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DNA Diaries in Congenital Anomalies

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From The President's Desk

Welcome back to SFM Bengal Chronicles.

Many of us came to fetal medicine offering to deal with many different shades of grey. Little did we know that we have to delve so much into the rich diversity of human genetics. The joyride on the pixels is getting more and more exciting with advancements of technology but it's genetics that is taking us to the final stop.

Thanks to our colleagues in genetics, we are managing pregnancy complications for the fetus and the mother with better precision. Also thanks to the SFM who took big strides for training in the subject at the right time. These days we can not think of our academic programmes without the valuable inputs from our colleagues in this fast growing branch of modern medicine.

Few years back, the cases of isolated clubfoot or facial clefts would have been passed without even discussing the options of genetic tests. Now our practice has changed for better for all the right reasons. But our knowledge base is perhaps getting outpaced with daily advancements in field of genetics. Majority of us are getting lost in the array of different emerging tests and their appropriate applications. The only way to close this potential information gap is regular updates through various instruments of continuing medical education. The Bengal chapter's current newsletter is one such step in this direction. I must thank the contributors for their valuable time in writing on these much useful topics. I hope you will enjoy reading and find them useful in your practice.

Happy reading.

Dr. Kanchan Mukherjee
President, Bengal Chapter
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Abdominal wall defects : is cascade testing the answer ?

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The abdomen is a portion of the trunk which connects the thorax and the pelvis. It is relatively deficient of skeletal support and comprises only the vertebral column and lower ribs posteriorly which results in flexibility of trunk as well as distensibility to accommodate dynamic changes in the volume of abdominal contents. The abdominal wall is the intermediate connection between the skeletal framework at the thoracic cage superiorly and pelvic bones inferiorly. It not only contains but also provides protection to the intra-abdominal organs. It is made of skin, fascia, and muscle encases the abdominal cavity and viscera. However this wall can distend, generate intra-abdominal pressure and move the vertebral column. Defects in the abdominal wall can affect the ability to contain the abdominal contents and results clinically as congenital or acquired hernias. It may result in an opening through which multiple abdominal organs may project.The abdominal wall defect is one the most common congenital anomalies with most frequent being gastroschisis and omphalocele. The incidence of gastroschisis is 3.09 per 10,000 births, with a live birth prevalence of 2.63 per 10,000 and for omphalocele 3.29 and 1.13 per 10,000, respectively. The incidence of omphalocele identified on second-trimester ultrasound is as high as 1 in 1,100 highlighting the significant rate of associated intrauterine fetal demise.

Gastroschisis takes place to the right side of the umbilicus and lacks the protective covering over the herniated abdominal contents. It is mostly found in isolation and not associated with other anomalies. The defect most likely occurs between the 5th and 8th week gestation and the pathogenesis is largely unknown.

Diagnosis & prognosis of Gastroschisis:

A detailed fetal imaging by ultrasonography is capable of identifying over 90% of the cases as early as 12th week of gestation. However, first trimester diagnosis can be interpreted with caution and confirmed later in gestation. Screening for alpha fetoprotein on the amniotic fluid is also recommended. Although the condition is not often associated with other major congenital or chromosomal anomalies, an accurate fetal anatomy assessment is required. The reported rate of the proportion of gastroschisis associated with major defects is about 10% [1], arthrogryposis being present in a minority of these fetuses [2], with a reported mortality rate of 5–10% in all cases of gastroschisis [3]. Others report a higher rate (14%) of additional associated anomalies, the central nervous system and cardiac malformations being the most common anomalies [4]. This has led to much research for gene association with the condition, however no definitive association has been reported till date.

Gastroschisis is typically thought to have a low empiric recurrence rate on the order of 3.5% for siblings. As per the study done by Kimberly J *et al.,* till now there total 9 cases of gastroschisis with familial recurrence have been reported with no definitive known genetic variant [5].

Prognosis of gastroschisis depends on the extra- and intra-abdominal bowel dilatation, stomach herniation, stomach dilatation, bowel matting, growth restriction, abnormal umbilical artery (UA) Doppler ultrasounds and abnormal amniotic fluid volume. Risk of intrauterine death (IUD) is reduced by 2.2% by intense surveillance by fetal imaging.

Omphalocele:

Omphalocele, also known as exomphalos is a congenital malformation due to a defect in closure of the anterior abdominal wall. This leads to midline herniation of the abdominal viscera covered by a membranous sac, into the base of the umbilical cord insertion[6]. The omphalocele defect can range widely in size and type of abdominal viscera present within the sac depending on when during gestation the arrest in bowel rotation occurs. Postnatal outcomes in infants with omphalocele are predominantly dependent on other concurrent anomalies or comorbidities associated with larger-sized defects.

In about 63-80% cases omphalocele is found to be associated with other congenital anomalies and genetic syndromes. In about 38-67% cases there is an underlying chromosomal defect. One of major chromosomal aberrations noted in Trisomy 13 (22-89% cases). As per the review done by Henriette Poaty *et al*., the average incidence of 77.2% for trisomy 18 and of 11.4% for trisomy 13. The other chromosomal aberrations found to be associated includes triploidy;
monosomy X (Turner syndrome); 47, XXY (Klinefelter syndrome); trisomy 16 and 21 (very low contributors); partial trisomy such as dup (1q), dup (3q), dup (4q), dup (5p), dup (6q), dup (11p), dup (15q23), dup (17q) or deletion like del (1q), del (9p); inv (11) [7-10].

Apart from the chromosomal aberrations, omphalocele is also caused due to genetic syndrome and is found to be familial. The most common genetic syndrome associated with omphalocele is Beckwith-Wiedemann syndrome (BWS),

seen in 3 to 22% of omphaloceles. BWS is caused by paternal uniparental disomy of the 11p15 imprinted chromosomal region. In case of BWS, along with omphalocele polyhydramnios, macrosomia, macroglossia, visceromegaly, abdominal wall defect, external ear abnormalities is detected in the prenatal ultrasound. Several other genetic syndromes including Miller-Dieker lissencephaly syndrome (microcephaly, lissencephaly, small brain, deletion on 17p13.3 band inherited in autosomal dominant mode); Pallister-killian syndrome (coarse dysmorphic facies, mental retardation, skin anomalies, tetrasomy 12p); Meckel-Grumber (occipital encephalocele, postaxial polydactyly, multicystic dysplastic kidneys, inherited on autosomal recessive mode); Goltz syndrome (X-linked dominant trait) and Marshallsmith syndrome has been reported.

Diagnosis & Prognosis of Omphalocele:

Omphalocele is typically diagnosed on prenatal ultrasound. The timing of prenatal diagnosis for omphalocele has shifted over the last two centuries from near-universal identification within the second trimester to almost half being identified during the late first trimester (11–14 weeks gestation). Comprehensive prenatal ultrasonography along with fetal echocardiogram allow for evaluation of other structural defects and are critical components of the prenatal workup for omphalocele. As per ACOG, a detailed chromosomal study by chromosomal microarray should be recommended along with detailed genetic counseling.

The prognosis of omphalocele largely depends on the size of the defect and associated congenital anomalies and syndromes. As such, large omphalocele with associated abnormalities have a higher mortality rate. Also, neonates with liver protrusion through the defect appear to have a poorer prognosis. The overall survival rate is close to 80%, which reflects the efficacy of prenatal diagnosis and the decision taken by many families to terminate pregnancies with severe anomalies.

Considering the etiology and outcome of both the conditions, prenatal diagnosis plays an extremely important role in order to understand the prognosis and further management. At present with the advanced fetal imaging the detection rate has been raised and association of genetic syndromes has to be ruled out in case of familial cases of gastroschisis as well for omphalocele. This has increased the number of prenatal mortality due to elective terminations. Serum markers like Maternal Serum Alpha Feto Protein (MSAFP) screening test is also a good marker indicative for abdominal wall defect. All the cases with gastroschisis and omphalocele shall be treated with multidisciplinary approach for both diagnosis and further management of pregnancy or in the neonatal period. Genetic Counseling helps to understand the familial prevalence and also helps to understand the recurrence risk. For any case with gastroschisis or omphalocele a detailed fetal imaging is highly recommended followed by fetal echocardiogram and chromosomal microarray in case of omphalocele. For the familial cases and with definitive association of other anomalies such as polycystic kidney, further exome sequencing should be considered.

