural Anomalies Detected by Ultrasonography (PAGE): A cohort study

Foetal Structural abnormalities (FSA) are identified in approximately 3% of all pregnancies. They display extensive genetic heterogeneity with causes including chromosomal, single gene disorders and less rarely

methylation defects. The PAGE STUDY (Prenatal Assessment of Genomes and Exomes) was a large scale exome-wide sequencing study for antenatally detected malformations. It was undertaken over a period of three years with a sample size of 610 foetuses. An important strength of the study was its prospective nature. After excluding the chromosomal copy number variations, a whole exome sequencing was performed on the DNA isolated from the foetus. The cohort comprised prenatally detected structural anomalies including increased nuchal translucency. The overall diagnostic yield (the number of foetuses which received a definitive diagnosis)as indicated by the total number of samples with a pathogenic and likely pathogenic variant was 8.5%. Additionally, clinically relevant variants of uncertain significance was present in 3.5% of the foetuses. The overall yield was much lower than the previous smaller studies and retrospective inclusions. The reasons for a lower diagnostic yield were the non-selective nature of the cohort especially the inclusion of foetuses with increased nuchal translucency. A higher diagnostic yield was present in foetuses with multisystem, cardiac and skeletal abnormalities while those of the spinal, neurological and increased nuchal translucency. An important limitation of the study was the lack of delivery of a real time diagnosis in pregnancy. Further a trio sequencing was not performed in all foetuses. A carefully curated cohort inclusive of foetuses with recurrent malformations, parental consanguinity and the multiple malformations or specific systemic malformations were shown to increase the diagnostic yield of exome sequencing and these factors should be born in mind while ordering this test. Given the ethical and the practical issues of exome sequencing in a prenatal setting, it is best done with a detailed phenotyping and on a specific group of foetuses. As more and more prenatal whole exomes are performed, it is important to share it in a confidential database in an anonymous manner so as to foster collaboration and better research opportunities, and most importantly to incorporate whole exome sequencing in a routine clinical setting.

Take home message: Exome sequencing in a prenatal setting should be ordered and interpreted with utmost caution. It is best suited for a carefully curetted cohort of foetuses in which a high diagnostic yield is expected.

Obstetrics

Pregnancy Outcomes in Nulliparous Women with Positive First-Trimester Preterm Preeclampsia Screening Test: The Great Obstetrical Syndromes Cohort Study

Amélie Boutin, PhD; Paul Guerby, MD, PhD; Cédric Gasse, MD, MSc; Sylvie Tapp, MSc; Emmanuel Bujold, MD, MSc

Am J Obstet Gynecol 2021;224:204.e1-7.

Background: The Fetal Medicine Foundation proposed a competing risks model for early identification of women at a high risk of preterm preeclampsia, typically associated with deep placentation disorders. The Great Obstetrical Syndromes include a spectrum of pregnancy complications (preeclampsia, intrauterine growth restriction, preterm birth, late spontaneous abortion, and abruptio placentae) that are also associated with deep placentation disorders.

Objective: This study aimed to estimate the rate of placentamediated pregnancy complications in nulliparous women with a positive first-trimester Fetal Medicine Foundation preterm preeclampsia screening test.

Study Design: We conducted a prospective cohort study of nulliparous women recruited at 11 to 14 weeks of gestation. Maternal characteristics, mean arterial blood pressure, levels of maternal serum biomarkers (pregnancy-associated plasma protein-A, placental growth factor, and soluble fms-like tyrosine kinase-1), and mean uterine artery pulsatility index were obtained to calculate the risk of preterm preeclampsia according to the Fetal Medicine Foundation algorithm. The predicted risks were dichotomized as a positive or negative test according to 2 risk cutoffs (1 in 70 and 1 in 100). The detection rate, false-positive rate, and positive and negative predictive values were calculated for placenta-mediated complications, including preeclampsia, small for gestational age (birthweight <10th percentile), fetal death, preterm

birth, and a composite outcome, including any of the foregoing. The same analyses were computed for a composite of severe outcomes, including preterm preeclampsia, severe small for gestational age (less than third percentile), and fetal death.

