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Non-immune Fetal Hydrops: An Update

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Introduction

'Hydrops fetalis' is a Greek term which refers to the pathological accumulation of fluid in fetal soft tissues and serous cavities. Non-immune fetal hydrops (NIFH) is defined as fluid accumulation in at least 2 fetal body compartments in the absence of red cell isoimmunisation (Moise, 2008). Abnormal fluid collection may be ascites, pleural effusion, pericardial effusion or generalised skin edema (skin thickness >5mm) (Figure 1). Other frequent sonographic findings associated with fetal hydrops include placental thickening and polyhydramnios. The placental thickness (in mm) is normally equal to the gestational age (in weeks) +/- 10 mm; if the placental thickness exceeds this range, it is considered as increased placental thickening (Moise, 2008). With the widespread use of routine antiD prophylaxis in Rh-negative mothers, prevalence of RhD alloimmunisation and associated hydrops has dramatically decreased and especially in developed countries, NIFH now accounts for almost 90% of cases of hydrops fetalis. The progressive fall in the incidence of immunologic hydrops fetalis has fostered growing interest in non-immune fetal hydrops. The world-wide prevalence of NIFH is estimated to range from 1 in 1500 to 1 in 3800 births (Bellini et al., 2015).

The identification of fetal hydrops by antenatal ultrasound requires extensive search for the etiology which includes a wide range of diseases including several genetic disorders. Even after undergoing numerous investigations, in a good number of cases the etiology remains unknown. In addition, the prognosis is usually poor with a perinatal loss of 70–90%, except in rare cases of spontaneous resolution of parvovirus B19 infection.

Etiology of Non–immune Fetal Hydrops

Non-immune fetal hydrops is a nonspecific finding and can be the manifestation of a wide variety of disorders (Bellini et al., 2009). The cause can be found in nearly 60% of cases prenatally and in around 85% of cases when postnatal tests are included. Identification of the exact etiology helps in accurate prognostication of the recurrence risk for subsequent pregnancies of the couple and definite prenatal testing can be offered in their future pregnancies (Moreno et al., 2013). The most common etiologies include cardiovascular causes, chromosomal anomalies and hematological abnormalities. Other conditions associated with NIFH include fetal infections, fetal malformations, inborn errors of metabolism, lethal skeletal dysplasias, numerous other single gene disorders, fetal tumours and placental abnormalities.

The important etiological associations of NIFH are listed in Table 1 (Moise, 2008).

Pathophysiology of non–immune fetal hydrops

The basic pathophysiological mechanism of fetal hydrops is imbalance in the regulation of fluid between vascular and interstitial spaces. Fluid movements between vascular and interstitial spaces are regulated by filtration of fluid across the capillary wall as described by the Starling equation which states that the fluid movement due to filtration across the wall of a capillary is dependent on the balance between the hydrostatic pressure gradient and the oncotic pressure gradient across the capillary. When these pressure gradients are disturbed due to various pathophysiological mechanisms, there is an increased fluid accumulation in the

interstitial spaces, which leads to fetal hydrops. Increased knowledge and understanding of the underlying mechanisms that disturb the fluid equilibrium would therefore be of great importance in identifying potential therapeutic interventions (Bellini et al., 2012).

The pathophysiology underlying the various causes of nonimmune fetal hydrops has been depicted in flow charts 1 and 2 (Bellini et al., 2012).