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Role of chromosomal microarray in the etiological diagnosis of congenital cardiac defects: a Systematic Review and Meta Analysis

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Introduction:

Congenital heart disease (CHD) is the most common type of birth defect and is a leading cause of infant mortality worldwide. The etiology of CHD is complex and can involve genetic, environmental, and multifactorial factors. Traditional diagnostic methods, such as karyotyping and fluorescence in situ hybridization (FISH), have limitations in detecting the genetic causes of CHD, particularly those involving submicroscopic chromosomal abnormalities. Chromosomal microarray (CMA) is a newer genetic test that can detect chromosomal imbalances at higher resolution and with greater accuracy than traditional methods. The objective of this review article is to evaluate the utility of CMA in the etiological diagnosis of CHD and to provide a comprehensive overview of the current evidence regarding the use of CMA in this population. Specifically, this review aims to answer the following research question: What is the diagnostic yield of CMA in the etiological diagnosis of CHD, and how does it compare to traditional diagnostic methods? The findings of this review will have important implications for clinical practice and public health policy, as it will inform the use of CMA in the diagnosis and management of CHD, ultimately improving patient outcomes.

Methods:

a. Search strategy and selection criteria:

A comprehensive literature search was conducted using the electronic databases PubMed, Embase, and Cochrane Library to identify studies published between January 2000 and December 2022 that evaluated the utility of chromosomal microarray (CMA) in the etiological diagnosis of congenital heart disease (CHD). The search strategy used a combination of relevant keywords and MeSH terms, including "chromosomal microarray", "congenital heart disease", "genetic testing", and "diagnosis". The search was limited to studies involving human subjects and published in English.

Two reviewers independently screened the titles and abstracts of all identified studies to assess their eligibility for inclusion in the review. Studies that met the following inclusion criteria were included: (1) included patients with CHD, (2) used CMA as a diagnostic tool, (3) reported on the diagnostic yield of CMA in the etiological diagnosis of CHD, and (4) were published in peer-reviewed journals. Studies that did not meet these criteria or were reviews, case reports, or conference abstracts were excluded.

b. Quality assessment of included studies:

The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) for case-control studies. This tool assesses the risk of bias in three domains: selection of cases and controls, comparability of cases and controls, and assessment of outcome. Studies were awarded a score out of 9, with higher scores indicating higher quality.

c. Data synthesis methods:

Data were extracted from each included study by two independent reviewers and entered into a standardized data extraction form. The following data were extracted: study design, sample size, patient characteristics, type of CHD, type of CMA used, diagnostic yield of CMA, and type of chromosomal abnormality detected. Any discrepancies in data extraction were resolved through discussion between the two reviewers.

A meta-analysis was conducted to estimate the overall diagnostic yield of CMA in the etiological diagnosis of CHD. Pooled estimates of diagnostic yield were calculated using a random-effects model, and heterogeneity was assessed using the I^2 statistic. Subgroup analyses were conducted to explore sources of heterogeneity. Sensitivity analyses were also conducted to assess the robustness of the results to different inclusion criteria and statistical methods. We assessed the quality of the included studies using the QUADAS-2 tool. We calculated pooled sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratio (DOR), and area under the receiver operating characteristic curve (AUC) with 95% confidence intervals (CI) using a bivariate random-effects model.

The results of the systematic review and meta-analysis were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Results:

A total of 23 studies met the inclusion criteria and were included in the systematic review and meta-analysis. These studies included a total of 10,784 patients with CHD who underwent CMA testing as part of their diagnostic evaluation.
The majority of the included studies were retrospective case-control studies, with sample sizes ranging from 41 to 4,300 patients. The types of CHD included in the studies varied widely, with the most common being ventricular septal defects, atrial septal defects, and tetralogy of Fallot.

The diagnostic yield of CMA in the etiological diagnosis of CHD ranged from 1.7% to 22.2%, with a pooled estimate of 7.7% (95% confidence interval [CI], 6.2%-9.3%). The pooled estimates for the sensitivity, specificity, PPV, and NPV of CMA in the diagnosis of chromosomal abnormalities in CHD were 21.9% (95% CI 19.0-24.9%), 99.5% (95% CI 99.2-99.8%), 56.6% (95% CI 48.1-65.1%), and 98.4% (95% CI 98.0-98.8%), respectively. The DOR was 231 (95% CI: 109-491), and the AUC was 0.97 (95% CI: 0.95-0.98). The most common chromosomal abnormalities detected by CMA were copy number variations (CNVs), which accounted for approximately 80% of all abnormalities detected.

CMA has also been shown to have a higher diagnostic yield than traditional cytogenetic methods in patients with CCDs. In a study by Breckpot et al., CMA was performed in 411 patients with CCDs who had a normal karyotype and FISH analysis. CNVs were identified in 16% of patients, and the diagnostic yield of CMA was significantly higher than that of karyotyping (16% vs. 3.7%, $p<0.0001$) and FISH analysis (16% vs. 5.4%, $p<0.0001$).

In addition to its diagnostic utility, CMA has also provided insights into the genetic architecture of CCDs. Several studies have identified novel CNVs and genes associated with CCDs, including recurrent CNVs involving genes such as GATA4, TBX5, and NKX2.5. These findings have led to a better understanding of the molecular mechanisms underlying cardiac development and have the potential to inform the development of new therapies for patients with CCDs.

Strengths and weaknesses of studies:

The strengths of the included studies were their large sample sizes and the use of a standardized diagnostic test (CMA) across all studies. The studies also had relatively high quality scores on the NOS, indicating low risk of bias.

However, there were several weaknesses of the studies that should be noted. First, there was substantial heterogeneity in the types of CHD and the patient populations included in the studies, which may have contributed to the variability in diagnostic yield. Second, there was variability in the type of CMA used across studies, with some studies using lower resolution arrays that may have missed smaller chromosomal abnormalities. Finally, there was a lack of standardized reporting of outcomes across studies, which made it difficult to compare results and conduct meaningful subgroup analyses.

Summary of findings:

The findings of this systematic review and meta-analysis suggest that CMA has a moderate diagnostic yield in the etiological diagnosis of CHD, with a pooled estimate of 7.7%. The most common chromosomal abnormality detected by CMA was CNVs. However, there was substantial heterogeneity in the types of CHD and patient populations included in the studies, which may limit the generalizability of the results. Further research is needed to better understand the factors that influence the diagnostic yield of CMA in the diagnosis of CHD and to develop standardized guidelines for the use of CMA in this population.

Discussion:

Interpretation of findings:

The results of this systematic review and meta-analysis indicate that chromosomal microarray (CMA) has a moderate diagnostic yield in the etiological diagnosis of congenital heart disease (CHD), with a pooled estimate of 7.7%. CNVs were the most common chromosomal abnormality detected by CMA. While these findings suggest that CMA may be a useful diagnostic tool in the evaluation of CHD, there was substantial heterogeneity in the types of CHD and patient populations included in the studies, which may limit the generalizability of the results.

Implications for clinical practice and public health policy:

The findings of this review have important implications for clinical practice and public health policy. Given the moderate diagnostic yield of CMA in the etiological diagnosis of CHD, clinicians may consider incorporating CMA into their diagnostic evaluation of patients with CHD. However, the heterogeneity in the types of CHD and patient populations included in the studies highlights the need for more research to better understand the factors that influence the diagnostic yield of CMA in this population.

In addition, the findings of this review suggest that there may be an opportunity for public health policies to promote the use of CMA as a diagnostic tool in the evaluation of CHD. Increased access to CMA testing may improve the accuracy of etiological diagnosis and may ultimately lead to more effective management and treatment of CHD.

Gaps in current knowledge and future research directions:

Despite the moderate diagnostic yield of CMA in the etiological diagnosis of CHD, there are still several important gaps in our current knowledge. First, the factors that influence the diagnostic yield of CMA in this population are not well understood. Further research is needed to identify patient and disease-related factors that may influence the diagnostic yield of CMA in the evaluation of CHD.

Second, there is a lack of standardized reporting of outcomes across studies, which makes it difficult to compare results and conduct meaningful subgroup analyses. The development of standardized reporting guidelines would help to improve the quality of research in this area.

Finally, there is a need for more research on the long-term outcomes of patients with CHD who receive a diagnosis through CMA testing. This information would help to determine the clinical significance of CNVs and other chromosomal abnormalities detected by CMA in the etiological diagnosis of CHD.