Results: We included 4575 participants with complete observations, of whom 494 (10.8%) had an estimated risk of preterm preeclampsia of ≥1 in 70 and 728 (15.9%) had a risk of ≥1 in 100. The test based on a risk cutoff of 1 in 70 could have correctly predicted up to 27% of preeclampsia, 55% of preterm preeclampsia, 18% of small for gestational age, 24% of severe small for gestational age, and 37% of fetal deaths at a 10% false-positive rate. The test based on a cutoff of 1 in 100 could have predicted correctly up to 35% of preeclampsia, 69% of preterm preeclampsia, 25% of small for gestational age, 30% of severe small for gestational age, and 53% of fetal deaths at a 15% false-positive rate. The positive predictive value of a screening test for preterm preeclampsia of ≥1 in 70 was 3% for preterm preeclampsia, 32% for the composite outcome, and 9% for the severe composite outcome.

Conclusion: Nulliparous women with a first-trimester positive preterm preeclampsia Fetal Medicine Foundation screening test are at a higher risk of both preterm preeclampsia and other severe placentamediated pregnancy complications. Approximately 1 woman of 10 identified as high risk by the Fetal Medicine Foundation algorithm developed at least 1 severe placenta-mediated pregnancy complication.

Key words: Fetal death, intrauterine growth restriction, placentamediated complications, preeclampsia, preterm birth, risk assessment, screening, small for gestational age, validation

AJOG at a Glance

Why was this study conducted?

The Fetal Medicine Foundation (FMF) algorithm for estimating the risk of preterm preeclampsia (PE) had not been validated in North America, and its ability to identify women at a high risk of other placenta-mediated complications had not been described.

Key findings

Almost a third of the women identified as high risk by the FMF algorithm developed at least 1 placenta-mediated pregnancy complication, and up to 10% of women had a severe complication.

What does this add to what is known?

This study validates the FMF algorithm for estimating the risk of preterm PE in a North American population of nulliparous women and demonstrates the utility of the algorithmin identifying women at a higher risk of other placenta-mediated complications.

Proceedings of Virtual AOGD Monthly Clinical Meeting held at UCMS & GTB Hospital New Delhi on 26th February, 2021

Idiosyncrasies of COVID 19 Infection: A case series

Rashmi, Himsweta S, Shilpa S, Kiran G, Amita S

Introduction: COVID-19 infection caused by SARS CoV 2 virus can cause severe morbidity and mortality. Diagnosis plays a key role in the management and containment of this disease. RT PCR test remains the gold standard for diagnosing SARS CoV2 infection. Dilemmas caused by RT PCR testing in cases of severe maternal outcome (Maternal near miss & maternal mortality) at GTB hospital are discussed.

Case Series: During the time GTB hospital was designated COVID only facility, 13 cases of maternal mortality and 7 cases of maternal near miss (MNM) occurred in the obstetric patients managed in the department of obstetrics & Gynecology. Out of these cases, in 5 cases (2 MNM & 3 maternal mortality) RT PCR test came out to be negative repeatedly. All these cases were referred from other hospitals as covid suspects due to severe acute respiratory illness symptoms. Time since symptom onset varied from 1 to 7 days. Three were antenatal while 2 were postpartum cases. Hospital stay duration ranged from 20 hrs to 24 days. At admission, general condition was poor and SPO2 was <80% on room air in all cases. Cases were managed in ICU wherein oxygen support was given along with low molecular weight heparin, Dexamethasone and broad spectrum antibiotics. Severe pre eclampsia was associated in one case. CT Chest was done in 2 cases and showed bilateral ground glass opacities suggestive of COVID. In other three cases Chest radiograph showed bilateral opacities suggestive of COVID pneumonitis. Naso pharyngeal swabs for SARS CoV2 were negative in all these cases and were repeated 2-4 times.

On other end of the spectrum, 2 obstetric cases of severe COVID 19 infection, continued to have positive RT PCR even after resolution of symptoms. Seroconversion on RT PCR occurred at day 31 and day 44 after symptom onset in these 2 cases.