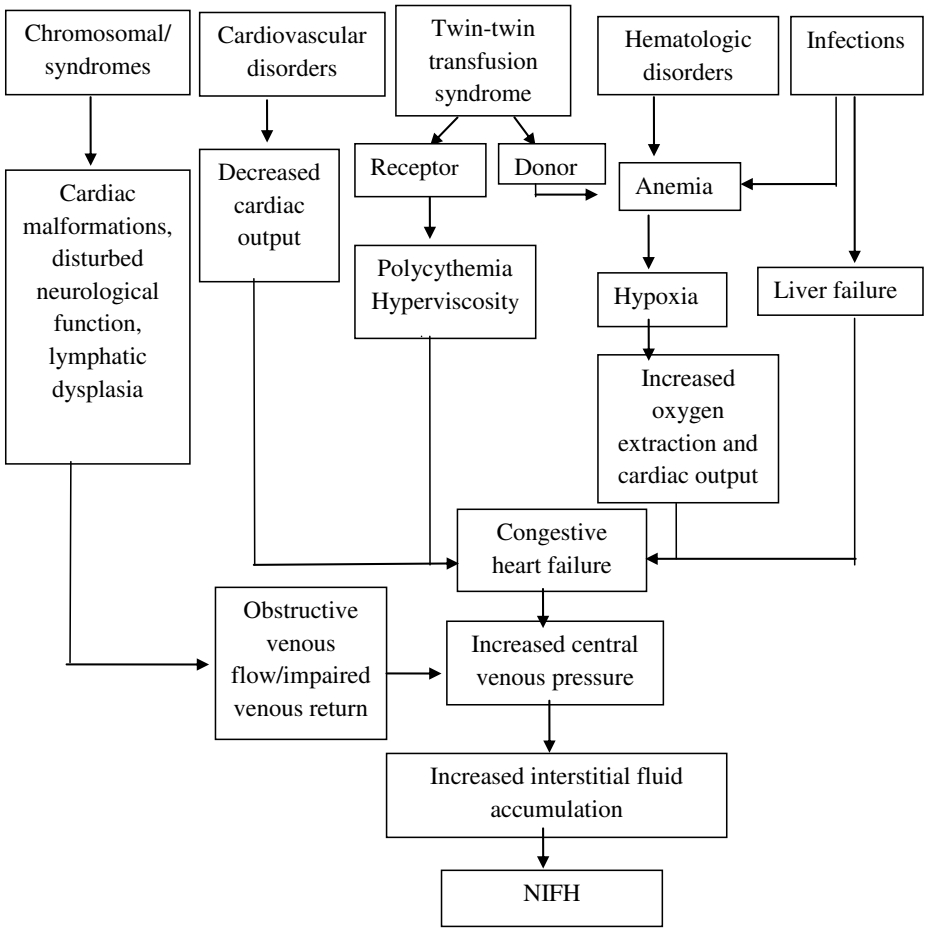
Table 1 Etiological associations of non-immune fetal hydrops.

Etiology	Proportion of cases
I Cardiac anomalies **i. Structural defects** - Atrioventricular septal defect isolated - Heterotaxy syndrome - Severe right or left ventricular outflow tract (RVOT/ LVOT) obstruction - Tricuspid dysplasia and Ebstein's anomaly - Absent pulmonary valve syndrome - Premature closure of foramen ovale - Truncus arteriosus with truncal valve insufficiency **ii. Cardiac tumours** - Rhabdomyoma - Hamartoma - Hemangioma - Intrapericardial teratoma **iii. Cardiomyopathy** - Dilated/restrictive - Myocarditis **iv. Arrhythmias** - Tachyarrhythmias / bradyarrhythmias **v. Idiopathic arterial calcification**	17-35%
II Chromosomal aberrations i. Monosomy X (Turner syndrome) (Figure 2) ii. Trisomy 13/15/16/18/21 iii. Triploidy and Tetraploidy iv. Partial duplications and Partial deletion of chromosomes (Figure 3)	7-16%
III Hematological disorders **i. Intrinsic hemolysis** - Alpha thalassemia - Erythrocyte enzyme disorders - Erythrocyte membrane disorders **ii. Extrinsic hemolysis** - Kasabach-Meritt sequence	4-12%