Conclusion:

In conclusion, this systematic review and meta-analysis provides evidence that chromosomal microarray (CMA) has a moderate diagnostic yield in the etiological diagnosis of congenital heart defects (CHD), with CNVs being the most commonly detected chromosomal abnormality. The study highlights the potential use of CMA as a useful diagnostic tool in the evaluation of CHD, but also identifies several limitations including heterogeneity in the types of CHD and patient populations included in the studies.

The strengths of this study include the rigorous search and selection criteria, the inclusion of studies from multiple databases, and the use of meta-analysis to estimate the overall diagnostic yield of CMA. However, the limitations of this study include the potential for publication bias and the heterogeneity of the included studies.

Based on the findings of this study, we recommend that clinicians consider incorporating CMA into their diagnostic evaluation of patients with CHD, and that public health policies should promote increased access to CMA testing for these patients. Further research is needed to identify patient and disease-related factors that may influence the diagnostic yield of CMA in the evaluation of CHD, to develop standardized reporting guidelines, and to investigate the long-term outcomes of patients with CHD who receive a diagnosis through CMA testing.

Overall, this study provides important insights into the use of CMA in the etiological diagnosis of CHD, and highlights the need for continued research in this area to improve the accuracy of diagnosis and ultimately improve the management and treatment of CHD.

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Prenatal Exome Sequencing: A Boon or a Bane?

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"**With great power comes great responsibility**" is an adage popularized by Spider-Man in Marvel comics, films, and related media The statement holds true today, for the use of the powerful diagnostic tool "Exome sequencing". Exome sequencing (ES) is a Next-generation sequencing (NGS) based test where the coding regions of all/ most genes are sequenced to find a variation. With the fast growing technology of sequencing, production of powerful machines with ever enhancing software solutions and mushrooming of NGS laboratories, this diagnostic technique is reaching the clinicians, paramedical staffs and patients with ease. Marketing teams of such NGS labs and their scientific personalities have started projecting exome sequencing as a "one stop solution" for genetic diagnosis. They are selling it with different new wrappers with different names. The clinicians who are unable to keep pace with recent knowledge and their semi-trained supportive staffs have started ordering the test indiscriminately, leading to wrong diagnosis, stigmatization and unnecessary pregnancy terminations. The uncertainties and adversities arising from such practice is causing immense distress to the end users, raising various ethical and legal concerns. It's a big enigma now: who is really authorized to order such test and when should it be used, especially in prenatal setup.

In pediatric and adult populations, clinical ES provides a diagnosis in 25–29% of individuals with disorders suggestive of a genetic aetiology [1, 2]. Given the success in children and adult patient populations, and the limitations of available genetic diagnostic tests, ES is now increasingly being applied in the clinical scenarios with fetuses with anomalies detected by ultrasound.

Significant fetal structural anomalies/ major congenital anomalies (MCA) affects 2–3% of all pregnancies and are responsible for significant pre- and perinatal mortality and neonatal morbidity [3,4]. The etiology is heterogeneous, ranging from non-genetic conditions like fetal infections and fetal teratology to genetic causes. Given respect for reproductive autonomy, all patients diagnosed with a fetal anomaly should be offered genetic counselling [5], including review and understanding of options for genetic testing like antenatal karyotype, QF-PCR, chromosomal microarray analysis (CMA) etc. CMA has been widely implemented in the analysis of invasively obtained prenatal samples (amniotic fluid or chorionic villi) for the genome wide detection of both aneuploidies and microdeletions/microduplications (copy number variants or CNVs). In up to 40% of pregnancies with a fetal structural anomaly, CMA is able to diagnose an aneuploidy or CNV [6], still leaving more than half of the cases undiagnosed. During prenatal testing, doing QF-PCR on the fetal and maternal samples is being widely practised now a days. Comparing the markers of the fetal with that of the maternal sample, maternal contamination is being ruled out, before proceeding for any further test. In that process QF-PCR also looks for major aneuploidies like that of chromosomes 21, 18, 13, X and Y. The diagnostic yield of QF-PCR and CMA has been depicted below (Figure 1) [7].

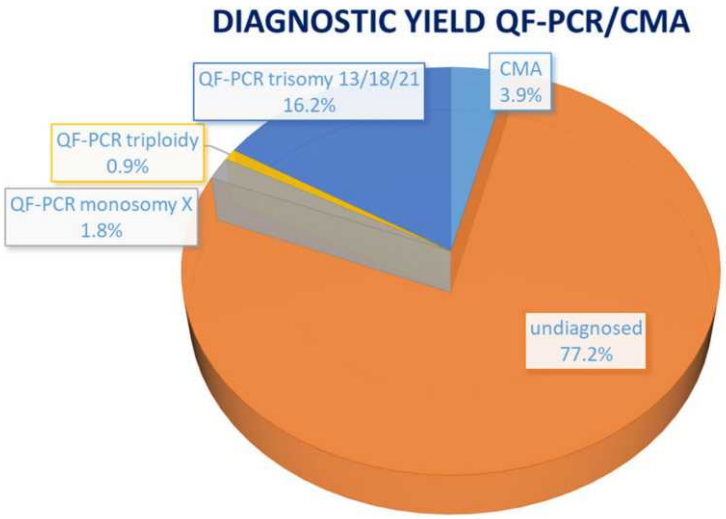

Figure 1: Diagnostic yield of different molecular cytogenetic tests (QF-PCR and CMA) on fetuses with structural anomalies (Janicki E et al)

Considering both structurally normal and abnormal fetuses, the diagnostic yield of CMA in a series of patients reported by our team was overall 5.5% [8]. In structurally abnormal foetuses yield was 8.8% and in foetuses with a high aneuploidy risk yield was 4.7%. A large part still remains undiagnosed. It is obvious that there would be various single gene disorders with small scale mutations which won't be picked up by screening the genome for copy number variations by CMA. For most such cases we need sequencing looking at nucleotide by nucleotide to find sequence variations. There would be various single gene disorders with small genes or few mutations at hotspots like that in achondroplasia or Apert syndrome [9]. In such situations, targeted mutation testing by PCR based methods or Sanger sequencing prevents unnecessary diagnostic delay and waste of resources.

However, there would be a huge number of cases where clinical diagnosis in the structurally abnormal fetus points towards a probable single gene disorder with a large gene or many putative genes. Here, NGS based test like exome sequencing in the prenatal sample is likely to be helpful in predicting outcome and help the family to take reproductive decision in the present and future pregnancies. In a recent paper we have shown how various ciliopathies like Meckel-Gruber syndrome with autosomal recessive pattern of inheritance can be inherited from unaffected parents. These ciliopathies are genetically heterogeneous (many genes are implicated) but might have very similar pattern of fetal malformations. Considering NGS (exome sequencing) as the preferred method of diagnosis in such situations is a prudent decision (Figure 2 and Figure 3) [10]. It is important to note that a good pedigree chart, clear clinical information and differential diagnoses need to provided to the NGS lab so that the lab personnel can easily deduce HPO (Human Phenotype Ontology) terms during data analysis and reporting.

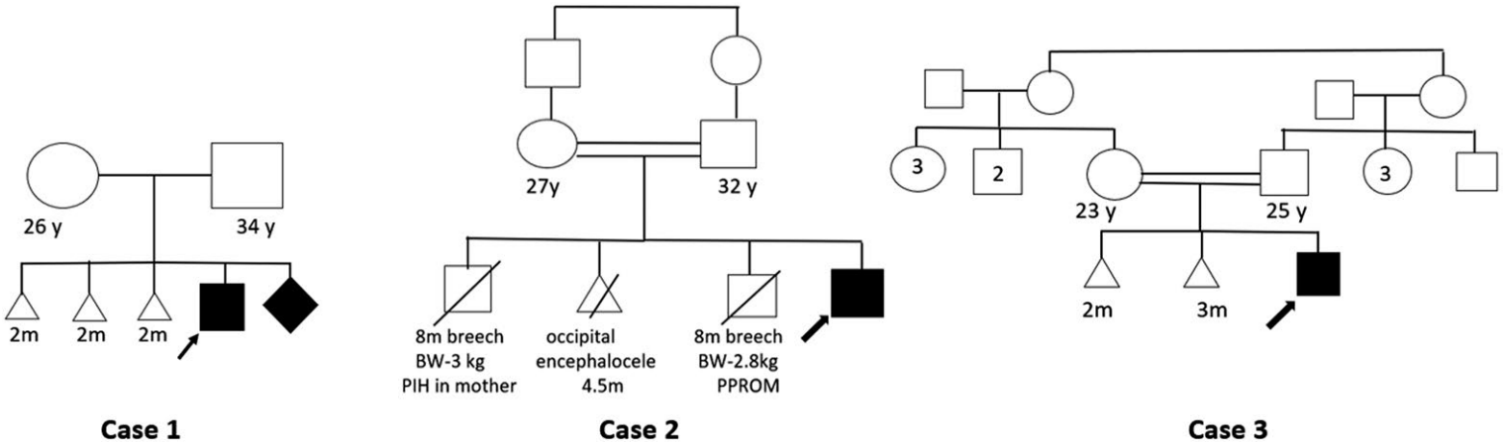

Figure 2: Typical autosomal recessive pedigrees of families with ciliopathies (Mandal K et al)