Discussion: RT PCR remains the gold standard for diagnosis of COVID 19. It is highly specific but sensitivity ranges from 60-70%. 54% of first sample can be false negative. Highest probability of positive testing is around symptom onset. Lower respiratory tract samples have higher virus detection rates. Lungs involvement can be detected on chest imaging and CT Scan has been found to be more sensitive than RT PCR for COVID 19, but specificity is low. X ray chest can also detect these abnormalities but sensitivity is low. Regarding prolonged RT PCR positive testing, it is not rare in severe illness. But prolonged shedding doesn't mean infectiveness. Culturable virus is not detected beyond 10 days in mild to moderate disease and beyond 20 days in severe disease. Only in immunocompromised patients, prolonged infectivity is seen and require negative RT PCR before stopping isolation.

Impact of COVID-19 Pandemic on Gynaecological Cancer Care Delivery: GTB experience

Bindiya Gupta, Amita Suneja, Kiran Guleria Shalini Rajaram, Yasmin

Background: Through out the world, many challenges have been faced in delivery of cancer care due to COVID-19 pandemic, like overwhelmed systems, lack of personal protective equipment, staff shortage etc. resulting in significant patient harm from interruption of cancer specific care.

Guru Teg Bahadur hospital had suspended elective work from March 2020 and was declared a designated COVID care facility from the beginning of June 2020 till February 2021. The major roadblock faced was complete suspension of oncology care services to patients throughout the year in our hospital. Moreover, the other tertiary care Delhi Government hospital was also a COVID facility and the nearby tertiary care cancer hospital had restricted chemo and radiation services.

Aim: To analyze impact of COVID-19 pandemic on gynecological cancer care delivery at University college of medical sciences and Guru Teg Bahadur Hospital during the pandemic

Methods: Records of new patients enrolled for gynae oncology care (preinvasive cancers excluded) between Jan- March 2020 were retrieved from oncology registers and patients were contacted telephonically in Jan 2021. Information was obtained regarding health condition, symptoms and completion of adjuvant treatment i.e. radiotherapy or chemotherapy.

Results: Out of 36 entries, 28 entries were complete, while 8 had missing/ wrong phone numbers. Four patients had died; one due to COVID, one had a sudden cardiac death while two died due to incomplete treatment. Out of 28, there were 9 patients of cancer cervix, 12 patients of cancer ovary, 6 patients of cancer endometrium and one patient of cancer vulva. Treatment was completed in only 17.8% (5/28). 35.7% (10/28) had partial/ interrupted adjuvant treatment, delayed follow up in 25% (7/28) while 21.4% (6/28) were unable to receive adjuvant chemotherapy and/ or radiotherapy.

Conclusions: Approximately 80% gynae oncology care services were impacted due to COVID -19 in our hospital. Many lessons have been learnt with this experience. Oncology should be made a part of emergency services. While assigning hospital for COVID care, provision should be made to arrange for proper treatment of other emergent diseases like cardiac, oncology etc. There should be implementation of an effective centrally coordinated response.

Rare Life-threatening Complication of a Common Life-Saving procedure

Archana Chaudhary, Rashmi Malik, Amita Suneja

Case: A 20 years old P3L3 was referred to GTB Hospital on post-op day 3 of LSCS with severe anaemia, uterine collection, multiorgan dysfunction with? Septicaemia. She underwent LSCS for obstructed labor and MSL. She developed atonic PPH which was managed by uterotonics, B/L uterine artery ligation and B-lynch compression sutures. She developed abdominal distension on day 2 which increased subsequently along with rise in uterine size upto 32 weeks. She was then referred to GTB Hospital for further management. On admission to GTBH, she had tachycardia (PR 130bpm), tachypnoea (RR 30/min) and pallor (corresponding to 6g%). BP was 110/80mmhg. Abdomen was distended with uterus enlarged upto 32 weeks size and extremely tender. Drain in situ contained 50 ml serosanguinous fluid. On P/V Cx was pulled up and uterus was enlarged to 32 weeks size. Investigations revealed low Hb% of 5.7g%, while others were WNL. USG showed multiple myometrial hematoma, empty uterine cavity with minimal free fluid in peritoneal cavity. On exploratory laparotomy uterus was grossly enlarged, congested and bluish black in appearance with B-lynch sutures in situ. Another circumferential stich was seen at the level of internal os encircling both round and infundibulo-pelvic ligaments. Ovaries were also congested, bluish black, friable and appeared to be necrosed. TAH with BSO was done. On HPE uterus, B/L ovaries and fallopian tubes showed marked congestion and haemorrhage with only scant viable tissue identifiable suggestive of gross necrosis.