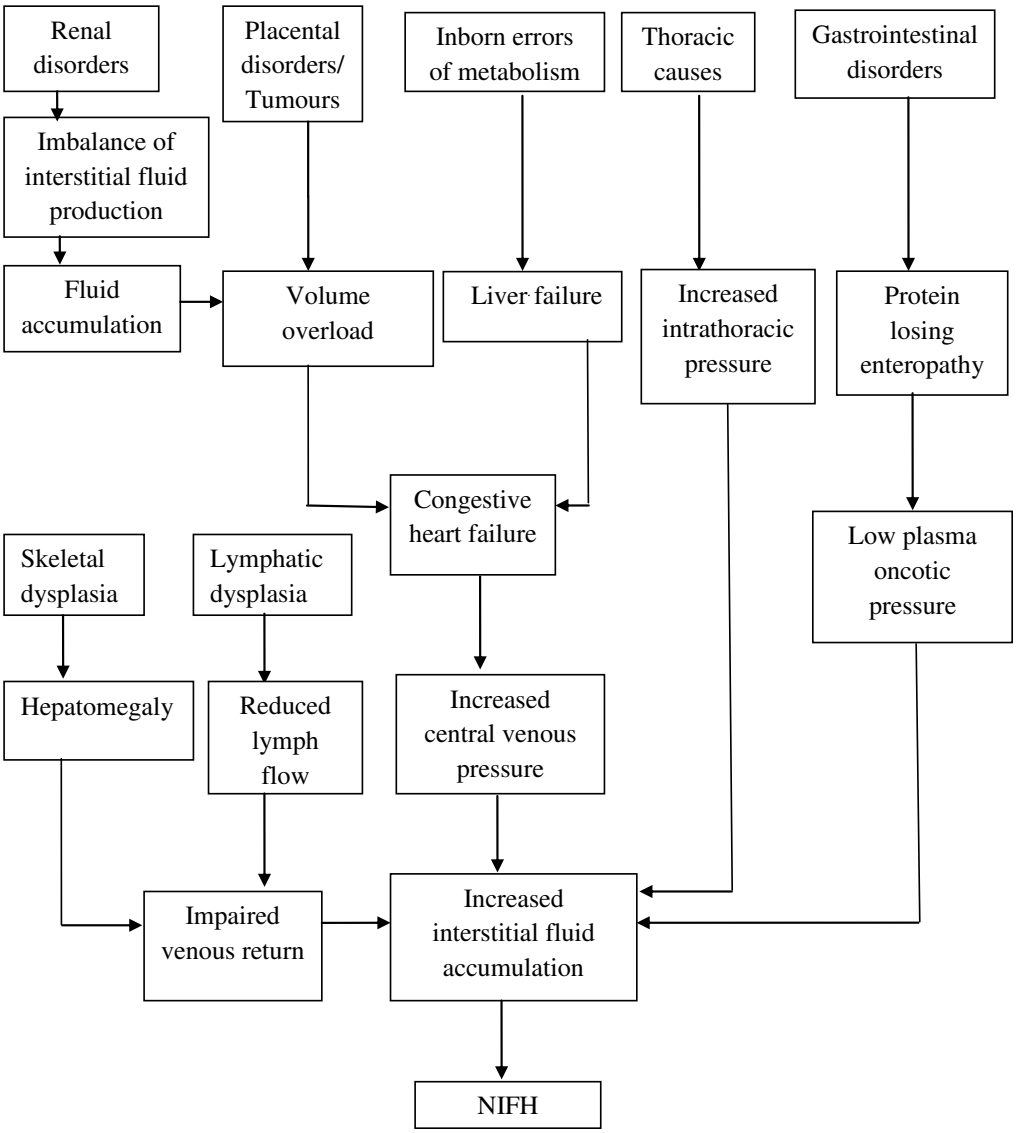
iii. Red cell aplasia - Parvovirus B19 infection - Diamond-Blackfan syndrome - Dyserythropoietic anemia - Congenital leukemia **iv. Fetomaternal hemorrhage**	
IV Twin–Twin transfusion	3-10%
V Infections TORCHES CLAP (Toxoplasma, Rubella, Herpes simplex, Echovirus, Syphilis, Cytomegalovirus, Coxsackie virus, Leptospirosis, AIDS, Adenovirus, Parvovirus)	5-7%
VI Syndromes **i. Autosomal dominant disorders** - Cornelia de Lange syndrome - Congenital myotonic dystrophy - Noonan syndrome - Tuberous sclerosis **ii. Autosomal recessive disorders** - Lethal multiple pterygium syndrome (Figure 4) - Neu Laxova syndrome - Cumming syndrome - Elejalde syndrome	3-4%
VII Skeletal dysplasias - Asphyxiating thoracic dysplasia - Short rib thoracic dysplasia with/ without polydactyly (Figure 5) - Achondrogenesis - Osteogenesis imperfecta type 2 - Lethal osteopetrosis - Lethal Kneist- like dysplasia - Chondrodysplasia punctate (Conradi-Hunermann variant) - Greenberg chondrodystrophy - Caffey syndrome	3-4%
VIII Gastrointestinal disorders **i. Intestinal haemorrhage and meconium peritonitis due to bowel perforation** **ii. Hepatic disorders** - Cholestasis /congenital portal hypertension - Hepatitis/hepatic fibrosis - Hepatic cirrhosis with portal hypertension - Polycystic liver disease	0.5-4%
IX Renal anomalies - Congenital nephrosis (Finnish type) - Polycystic kidney disease - Renal vein thrombosis	2-3%