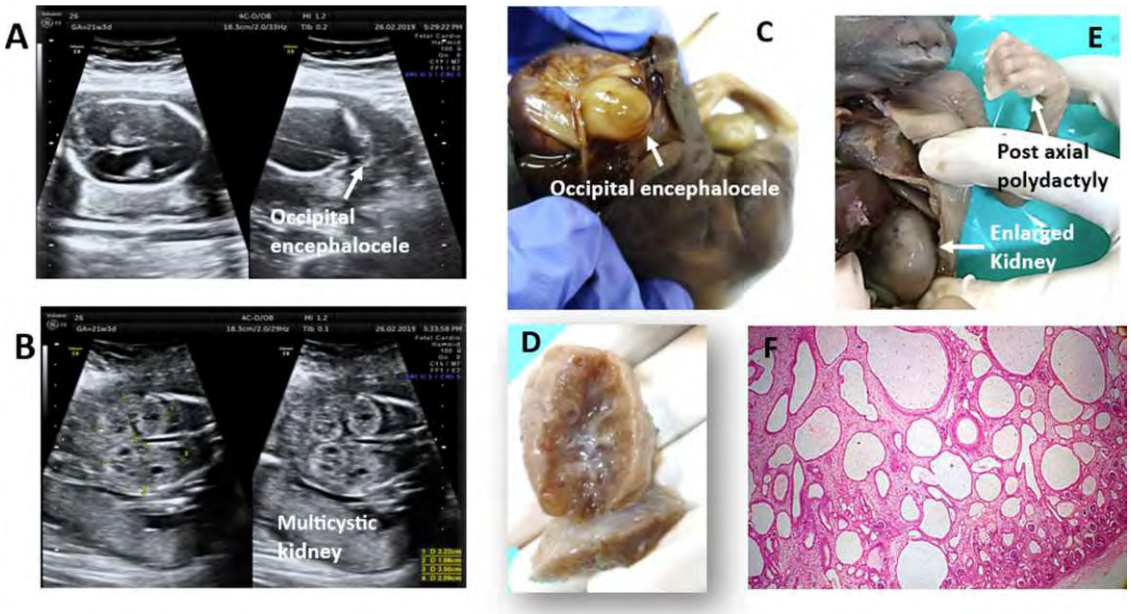

Figure 3: Ultrasound, fetal autopsy and histopathology images in a case with Meckel-Gruber syndrome (Mandal K et al)

Several recent meta-analyses have demonstrated diagnostic yield of 1.8–68% for prenatal whole exome sequencing (WES), with the yield largely depending on the inclusion criteria and organ system affected. With increasing evidence of the relevance of WES in the prenatal context, revision of the guidelines of the International Society for Prenatal Diagnosis (ISPD) offers directions on how to implement it [11].

A useful guideline for considering fetal exome can be obtained from the American College of Medical Genetics and Genomics (ACMG) position statement [12]. Some pre-test considerations are as below:

- Exome sequencing may be considered for a fetus with ultrasound anomalies when standard CMA and karyotype analysis have failed to yield a definitive diagnosis. If a specific diagnosis is suspected, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test.

- Clinicians should seek guidance from the laboratory (or medical geneticist) regarding the methods and choice of available testing.
- Exome sequencing is a phenotype-driven test, therefore, the ordering health-care professional should provide the testing laboratory with adequate information required to generate the most accurate interpretation of results.
- Trio analysis consisting of the proband and both biological parents is preferred to singleton (fetus only) or duo (fetus and one parent) analyses. Trio analysis consistently shows higher diagnostic yields compared with nontrio analysis.
- Pretest counseling is ideally provided by a genetics professional during which the types of variants that may be returned in a laboratory report for all tested family members would be reviewed.
- With the use of prenatal ES, the turnaround time has to be rapid to maintain all aspects of reproductive choice.
- Sufficient specimen quantity is required for a rapid turnaround time, and ordering providers should be considerate of specimen requirements established by the testing laboratory.
- As with all prenatal genetic studies, the presence of maternal cell contamination that may interfere with the interpretation of fetal results must be excluded.

Considering the easy availability of exome sequencing facility we should be extremely cautious about the use of this powerful tool for fetal testing. We need to keep us updated with the recent advances and guidelines, or else there can be devastating outcome both in terms of patient care and legal consequences.

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Echogenic Kidney: Investigations and Implications

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The science of Fetal Medicine has enabled the enhanced care of a baby right from the antenatal period. Advances in technology and expertise in the field of Fetal Medicine have assisted in the improvement of fetal surveillance to help detect high risk pregnancies where potentially compromised foetuses may be identified.

Advances in ultrasound technology have had a significant contribution to the surveillance during the antenatal period. It has also helped in the improved evaluation and diagnosis of fetal anomalies, which may further be evaluated through rapid testing options to understand the diagnosis, prognosis and assist in parental counseling by multidisciplinary healthcare teams regarding the way forward for the pregnancy. Ultrasound imaging is commonplace in the fetal medicine setup which is used to monitor the growth and development of fetal organs and identify possible structural anomalies at crucial time points during the gestational period.

Fetal echogenic kidneys are a type of renal finding which is detected during an antenatal ultrasound, which may be suggestive of a possible kidney condition. During the ultrasound scan, the brightness or echogenicity of the fetal kidney is quantified as equal to that of the liver. If the kidneys are noted to appear brighter than the liver, they are considered to be echogenic or hyperechogenic.

Echogenic kidneys in the foetus may be considered as a non-specific finding in cases where they may be the sole finding. However, in certain cases it may be indicative of a possible kidney anomaly. Echogenic kidneys may be associated with a wide spectrum of kidney disorders with variable outcomes.

An echogenic kidney may be caused due to multiple factors such as:

- Physiologic variation
- Obstructive uropathy
- Renal vein thrombosis
- Cytomegalovirus infections
- Genetic conditions- Chromosomal disorders or single gene disorders

Genetic syndromes associated with echogenic kidney findings:

- Chromosomal Disorders: Trisomy 21, Trisomy 18 and Trisomy 13
- Intrinsic renal disease
    - Autosomal dominant polycystic renal disease
    - Autosomal recessive polycystic renal disease
- Meckel-Gruber Syndrome
- Bardet-Biedl syndrome
- Congenital nephrotic syndrome

As it is difficult to ascertain the fetal renal function during pregnancy, the diagnosis and prognosis establishment poses a significant challenge in the clinical treatment and prenatal counseling for such cases. Further clinical evaluation and testing may enable clinicians in providing a diagnosis, prognosis and possible management and treatment options based upon the type of disorder noted.

Clinical Evaluation and Investigations for echogenic kidneys:

A detailed clinical evaluation and family history is important for further testing. The clinical evaluation for the fetus/ gestation may include:

1. Presence of bilateral or unilateral echogenic kidney
2. High risk on combined first trimester screening, quadruple marker test or non-invasive prenatal screening test (NIPT)
3. Presence of other ultrasound soft markers such as nuchal translucency, absent nasal bone, intracardiac echogenic focus, echogenic bowel, short femur and humerus, pyelectasis, or choroid plexus cysts
4. Structural anomalies in the genitourinary tract such as an enlarged kidney, hypoplastic or dysplastic kidneys
5. Amniotic fluid index (oligohydramnios or polyhydramnios)

Congenital anomalies in other organs systems such as the central nervous system (CNS), cardiovascular system et

When to consider genetic testing?