Discussion: PPH is still the major cause of maternal morbidity and mortality. Various uterine conserving and fertility preserving techniques to tackle atonic PPH include balloon tamponade, step wise uterine devascularization, uterine compression sutures and selective radiological arterial embolization. The concept of UCS was pioneered by B-lynch et al in 1997. Since then, different techniques and modifications have been introduced with claimed added advantages, but the evidence on efficacy and safety of UCS is weak, as the data is limited by case series reported by proponents themselves and lack of controlled trials or RCT available in literature. Various complications reported include uterine necrosis, hematoma, pyometra, uterine synechia and uterine rupture in subsequent pregnancy. Uterine necrosis in our case may be attributable to too tight sutures, concomitant devascularization and wrong technique (circumferential stich compressing the vessels in IP and broad ligaments). Obstetricians must be aware of the potential risk and complications of compression sutures.

A Wriggly Problem of Cervix

Richa Aggarwal, Preeti Sharma
Abha Sharma, Amita Suneja

Case Summary: A 27 years old female presented to gynae OPD with complaints of foul smelling

discharge per vaginum for a year. A diagnosis of PID with cervicitis was made and she was treated with a 14- day course of Doxycycline and Metronidazole along with Clotrimazole vaginal pessary for 6 days. Despite full antibiotic course, there was only mild relief in symptoms. Pap smear was reported unsatisfactory due to inflammation. Colposcopy was planned later due to her bothersome complaints and suspicious looking cervix. A white worm popped out of cervix which turned out to be Trichuris trichiura. Deworming was done for the patient and her family members. The Patient reported again with similar complaints and this time colposcopy and guided biopsy done that showed Tubercular granulomatous cervicitis hence, she was put on category I ATT that relieved her symptoms and improved cervical findings.

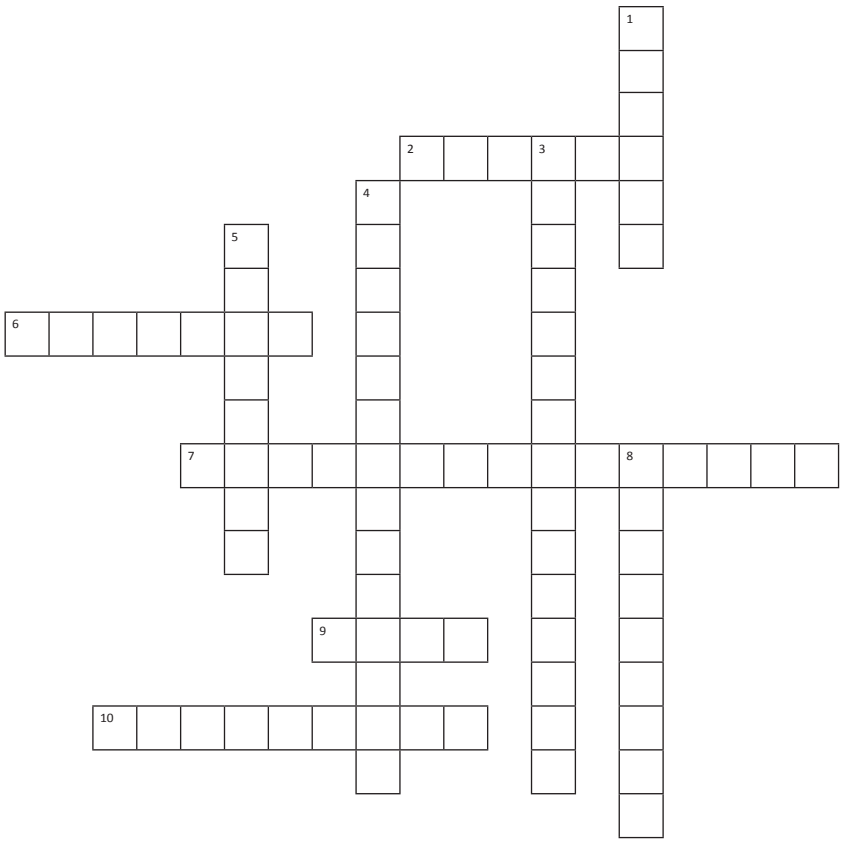
This is the first case of T. trichiura in ectopic location and the first case of live worm in genital tract. Previously reported case reports involving genial helminthiasis involve accidental findings of ova on cervical / vaginal smears or granuloma containing parasite or ova in histopathology, Studies have shown that helminthic infection can reactivate latent TB and aggravate the disease expression. People living in tropical and subtropical areas are at highest risk of infection by T. trichiura.