X Inborn errors of metabolism **i. Lysosomal storage disorders** (Figure 6) - Mucopolysaccharidosis types 1, 4, 7 - Sphingolipidoses (GM1 gangliosidosis, Galactosialidosis, Farber disease, Gaucher disease, Niemann-Pick disease type A) - Mucolipidosis type 1 (Sialidosis) and type 2 (I cell disease) - Transport defects (Niemann-Pick disease type 3 and Sialic acid storage disease) **ii. Non-lysosomal disease** - Glycogen storage disease type 2 - Long- chain hydroxyl- acyl CoA dehydrogenase deficiency - Carnitine deficiency - Congenital disorder of glycosylation type I/IX	1-2%
XI Placental causes - Chorioangioma of placenta/ Subchorial placental hematoma - Umbilical cord abnormalities (true knots of cord, umbilical cord torsion, angiomyxoma of umbilical cord, umbilical vein thrombosis)	1%
XII Miscellaneous	3-15%
XIII Unknown	15-25%

Flow chart 1:



Flow chart 2:



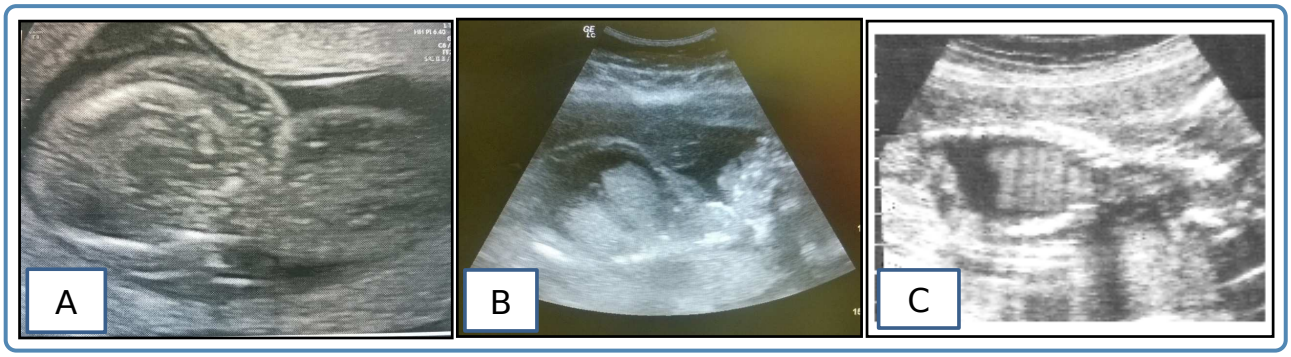


Figure 1 Antenatal ultrasound findings suggestive of fetal hydrops. A: Fetal scalp edema, B: Fetal ascites, C: Fetal pleural effusion.

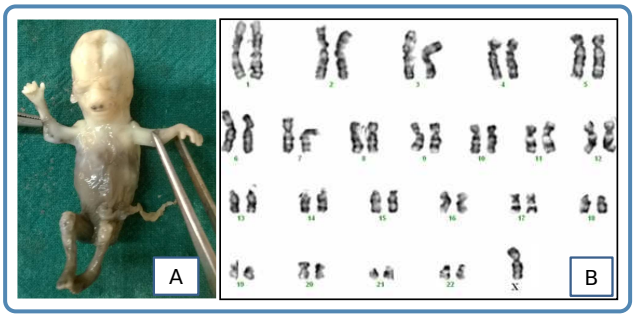


Figure 2 Fetus with Turner syndrome. A. Autopsy findings of webbing of neck, subcutaneous edema and joint contractures. B. Karyotype showing 45,X.

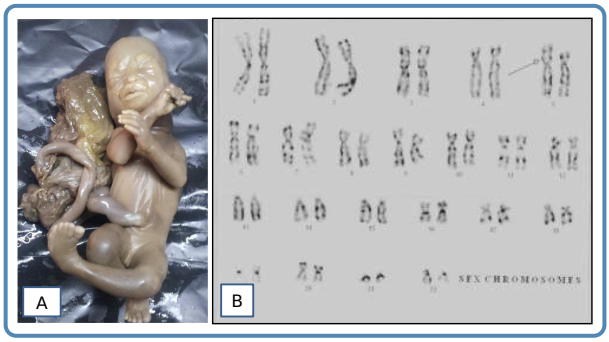


Figure 3 Fetus with unbalanced chromosomal translocation. A. Autopsy findings of facial dysmorphism and generalised subcutaneous edema. B. Karyotype showing 46, SC, der 5, t(5;10) (p15.3; q24.3) mat.