Genetic conditions with an associated renal presentation may be due to chromosomal changes such as aneuploidies or microdeletions/ microduplications or due to single gene disorders in the fetus. The clinical evaluation and phenotyping is useful in choosing the right genetic test.

a. Based upon the clinical presentation, if the echogenic kidney finding is associated with another soft marker(s) or high risk biochemical screening test, indicative of possible chromosomal aneuploidy, further genetic testing may be recommended for the fetal sample. To rule out chromosomal aneuploidies, testing options may include cytogenetic tests such as karyotype and/or fluorescence *in situ* hybridization (FISH) or molecular tests such as QF-PCR or Chromosomal Microarray (CMA) may be recommended.

b. In cases with a prenatal finding of hyperechogenic kidneys with genitourinary tract anomalies or other congenital anomalies, there may be suspicion of a chromosomal microdeletion/ microduplication syndrome such as the 17q12 microdeletion syndrome or Williams Beuren Syndrome (chromosome 7q11.23 microdeletion). Prenatal Chromosomal Microarray may be recommended to rule out such microdeletion/ microduplication syndromes.

When the echogenic kidney finding is associated with renal or other congenital anomalies, these may be suggestive possible Copy Number Variations (CNVs) such as microdeletion/ microduplication syndromes or may be indicative of single gene disorders.

c. In cases where CNVs have been ruled out using CMA or there is a high degree of suspicion of single gene disorders, further advanced genetic testing may be recommended. Single gene disorders may include disorders such as Bardet Bieldl Syndrome, Meckel Gruber Syndrome, Polycystic Kidney Disease (autosomal recessive or autosomal dominant), Congenital Nephrotic Syndrome. In such cases prenatal Next Generation Sequencing (NGS) such as Exome Sequencing may be recommended. There are currently a number of NGS panels available based upon the type of disorder being screened. The most commonly sought panel is the Clinical Exome Sequencing panel which looks into a subset of clinically relevant genes associated to known single gene disorders. However, with the advancement and cost effectiveness of the NGS technology, there is a gradual shift towards the option for prenatal Whole Exome Sequencing leading to a possible increase in diagnostic yield.

The choice of genetic test for a case is dependent upon the clinical presentation of the fetus, and may vary based upon the differential diagnosis. In certain cases, multiple genetic tests may be recommended to help rule out the suspicion of different genetic conditions in the fetus

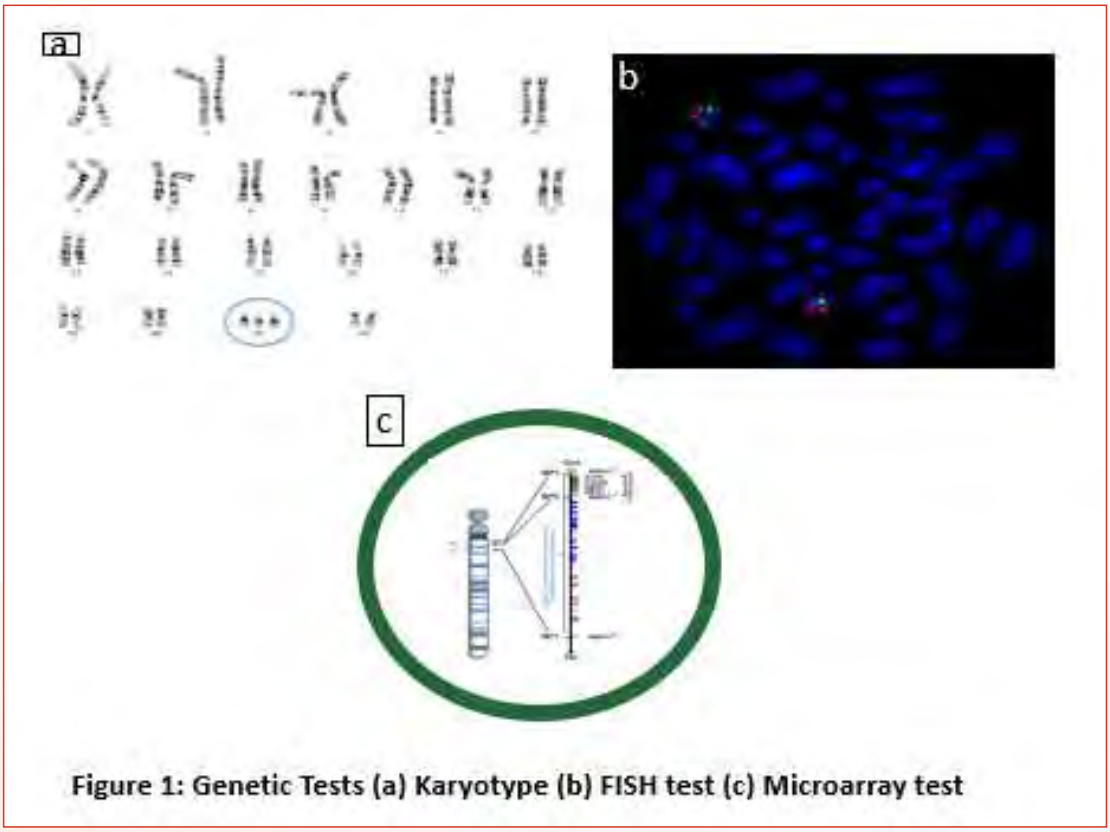

Figure 1: Genetic Tests (a) Karyotype (b) FISH test (c) Microarray test

Implications and counseling:

Genetic testing may assist clinicians in establishing a diagnosis for the fetus. This further helps in counseling the parents to understand the condition identified, its associated symptoms, and prognosis during the prenatal period itself. Often there are multiple rounds of counseling by multidisciplinary healthcare teams, to help parents understand the possible options for management or treatment, if available. Such extensive testing and counseling may help the potential parents to take an informed decision regarding the pregnancy and their future baby. A genetic diagnosis may also assist couples in understanding the risk of recurrence in subsequent pregnancies.

Summary:

The lone echogenic kidney finding may not be significant in itself, but when associated with other findings in the prenatal setting may help uncover a significant diagnosis in the fetus at an early stage. Such a finding when detected, may warrant further evaluation and testing to rule out any potential medical conditions and help to-be parents take an informed decision regarding the management of their pregnancy.

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Approach to Persistent Increased Nuchal Translucency : A Diagnostic Dilemma

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Nuchal translucency (NT) is the measurement of the widest thickness of fluid collection under the skin in fetal neck, which is evaluated by an ultrasound scan between 10 and 13 weeks of gestation, with crown to rump length of the fetus corresponding between 45-84mm. NT is measured in a mid-sagittal view of the fetal face, defined by the presence of the echogenic tip of the nose and rectangular shape of the palate anteriorly, the translucent diencephalon in the center and the nuchal membrane posteriorly.

More than 90% of fetuses with the major aneuploidies have been identified by measuring fetus NT value during the first trimester at a false positive rate of 5% in combination with maternal serum markers . Therefore, first-trimester NT value is an important indication in prenatal diagnosis, which has been widely used clinically. Up till now, more than one hundred of diseases have been confirmed to be related to increased NT, whereas such association remain vague and more diseases are expected to be discovered in future.

Thus, fetal nuchal translucency (NT) has brought about a paradigm shift in predicting the health of fetus and inverting the pyramid of antenatal care where first trimester now offers an array of opportunity to evaluate the fetus. However, the association of persistent increased NT has been a molecular diagnostic dilemma in view of the spectrum of diseases that need to be screened. Previous publications demonstrated that fetuses with increased NT are at high risk of chromosomal abnormalities , fetal structure defects and other pathological conditions, including congenital heart disease , infection in utero and genetic syndromes .

NT ≥ 99th centile (≥3.5 mm), found in around 1% of pregnancies, is associated with chromosomal anomalies, monogenic conditions , heart malformations and other malformations in euploid fetuses . NT ≥ 3 mm, found in around 5% of screened fetuses , is considered a high risk factor for aneuploidies, while the interpretation of the 2.5–2.9 mm NT is controversial.