Keywords: Trichuris trichiura, Vaginal discharge, Granulomatous cervicitis, Tuberculosis.

Cross Word Puzzle

Ruma Satwik
Consultant, Centre of IVF and Human Reproduction, Sir Gangaram Hospital, New Delhi

CROSSWORD



Across

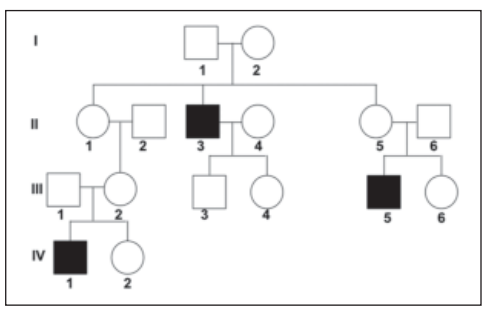
2. A fetal condition responsible of fetal growth restriction, neurologic sequelae(6)
6. A foetal congenital anomaly almost always detectable by end of first trimester (7)
7. --- anastomosis that are protective against TTTS (15)
9. Large inter-twin haemoglobin difference without oligo-poly sequence (4)
10. Case of Trisomy 13 on FISH at CVS, and normal 24-chromosome karyotype on amniocentesis (9)

Down

1. Fetal loss rate in percentage in untreated TTTS (6)
3. Fetal heart rate more than 210 beats per minute (15)
4. An autosomal dominant genetic disorder leading to short stature (14)
5. A pictorial representation of family members with and without illness and their relationships (8)
8. One non-pathologic cause of non-visualization of nasal bone in first trimester (9)

PICTORIAL QUIZ

Veronica Arora
Associate Consultant, Sir Ganga Ram Hospital, New Delhi



Questions

Q 1. What is the mode of inheritance in this pedigree?

Q 2. Give 2 examples of disorders which follow this type of inheritance.

Q 3. Individual III. 6 comes to you for pre-marital counselling?

a. What is her risk of being a carrier

b. If she is a carrier, will all her children have the disorder?

c. She is also interested to know if she can develop the disorder, if yes, what is the mechanism?

Q 4. What are the options for III.6 in pregnancy to avoid having a children with the given disorder?

Announcement

Calendar of Virtual Monthly Clinical Meetings 2020-21

29th May, 2020	B L Kapoor Hospital
26th June, 2020	VMMC & Safdarjung Hospital
31st July, 2020	AIIMS
14th August,2020	Lady Hardinge Medical College & Smt. Sucheta Kriplani Hospital
28th August, 2020	Army Hospital- Research & Referral
11th September, 2020	Indraprastha Apollo Hospital
25th September, 2020	DDU Hospital
27th November, 2020	MAMC Hospital
18th December, 2020	Sir Ganga Ram Hospital
1st January, 2021	ESIC PGIMSR Hospital
29th January, 2021	Dr RML Hospital
26th February, 2021	UCMS & GTB Hospital
26th March, 2021	Lady Hardinge Medical College
23rd April, 2021	Apollo Hospital