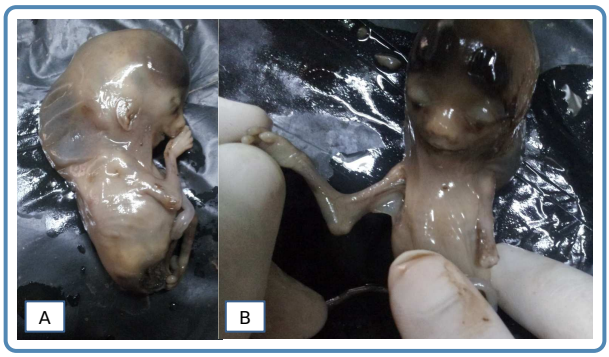


Figure 4 Fetus with autopsy findings suggestive of lethal multiple pterygium syndrome. A & B: Cystic hygroma, webbing of neck, contractures of joints and pterygia across joints.

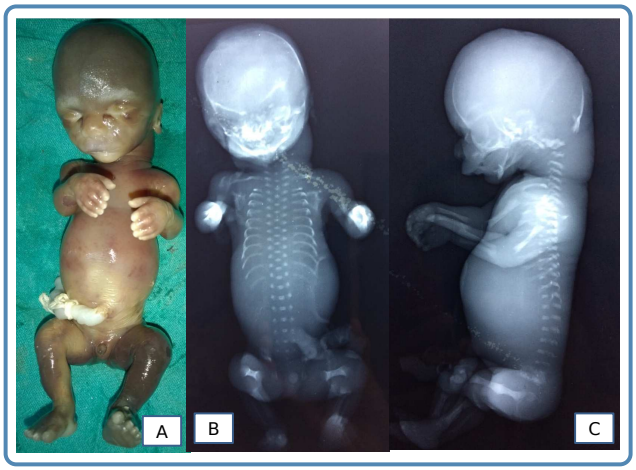


Figure 5 Fetus with short rib thoracic dysplasia. A. Autopsy findings of generalised subcutaneous edema, rhizomelic limb shortening and narrow and short thorax. B. Skeletal radiograph showing narrow thorax with short ribs and shortening of humerus and femur.

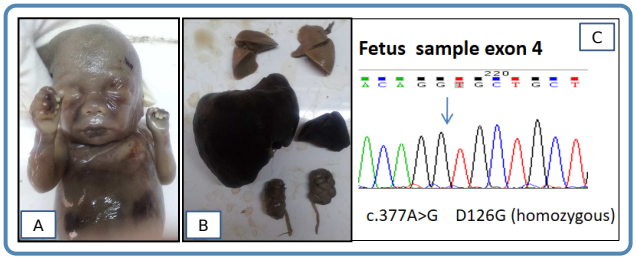


Figure 6 Fetus with Gaucher disease. A & B: Autopsy findings of generalised subcutaneous edema and enlarged liver and spleen. C: Sequence chromatogram of the GBA gene showing the homozygous pathogenic mutation.

Detection of Non-immune fetal hydrops

Detailed antenatal ultrasonography (USG) is the initial diagnostic modality for any case with non-immune fetal hydrops, and apart from detecting the hydrops per se also helps to assess the severity of hydrops and to detect associated malformations (Figure 1). Sonography can even provide important clues to the underlying cause of the hydrops in many cases. Increased nuchal translucency is often

the first sign of NIFH due to chromosomal abnormalities. Cases secondary to cardiac abnormality usually show significant cardiomegaly (Skoll et al., 1991). A fetus with anemia-related hydrops is likely to demonstrate the presence of pleural fluid and skin edema (Skoll et al., 1991). The middle cerebral artery peak systolic velocity (MCA PSV) >1.5 MoM (multiples of median) indicates fetal anemia in fetuses of more than 16 weeks of gestation. Fetal hydrops associated with metabolic disorders is usually severe with massive ascites and significant thickening of the skin. Additional USG findings in various fetal infections associated with fetal hydrops include intrauterine growth retardation, polyhydramnios/ oligohydramnios, microcephaly, ventriculomegaly, intracranial calcification, cardiac anomalies, liver calcifications and echogenic bowel (SOGC clinical practice guidelines, 2013). However, in most cases, further investigations are required to clearly diagnose the etiology.