Though NT is a gold standard with maternal serum screening , standalone NT measurements do not do much justice. This was depicted in a study by Lisa Hui et al,in 2007, where more than 80,000 fetuses with NT <3.0 mm were presented, to illustrate that most of the atypical chromosome conditions occur in fetuses with a “normal” NT. Clinicians need clear, evidence-based recommendations for the diagnosis and management of an increased NT, even though most of the atypical chromosome conditions are ascertained through other means. There are still remarkable inconsistencies in the definition of an increased NT. In view of this population-based data set, suggested that a 3.0 mm threshold for an increased NT proposed by the American College of Obstetricians and Gynecologists overcalled the risk of an atypical chromosome condition and that 1.9 multiples of the median is a more appropriate threshold for this purpose.

Established there is isolated risk or a presentation of NT with other anomalies, diagnostic testing becomes mandatory . In the present diagnostics platter , test choices range from Karyotype to exome sequencing. It is important to understand the yield of genetic diagnostics while offering such tests to have information that might be valuable.

Meta analysis have established , that diagnostic yield for aneuploidy for fetus with isolated NT measuring at 2.5 - 3.0mm , is about 1-4 % . Non invasive prenatal screening has strong concordance and with karyotype and detection rate, in this subgroup . In this range, the rearrangements identified with CMA appear to be not frequent and the main chromosomal abnormalities responsible can also be identified by NIPS. NIPS in this group might be beneficiary but not conclusive.

4. Structural anomalies in the genitourinary tract such as an enlarged kidney, hypoplastic or dysplastic kidneys
5. Amniotic fluid index (oligohydramnios or polyhydramnios)

Congenital anomalies in other organs systems such as the central nervous system (CNS), cardiovascular system et

A recent publication by Gioia Mastromro et al , 2021, analyzing a cohort of 96 fetuses with isolated NT ≥ 2.5 mm showed karyotype anomalies in 22.76% of cases and CMA presented an incremental detection rate of 2.35%. Fetuses with isolated NT ≥ 3 mm presented aneuploidies in 14.36% of cases and CMA had an incremental detection rate of 3.89%. When the isolated NT measured at least 3.5 mm the diagnostic yield of karyotyping was 34.35%, the incremental CMA detection rate was 4.1%, the incremental diagnostic rate of the RASopathy panel was 1.44% and it was 2.44% for exome sequencing. Interestingly, CMA presents a considerable diagnostic yield in the group of fetuses with NT ≥ 3.5 mm.

The causes for persistent increased NT with other anomalies can be due to chromosomal aneuploidy in 20- 50 % of the fetus , thus indicating chromosomal analysis as the first line of investigation in such cases. However, euploid fetus with persistent increased NT> 4.5 mm, could also indicate single gene disorders .

Since the 1990's, extensive studies have established that euploid fetuses with increased nuchal translucency has subsequently a possible congenital heart defect. L Orosz et al described higher risk for wide range of fetal structural defects especially for congenital heart defects (CHDs) and also for specific genetic syndromes. The prevalence of CHDs, including the defects of the great vessels, stood out among the others. In a cohort of 36 fetuses published by L Orosz et al ,2009, it was reported , 11 % of that fetuses with NT between 95th and 99th centiles had minor heart problems . The rate of major cardiac defects proved to be 13.3% in the group with NT between 3.5-4.4 mm, and 17.3% in the group with NT > 4.5 mm. About 9.8% resulted in IUFD

For pregnancies enrolled in the first trimester with an increased NT of at least 3.5 mm, a relatively low rate of diagnostic variants (1.8%) from prenatal exome seqeuning for isolated increased NTs that remained isolated throughout the pregnancy was reported. However, there was an increased diagnostic rate where fetuses had additional structural anomalies or hydrops, either at presentation (22.2%) or developing later in pregnancy (32.4%). It was also illustrated that there was significantly higher diagnostic rates where the size of the isolated increased NT was larger at presentation.

Among genes identified, up to 29% of cases, pathogenic variants had been identified, including RASopathies, inborn errors of metabolism, musculoskeletal disorders, lymphatic, cardiovascular, neurodevelopmental and hematologic diseases. Such variants also help to predict the prognosis , and the recurrence risk and action can be taken by the parents accordingly.

Although exome has significantly increased the rate of diagnoses in such cases , with a diagnostic yield of only 25–30%, trio analysis that are recommended are often not a viable option in India. Sequencing of the fetal DNA in isolation , makes interpretation difficult as reporting a variant of uncertain significance (VOUS) becomes a challenge. VOUS are hard to interpret in absence of India-specific normal population data, and absence of functional analysis. Parental targeted sequencing of the variant often is not possible due to advanced gestational age of presentation and / financial limitations.

Also imperative is reporting of likely pathogenic/pathogenic variants that might not explain the fetal phenotype. In line with the ACMG protocols, these variants should not be initially reported to the parents. Not all phenotypes are known to be found in a fetus, thus additonal parental / family member testing might be needed. This dilemma of reporting also raises ethical issues in reporting for fetal exomes highlighting the limitations of fetal phenotyping, and emphasizes how with prenatal sequencing we are expanding our understanding of fetal phenotype–genotype relationships that were previously only recognized postnatally. Documenting this growing knowledge is essential for accurate prenatal interpretation and complete reproductive genetic counselling in future cases. These issues highlight the need for careful review of family and past obstetric history, as well as careful, expert parental examination when considering the underlying aetiology of increased NT to guide molecular testing, particularly where genes exhibit variable penetrance or expression. While such consults it is also important to mention that a genetic cause might not be identified. A negative genetic report might not rule out underlying genetic etiology. In view of the complexity of such testing , every genetic test should be done by a pretest and post counselling to prepare the couples for the cascade testing and set their expectations.

Since the prevalence of CHD is 100 times higher in the population of fetuses with NT above 4.5 mm, specialist fetal echocardiography should be offered in the second trimester together with other follow-up investigations especially in cases where no genetic etiology has been established. Among the euploid fetus with a negative genetic report , postnatal follow up indicated that though children were without any major abnormalities, a high number of minor anomalies were revealed during the long-term. These anomalies do not have significant disadvantage to the quality of life, but some of them necessitates short or long-term medical treatment and this should also be leveled with the future parents.

Approach to Investigation for Hydrops Fetalis

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Introduction:

Hydrops fetalis is a condition characterized by the accumulation of excessive fluid in two or more fetal compartments. It is a rare and serious condition that can lead to fetal death or long-term complications. These features can be detected sonographically and include scalp and body wall edema (defined as skin thickness greater than 5 mm), ascites, pleural effusion, pericardial effusion, presence of polyhydramnios)

In the past, the diagnosis was obtained upon the delivery of a severely emphysematous neonate, frequently stillborn. Hydrops fetalis is now a recognised prenatal diagnosis because to sonography. Edema is almost always present as a disorder progresses, frequently together with placentomegaly and hydramnios. Many conditions with diverse pathophysiologies that have the potential to have a negative impact on the baby might cause hydrops fetalis. It is separated into immune and non-immune types. Immune hydrops fetalis is the term given to it if it is observed in conjunction with red cell alloimmunization. If not, it is referred to as non-immune hydrops fetalis. The cause of immune hydrops, also known as erythroblastosis fetalis, is a reaction between maternal blood antibodies and foetal antigens that causes foetal hemolysis. There are factors other than antigen-antibody interactions that lead to non-immune hydrops.

Etiology:

Many disorders cover an extensive spectrum and are linked to hydrops. The two primary types of hydrops fetalis are as follows:

Immune hydrops fetalis is brought on by isoimmunization, including Rh isoimmunization, ABO incompatibility, anti-c, C, e, E, Duffy antibodies, and Kell alloimmunization. It is outside the scope of this article to explore the immunological hydrops in depth.