Answer: March 2021 Issue

Crossword

Across

2. Goitre
6. Acrania
7. Arterioarterial
9. Taps
10. Mosaicism

Down

1. Ninety
3. Tachyarrhythmia
4. Achondroplasia
5. Pedigree
8. Ethnicity

Pictorial Quiz Answers

1. X-linked recessive inheritance
2. Hemophilia A, Glucose-6-phosphate dehydrogenase deficiency
3. a. Her risk of being is a carrier is 50% as her mother is an obligate carrier.
   b. No. 50% of her sons will be affected and 50% of her daughters will be carriers.
   c. She could have symptoms. This could happen by the following mechanisms- Skewed X-inactivation, Monosomy X, Mutation or deletion on the other allele
4. Ways to prevent having
   - Prenatal diagnosis at 11 weeks by CVS or at 16 weeks by amniocentesis
   - In-vitro fertilization with Pre-implantation genetic diagnosis
   - Donor ovum

AOGD Events Held

1st February 2021 - FAQ on **"GDM"** under the aegis of AOGD.

2nd February 2021 - Webinar on **"Recurrent Hydrops Fetalis"** under the aegis of fetal Medicine & Safe Motherhood Subcommittee of AOGD.

5th, 6th & 7th February 2021 - Virtual **"Gurukul Classes"** under the aegis of ISOPARB & AOGD.

6th February 2021 - E-workshop on **"Hypertensive Disorders of Pregnancy"** by Delhi Gynaecologist Forum (South West Delhi), Safe Motherhood Committee & Manipal Hospital Dwarka, New Delhi.

9th February 2021 - Webinar **"FAQ on Menopausal Medicine"** under the aegis of AOGD.

13th February 2021 - Webinar on **"Thyroid & Menopausal and GDM Management"** by Shield Healthcare in collaboration with Reproductive Endocrinology Committee of AOGD and DGF South West Delhi.

13th February 2021 - Webinar on **"Updates on Threatened Miscarriage"** under the aegis of AOGD, NARCHI Delhi and ISCCP.

17th February 2021 - FAQ on **"Uterine Fibroid"** under the aegis of AOGD.

17th February 2021 - Webinar on **"Fetal Autopsy Unrevealing The Mystery"** by Department of Obstetrics and Gynaecology & Department of Anatomy, Maulana Azad Medical College, New Delhi under the aegis of NARCHI & AOGD Fetal Medicine Committee.

17th February 2021 - Webinar on **"AUB"** by AOGD Infertility Committee.

On 18th February 2021 - **"AOGD Virtual Monthly Clinical Meeting"** organised by Sir Ganga Ram Hospital,, New Delhi, 04:00-05:00 pm.

21st February 2021 - CME on **"Emergencies in Clinical Obstetrics"** by Department of Obstetrics and Gynaecology ESI-PGIMSR, Delhi.

22nd February 2021 - eCME on **"Exploring A Approach to Management of Threatened Miscarriage and Complications of Recurrent Pregnancy Loss: Case Based Discussion"** by Reproductive Endocrinology Committee of AOGD with Abbott.

25th February 2021 - FAQ on **"Cervical Cancer"** under the aegis of AOGD.

26th February 2021 - **"AOGD Virtual Monthly Clinical Meeting"** organized by University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi.

27th February 2021 - Webinar CME on **"Women's Health"** by Institute of Obstetrics and Gynaecology, Sir Ganga Ram Hospital, New Delhi.

27th February 2021 - Webinar on **"Breast and Cervical Cancer Prevention"** by Breast cancer and cervical cancer screening & screening committee AOGD in association with Asian Society of Mastology and American College of Surgeons.

6th March, 2021 - Webinar on **Hormones and Cancer Interplay & Borderline Ovarian Tumours How Clinical Decisions Improve Outcome?** Under the aegis of Reproductive Endocrinology Committee AOGD and DGF-South-West.

8th March, 2021 - FAQ on **Screening of Cervical Cancer** under the aegis of AOGD.

6th to 8th March, 2021 - **FOGSI Screening Camp and Awareness Drive on Preventable Cervical & Brest Cancer** under the aegis of AOGD.