Stepwise evaluation for non–immune fetal hydrops

As NIFH is an etiologically heterogeneous condition, each case of NIFH would require stepwise evaluation for all the known causes, in order to ascertain the exact etiological diagnosis. As a significant proportion of cases have a genetic etiology, identification of the exact cause in each case is very important for accurate counseling regarding the recurrence risk and prenatal diagnostic testing for future pregnancies.

Step 1: Fetal imaging

i. Detailed obstetrical ultrasound which should include a detailed survey for anomalies of the fetus, placenta, umbilical cord and amniotic fluid and assessment of the fetal Doppler (Middle cerebral artery) and fetal echocardiogram.

Step 2: Tests in the mother

i. VDRL test for syphilis and TORCH serology. Maternal TORCH serology should be done in all cases of NIFH occuring for the first time in the family.
ii. SS-A and SS-B antibodies to be tested in the mother in cases of fetal bradyarrhythmia.

Step 3: Invasive testing

i. Amniocentesis: For fetal karyotyping or chromosomal microarray analysis; PCR for Cytomegalo virus/PCR for parvovirus-B19/toxoplasmosis in selected cases; DNA extraction for further molecular genetic studies; enzyme assay for lysosomal storage disorders.
ii. Fetal blood sampling: Complete blood picture with red blood cell count, white blood cell count and platelet count; TORCH serology/ PCR for viral infections and viral and bacterial cultures; liver function tests including serum total protein and albumin in some cases.

In case of antenatal doppler evidence of fetal anemia, PCR for Parvovirus B19 and molecular genetic testing for alpha thalassemia should be done in the fetal sample.

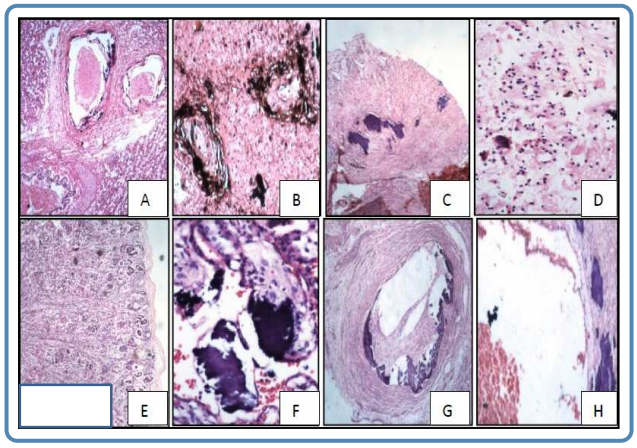


Figure 7 Histopathology showing abnormal calcific deposits in various fetal organs in a fetus with Idiopathic infantile arterial calcification. A & B: Haematoxylin-eosin & Vonkossa stain for pulmonary vessel calcification, C: Myocardial calcification, D: Cerebral calcification, E: Renal cortex calcification, F: Placental villi calcification, G: Aortic calcification, H: Renal arterial calcification.

Step 4: Postnatal evaluation

i. If the fetal samples have not been procured antenatally or the antenatal samples are inadequate, fetal cord blood/ skin biopsy/ umbilical cord sample should be collected after delivery for karyotyping/ enzyme assays/ DNA extraction. If fetal cord blood is being collected, about 2-3 ml of fetal cord blood should be collected in a heparinized vacutainer and about 5ml in an EDTA vacutainer. If fetal autopsy is

planned, the fetal body and placenta should be preserved in 10% formalin.
ii. Whole body skeletal radiographs of the fetus- anteroposterior and lateral views
iii. Head to foot external dysmorphology evaluation
iv. Internal dissection of fetal organs
v. Histopathology of fetal organs, placenta and umbilical cord (Figure 7)
vi. Immunohistopathology, as required, for detection of lymphodysplasia; lymphodysplasia may be the underlying pathophysiological mechanism in a number of cases including chromosomal abnormalities. Immunohistochemical studies with CD31 and CD34 are helpful especially if the lymphodysplasia lesional areas are small and not visible on gross examination (Bellini et al., 2010).