Non-immune hydrops (NIHF): This type of hydrops fetalis accounts for approximately 80 to 90 percent of all cases of the condition. The prevalence of nonimmune hydrops fetalis (NIHF) in the general population is estimated to be 1 in every 2500–3500 neonates and 1 in every 1600–7000 foetuses. Non-immune hydrops fetalis (NIHF) is a condition that results from fetal or maternal disorders that cause abnormal fluid accumulation in the fetal body. It occurs when an underlying disease, genetic disorder, or birth defect interferes with the fetal body's ability to manage fluid. NIHF can result from many various underlying conditions, such as:

- **Cardiac causes:** Paroxysmal supraventricular tachycardia, hypoplastic left heart, endocardial cushion defects, and congenital pulmonary airway malformation
- **Lymphatic causes:** Congenital lymphatic dysplasia
- **Infections:** Parvovirus B19 (fifth disease), cytomegalovirus, and syphilis infections in pregnant women
- **Chromosomal anomalies:** Turner syndrome, Down syndrome, Edward syndrome or by deletion/duplication of particular chromosomes.
- **Metabolic diseases:** Lysosomal storage disorder, Niemann-Pick disease type-C (NPC), Gaucher disease type 2, beta-glucuronidase enzyme deficiency and Farber disease
- **Tumors:** Teratoma (sacrococcygeal teratoma), hepatic tumors, and neuroblastoma
- **Maternal diseases:** Diabetes mellitus and hyperthyroidism
- **Urinary causes:** Congenital nephrosis and prune belly syndrome
- **Digestive causes:** Volvulus and meconium peritonitis
- **Hematologic causes:** Alpha-thalassemia, twin-to-twin transfusion syndrome (TTTS) in monochorionic twin pregnancies, and leukemias
- **Disorders of red blood cell (RBC) metabolism:** Glucose phosphate isomerase deficiency, pyruvate kinase deficiency, and glucose-6-phosphate dehydrogenase (G6PD) deficiency
- **Disorders of RBC production:** Congenital dyserythropoietic anemia, Diamond-Blackfan syndrome, and Fanconi anemia
- **Disorders of RBC membrane:** Hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, and hereditary stomatocytosis syndromes.

Cause	Cases	Mechanism
Cardiovascular	17-35%	Increased central venous pressure
Chromosomal	7-16%	Cardiac anomalies, lymphatic dysplasia, abnormal myelopoiesis
Hematologic	4-12%	Anemia, high output cardiac failure; hypoxia (alpha thalassemia)
Infectious	5-7%	Anemia, anoxia, endothelial cell damage, and increased capillary permeability
Thoracic	6%	Vena caval obstruction or increased intrathoracic pressure with impaired venous return
Twin-twin transfusion	3-10%	Hypervolemia and increased central venous pressure
Urinary tract abnormalities	2-3%	Urinary ascites; nephrotic syndrome with hypoproteinemia
Gastrointestinal	0.5-4%	Obstruction of venous return; gastrointestinal obstruction and infarction with protein loss and decreased colloid osmotic pressure
Lymphatic dysplasia	5-6%	Impaired venous return
Tumors, including chorioangiomas	2-3%	Anemia, high output cardiac failure, hypoproteinemia
Skeletal dysplasias	3-4%	Hepatomegaly, hypoproteinemia, impaired venous return
Syndromic	3-4%	Various
Inborn errors of metabolism	1-2%	Visceromegaly and obstruction of venous return, decreased erythropoiesis and anemia, and/or hypoproteinemia
Miscellaneous	3-15%	
Unknown	15-25%	

SMFM. Nonimmune hydrops fetalis. Am J Obstet Gynecol 2015.

Evaluation:

The investigation of NIHF requires a multidisciplinary team approach involving fetal medicine specialists, neonatologists, obstetricians, hematologists, and geneticists. The goal of investigation is to identify the underlying cause of NIHF, provide prognostic information, and guide management decisions.

In the first 18 to 22 weeks of gestation, a thorough ultrasound is performed. In the foetal head, back of neck, thorax, and belly, ascites and skin edoema (>5 mm thickness) are the most frequently found abnormalities during early pregnancy. Aneuploidy or related anatomical abnormalities are most likely the cause of generalised skin edoema. Plural and pericardial effusions in the foetus are uncommon before 15 weeks of gestation, although polyhydramnios and placental edoema are most frequently encountered before 20 weeks.

Maternal toxoplasmosis, rubella, CMV, herpes (TORCH), and parvovirus B19 must all be considered as possibilities. The congenital abnormalities ventriculomegaly, microcephaly, and hyperechogenic bowel are related with CMV and toxoplasmosis, but foetal anaemia and ascites are most frequently associated with parvovirus B19 infection. Thus, it is usually advisable to consider an antibody test for TORCH infections.

Finding the underlying reasons of hydrops may also be done using the foetal heart rate, umbilical artery pulsatility index, end-diastolic flow, and middle cerebral artery peak systolic velocity (MCA-PSV). Trisomy 21 results in an increase in the average foetal heart rate. Trisomy 18 and triploidy cause the umbilical artery to have more blood flow resistance. Fetal anaemia may be accurately identified by measuring MCA-PSV. Fetal anaemia is deemed to exist when the MSA-PSV ratio is more than 1.5. Cordocentesis aids in determining the origin of foetal anaemia.

If hydrops fetalis is discovered before 15 weeks of gestation, chorionic villous sampling (CVS) is most usually performed for karyotyping or chromosomal microarray. Chromosomal aberrations mostly fall into two categories: numerical and structural aberrations. Chromosome division failures are typically the root cause of numerical aberrations, which result in the presence of extra chromosomes or the absence of certain chromosomes (s). Triploidy, trisomy, and monosomy are typical forms of numerical aberration (also called aneuploidies). The cause of 7% to 16% of NIHF cases is aneuploidies. Every aberration occurs occasionally at different rates. The most frequent aneuploid condition is monosomy X (Turner syndrome), which accounts for 42% to 67% of cases. Other aneuploidies associated with hydrops include trisomy 21—Down syndrome (23% to 30%); trisomy 13, 18 and 12 (10%); and ploidies (triploidies and tetraploidies)

Loss, gain, or rearrangement of genetic material inside a certain chromosome causes structural abnormalities. These anomalies include translocations, inversions, ring formations, duplications, and deletions. Modern genomic medicine makes it possible to detect these aberrations. One of the tools designed for chromosomal imbalance identification is a chromosomal microarray analysis (CMA). These abnormalities may or may not be detected in routine karyotyping, depending on the size of the deleted or duplicated fragment. Only a chromosomal microarray can detect copy number variations (CNVs), which are tiny (5 Mb, submicroscopic chromosomal imbalances).

In cases of hydrops fetalis presenting with possible single gene etiology, monogenetic disorders need to be rules out. The largest review found that 5% to 10% of NIHF patients are caused by syndromes such as lysosomal storage disorders, mucopolysaccharidosis (MPS) VII and IVA, type 2 Gaucher disease, sialidosis, GMI gangliosidosis, galactosialidosis, Niemann-Pick disease type C, disseminated lipogranulomatosis (Farber disease), infantile free sialic acid storage disease (ISSD), and mucolipidosis II (I-cell disease). Gene sequencing or phenotypic/clinical results from ultrasounds, physical exams, or autopsy are the bases for this classification. It is justified to do gene panel or even whole exome sequencing in order to accurately determine the prevalence of monogenic diseases in the aetiology of NIHF, particularly in foetuses with developmental abnormalities.

Maternal Sjogren syndrome can result in bradyarrhythmias and total heart block in foetuses, which can lead to the development of hydrops. Yet, during the first trimester, congenital heart block is the first symptom to be seen on ultrasonography. This has a pretty dismal prognosis.

Fetal anaemia and nonimmune hydrops are caused by fetomaternal haemorrhage, which is indicated by elevated alpha-fetoprotein (AFP) throughout pregnancy. As a result, AFP levels are carefully monitored.

Immune hydrops fetalis can be found using the direct and indirect Coomb tests, however the two do not correlate with the severity of the condition. Other causes of NIHF after delivery are also found using the echocardiogram, thyroid hormone levels, complete blood count, and metabolic panel.

Flow chart for Diagnostic evaluation of nonimmune hydrops fetalis

Fig credit: SMFM. Nonimmune hydrops fetalis. Am J Obstet Gynecol 2015.
Severe fetal anemia can be treated by in utero fetal transfusion.
Abbreviations: CMV: cytomegalovirus; MCA: middle cerebral artery; PSV: peak systolic velocity; MoM: multiples of the median; IgM: immunoglobulin M; IgG: immunoglobulin G; MCV: mean corpuscular volume; RBC: red blood cell.

Conclusion:

The cause of NIHF affects both the short- and long-term prognosis. When there is a treatment option, the prognosis is based on how well the patient responds to it. Neonatal survival rates are frequently less than 50%, and aneuploidy is associated with a poor prognosis. Only rare instances or possibly curable causes of NIHF, such as foetal arrhythmia or infection with parvovirus B, have a better prognosis, according to the data from a cohort and the literature that is currently accessible. Genetic examination is essential since there are many potential genetic causes of NIHF. It should involve traditional karyotyping or CMA (to rule out chromosomal abnormalities, particularly when internal organ anomalies are present), as well as gene sequencing to identify monogenic disorders. Monogenic abnormalities appear to contribute to disease that present with NIHF to a higher extent than chromosomal aberrations, according to the literature; as a result, the detection of such abnormalities is crucial. This is crucial, especially in families with recurrent NIHF because chromosomal aetiology is unlikely to be the cause.