Forthcoming Events

3rd March 2021 - Virtual Quiz on **"Critical Care in Obstetrics"** by Institute of Obstetrics and Gynaecology, Sir Ganga Ram Hospital, New Delhi under the aegis of AOGD.

On 5th March 2021 - FAQs on **"Multi Micro Nutrients in Pregnancy"** under the aegis of AOGD.

On 6th March 2021 - Webinar **"Providing Quality Care in Abdominal Malformations"** by Fetal medicine sub-Committee & QI committee of AOGD And Delhi Association of Paediatric Surgeons.

9th March 2021 - FAQs on **"Ovulation Induction"** under the aegis of AOGD.

16th March 2021 - FAQs on **"Female Sexual Disorders"** under the aegis of AOGD.

18th March 2021 - FAQs on **"HIV in Pregnancy"** under the aegis of AOGD.

25th March 2021 - FAQs on **"Care of Pregnant Women"** under the aegis of AOGD.

26th March 2021 - **AOGD Virtual Monthly Clinical Meeting** will beorganized by Lady Harding Medical College, New Delhi.

Congratulations to
Newly Elected AOGD Sub - Committee Chairpersons & Co Chairperson (2021-2023)

2021-2023			
Sub-Committee	**Chairperson & Co-chairperson**	**Contact No.**	**Email**
Endometriosis Committee	Dr Anjila Aneja	9810059519	anjilaaneja1966@gmail.com
QI Obst & Gynae Practice Committee	Dr K Aparna Sharma, *Chairperson* Dr Jyoti Bhaskar, *Co-chairperson*	9711824415 9711191648	kaparnasharma@gmail.com jytbhaskar@yahoo.com
Oncology Committee	Dr Sunita Malik	9818914579	svmalik@yahoo.com
Urogynaecology Committee	Dr Geeta Mediratta, *Chairperson*	9810126985	gmediratta@yahoo.com
Adolescent Committee	Dr Anita Rajorhia, *Chairperson* Dr Sujata Das, *Co-chairperson*	9711177891 9971346064	anitarajorhia716@gmail.com drdassujata2110@gmail.com
Reproductive Endocrinology Committee	Dr Surveen Ghumman, *Chairperson* Dr Deepti Goswami, Co-chairperson	9810475476 9968604348	surveen12@gmail.com drdeeptigoswami@hotmail.com
Safe Motherhood Committee	Dr Manju Puri	9313496933	drmanjupuri@gmail.com
Fetal Medicine and Genetics Committee	Dr Seema Thakur, *Chairperson* Dr Sangeeta Gupta, Co-chairperson	9818387430 9968604349	seematranjan@gmail.com drsangeetamamc@gmail.com
Endoscopy Committee	Dr Kanika Jain	9811022255	dr.kanika@gmail.com

Existing AOGD Sub-committee Chairpersons 2020-2022			
Sub-Committee	**Chairperson**	**Contact No.**	**Email**
Breast and Cervical Cancer Awareness, Screening & Prevention Committee	Dr Sushuma Sinha	9717691898	sushmasinha@gmail.com
Infertility Committee	Dr Kavita Aggarwal	9990167888	drku93@gmail.com
Rural Health Committee	Dr Seema Prakash	9818225007	seemaprakash2502@gmail.com
Multidisciplinary Patient Sub-committee	Dr Shashi Lata Kabra Maheshwari	9718990168	drshashikabra@gmail.com

AOGD members are invited to become members of various Sub-committee.

Please contact respective Chairperson.

Membership of Maximum two Sub-committee can be taken at a time.

2 winners have qualified for FOGSI USHA KRISHNA QUIZ
from Association of Obstetricians & Gynaecologists of Delhi
in the AOGD Virtual Quiz on Critical Care in Obstetrics

organised on 3rd March, 2021
Institute of Obstetrics & Gynaecology, Sir Ganga Ram Hospital, Delhi

Dr Harshiba & Dr Neha Khatri
Maulana Azad Medical College & Lok Nayak Jai Prakash Narayan Hospital, Delhi