Whole body fetal radiographs, detailed fetal dysmorphology evaluation, internal organ dissection and histopathological examination of the fetal organs and the placenta should done in every case. If a specific etiology is identified with this first-tier evaluation, specific cytogenetic or molecular genetic testing can be done in the fetal sample for confirmation of the same. As per various literature reports, perinatal autopsy provides important additional information or changes the ultrasonography-based diagnosis in 22-76% cases.

In cases where autopsy evaluation does not reveal a specific etiology, karyotype and enzyme assays for common NIFH-associated lysosomal storage disorders can be done. In cases where the above evaluation is inconclusive and the cause remains unknown, higher resolution genetic testing techniques i.e. chromosomal microarray and exome sequencing can be done in the fetal DNA sample, for copy number variations and single gene etiologies respectively. Both parents can be tested further, as relevant, for the genetic etiology identified in the fetus.

Prognosis

Prognosis depends upon the etiology, the gestational age at onset and whether pleural effusions are present. In general, the earlier the hydrops occurs, the poorer the prognosis. In particular, pleural effusions and polyhydramnios prior to 20 weeks of gestation are poor prognostic signs because of increased risks of pulmonary hypoplasia and preterm labour/ premature rupture of membranes, respectively. On the other hand, absence of aneuploidy and absence of major structural abnormalities confer a better prognosis. Despite continued advances in perinatal care NIFH continues to be associated with significant mortality (Simpson et al., 2006).

Therapeutic options

Fetal treatment for NIHF depends on the etiology and gestational age. Some of the therapies for selected etiologies are listed in Table 2 (SOGC clinical practice guidelines, 2013).

NIFH related to fetal toxoplasmosis treated with maternal administration of pyrimethamine and sulfadiazine and NIFH related to fetal syphilis treated with penicillin resolves but the overall prognosis due to cerebral complications remains high. Fetal cytomegalovirus infection has been treated with maternal and direct fetal administration of hyperimmune globulin. However there are only a few reported cases where this therapy was attempted and they did not resolve with this therapy.

Genetic counseling

Genetic counseling is an integral component of the management of any family with non-immune fetal hydrops. If the cause of hydrops is identified, the nature of abnormality, pattern of inheritance and recurrence risk in future pregnancies can be determined. In cases of hydrops due to cardiovascular anomalies, the recurrence risk depends on the type of anomaly and varies from 3-50%. Hydrops due to infections is less likely to recur. Hydrops due to chromosomal abnormalities usually have a recurrence risk of around 1%, unless they are associated with a familial chromosomal rearrangment, in which case the recurrence risk would be higher, depending on the nature of the chromosomal anomaly. If the fetal hydrops is due to autosomal recessive disorders there is 25% risk of recurrence in the subsequent pregnancies of the couple. If NIFH is due to an autosomal dominant condition, most often it would be due to a *de novo* mutation, but there would be a small but significant risk of recurrence in subequent pregnancies due to the possibility of gonadal mosaicism for the pathogenic mutation in either parent. Idiopathic NIFH generally has a low recurrence risk. Prenatal testing, as required, can be offered for subsequent pregnancies of the couple through targeted cytogenetic/molecular genetic testing, based on the etiology identified in the affected fetus.

Table 2 Therapeutic modalities for some causes of non-immune fetal hydrops.

Etiology	Therapy
Twin to twin transfusion syndrome	Laser ablation of placental anastomoses or selective termination
Twin-reversal arterial perfusion	Percutaneous radiofrequency ablation
Cardiac arrhythmias	Maternal transplacental administration of antiarrhythmic medications
Fetal anemia	Fetal blood sampling followed by intrauterine transfusion
Fetal hydrothorax/ pleural effusion associated with bronchopulmonary sequestration	Placement of thoracoamniotic shunt/ needle drianage of effusion
Fetal CPAM - (Congenital pulmonary airway malformation) Macrocystic Microcystic	 Needle drainage/ Thoracoamniotic shunt Corticosteroid therapy
Large bronchopulmonary sequestration	NdYAG Laser of the feeding vessel
Fetal thyrotoxicosis	Antithyroid drugs

Conclusion

Non-immune fetal hydrops is a significant cause of prenatal and perinatal morbidity and mortality. With the use of advanced genetic testing technologies such as chromosomal microarray and whole exome/ whole genome sequencing, we are likely to identify the underlying genetic basis in a greater proportion of cases with NIFH. This in turn would help to provide a greater insight into the etiopathogenesis of NIFH and help to identify potential therapeutic targets for this condition.

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