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Skeletal Dysplasia – The Underlying Genetics

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Skeletal dysplasia (SD) can be explained primarily as poor skeletal growth. A person can be classified as suffering from skeletal dysplasia when he/she is below the 2.5th percentile of what his/her mean age group and sex normally belongs to (Krakow, 2009). The condition can be detected through an ultrasonographic investigation at around 20 weeks of gestation colloquially called as anomaly scan. Although the parents are expecting positive feedback on how their pregnancy is progressing, sometimes the radiologist notices that the fetal long bones and ribs are significantly shorter than expected or the fetal head is larger; other concerns may arise as well. These conditions are called as Fetal Skeletal Dysplasia (FSD). SD is a rare disorder with comprehensive global prevalence rate of 1 in 4000 births out of which 25 percent are stillborn and 30 percent die within neonatal period.

Symptoms

SD can have several overlapping as well as nonoverlapping symptoms and thus can be sub-categorized into few groups based on associated primary deformity type, as follows-

Deformity of long bones- shortening of long bones, shape abnormality, absence of extremities as well as echogenicity reduction are the primary symptoms in this type of SD.

Deformities of hand and feet- Numerical and/or structural alterations of digits of both hand and feet like polydactyly, syndactyly and clinodactyly as well as disproportion between feet and hand can also be symptoms of SD.

Deformities of fetal head and thorax – Reduction in skull bone ossification and/or reduction in thorax size are also associated symptoms of SD.

Individuals with less pathogenic mutations carry biochemical parameters in normal range except that of a smaller height and stature. Therefore, human society always have a community of short stature people. Eternally it has been the human psychology to believe themselves as healthy until they are feeling ill, though there may exist several complications. Along with these, short stature community are psychologically separated from the normal statured ones due to huge height difference. In such psycho-social scenario the practice of nonassortive marriage has been very high and fetus usually remain affected with multiple mutations from already affected parents

Causes of Skeletal Dysplasia

Summarily, skeletal dysplasia is a group of more than 450 rare, heritable disorders of bone that can be lethal even in majority types involving mutations in at least 60 genes and counting (Krakow, 2015). The pattern of inheritance is wide, including autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive (Warman, 2011). Based on the affected gene the symptoms and pathology vary from lethal to near-normal level. Table 1 summarizes the common forms of skeletal dysplasia along with their prevalence, mode of inheritance and gene(s) mutation associated with the specific of disorder. The variability of prevalence is found among some populations in a non-random distribution due to may be founder effect.

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Dysplasia	***Prevalence***	***Sub Type***	***Aetiology***	***Prognosis***	***Genes Reported Involved***
Thanatophoric Dysplasia	1 in 20,000 to 1 in 50,000	Type I Type II	Autosomal dominant	Lethal	FGFR3 (Trantirkova, 2012)
Osteogenesis Imperfecta	1 in 20,000 live births	*Type II Type III*	Autosomal Dominant	Lethal	COL1A1 COL1A2 CRTAP (Type VII) P3H

Achondroplasia	1 in 20,000	-	Autosomal Dominant	Lethal	FGFR3 (Horton, 2012)
Achondrogenesis	UNknown	Type Ia	Autosomal recessive	Lethal	TRIP11 (Grigelioniene, 2013)
		Type Ib	Autosomal recessive		SLC26A2 (Rossi, 2001)
	1 in 40,000 to 1 in 60,000	Type II	Autosomal Dominant		COL2A1 (Korkko, 2000)
Asphyxiating Thoracic Dystrophy	1 in 100,000 to 1 in 130,000	-	Autosomal recessive	General	IFT80 DYNC2H1 and others (Baujat, 2013)
Ellis–Van Creveld Syndrome	1 in 60,000 to 200,000 newborns	-	Autosomal recessive	Lethal	EVC or EVC2 (Baujat, 2007)
Hypophasphatasia	1 in 100,000	-	Autosomal recessive	Lethal	ALPL (Mornet, 2000)
Campomelic dysplasia	1 in 40,000 to 200,000	-	Autosomal dominant	Lethal	SOX9 (Barone, 2014)
Spondylothoracic Dysostosis	1 in 200,000	-	Autosomal recessive	Lethal	MESP2 (Cornier, 2004)
Spondylocostal Dysostosis	Unknown	-	Autosomal recessive	Not lethal	DLL3 (Bulman, 2000)
Diastrophic Dysplasia	1 in 500,000		Autosomal recessive	Normal	SLC26A2 (Rossi, 2001)

Table 1 – Skeletal dysplasia – types, prevalence and gene(s) responsible.

Till the availability of high throughput technologies for molecular diagnosis of skeletal dysplasia, it was reported that genetic mechanisms like deletions and/or duplications, germline mosaicisms, uniparental disomy and compound heterozygosity were the mutation types associated with SD (Rimoin, 1996; Superti-Furga, 2001; Edwards, 1992; Sevenson, 2004 & 2007). However, applications of NGS technologies not only increased the SD associated number of mutations but also have found that SNPs in the genes and microRNAs also play crucial roles in SD pathogenicity. It has also been found that SD associated SNPs may be inherited or even denovo.

Therefore, it is very crucial for better clinical management of SD that the mutation is specifically diagnosed. Since, majority of the SD types are lethal and will lead to Intra Uterine Fetal Demise (IUFD) therefore, timely detection of mutation may help the parents and clinician for Medical Termination of Pregnancy decision. In such cases, Chorionic Villus Sampling (CVS) and/or Amniocentesis may be very helpful.

Below is the recommended test types for specific detection of SD associated mutations as Table 2

Mutation Type	Preferred Diagnostic Test
Large Deletion / Duplication	**MLPA / CMA**
Small Deletion / Duplication	**CMA**
Uniparental Disomy	**MS-MLPA**
Inherited SNPs	**WGS / WES**
DeNovo SNPs	**WGS / WES**
microRNA associated	**WGS**

Table 2 – Mutation types and specific diagnostic tests for detection; MLPA – Multiplex Ligation-dependant Probe Amplification; CMA – Chromosomal Microarray; MS-MLPA – Methylation Sensitive MLPA; WGS – Whole Genome Sequencing; WES – Whole Exome Sequencing.

Remarks

Earlier accurate diagnosis has remained medical boon in identification of fetal as well as neo-natal genetic pathologies. Therefore, correlated genetic testing is highly recommended along with radiological and biochemical parameters for accurate identification of SD subtype for better clinical management.

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Secretary Annual Report

SFM Bengal Chapter 2022-23

Dear respected members of Bengal chapter, it gives me immense pleasure and pride to write the annual report of our chapter for the year 2022-23. Its been a year since I have been given the responsibility of serving our chapter as its secretary under the able leadership of our president, Dr. Kanchan Mukherjee.

Fetal Medicine as a subspeciality is growing in Bengal. We have many juniors taking up this field of medicine under the guidance of our respected seniors. This society is a unique combination of specialists from different fields of medicine. Getting them together under a common platform is a difficult task, which has been successfully done by the Society of Fetal Medicine.

Presently we have 180 members in the Bengal Chapter. We are constantly trying to motivate others to join SFM and strengthen our chapter. In this process of doing so, we have organised 3 onsite programmes in the last year. 1 outreach programme in Durgapur, 1 evening CME in Kolkata, 1 Annual Conference in Kolkata.

In addition to this, Bengal chapter became the 1st chapter of SFM to publish a newsletter. We have already released 3 issues in the last year, this being the 4th.

Bengal chapter became the 1st chapter to introduce national image competition, which was published in our newsletters. Members from all over the country submitted images and took part in the competition.

Our vision for 2023-24 is to increase the membership of our chapter. We plan to do multiple outreach programmes in Purulia, Midnapore, Bardhaman, Siliguri and Baharampur to spread awareness regarding SFM and to increase our membership.
I sincerely thank everyone for being part of SFM and for the support we have received from all of you.
Thanking you
Prasanna Roy
Secretary, Bengal chapter, SFM

We thank our esteemed authors who despite their busy schedules spent valuable time to write for our newsletter.

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