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CASE REPORT

Lysosomal Storage Disorders in Nonimmune Hydrops Fetalis (NIHF): An Indian Experience

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Abstract Lysosomal storage disorders (LSD) are rare inherited neurovisceral inborn errors of metabolism which may present as nonimmune hydrops fetalis (NIHF) during pregnancy. Although causes of NIHF are highly diverse, LSDs are one of the underlying causes of NIHF. The aim of this study was to elucidate most frequent causes of LSDs presenting as NIHF in Indian population. Several fetal tissues were investigated for enzymatic diagnosis of LSDs using modified fluorometric assays in the current prospective study carried out at our national tertiary center from 2006 through 2016. Other general causes of NIHF were ruled out. Twenty-one percent (7/33) of cases were confirmed to have LSDs. Two patients were diagnosed with Hurler syndrome; two had Sly syndrome and one each of Niemann-Pick disease type A/B, Gaucher's disease, and mucolipidosis. Four of eleven cases (36%) with recurrent NIHF were found to have LSDs. In spite of extreme rarity of LSDs, they should be considered as a potential cause of NIHF, especially with recurrent NIHF. Specific investigations of LSD leading to definitive diagnosis may aid the clinician in providing accurate genetic counseling and prenatal diagnosis to the patients and help in subsequent pregnancies to the families. Furthermore, early intervention and management with enzyme replacement therapy may be planned for the lysosomal storage disorders where available.

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Introduction

Nonimmune hydrops fetalis is defined as the accumulation of excess pathological fluid in fetal soft tissues and serous cavities detected by ultrasonography where isoimmunization has been excluded (Machin 1989). During intrauterine life, it presents with subcutaneous edema and effusion in two or more serous cavities such as pericardial, pleural, and ascites and is associated with polyhydramnios or placental thickening (>4 cm in second trimester and >6 cm in the third trimester) (Society for Maternal-Fetal Medicine (SMFM) et al. 2015). The varied pathophysiologic mechanisms involved in NIHF are intrauterine anemia and heart failure, hypoproteinemia, and various structural anomalies that interfere with fetoplacental circulation (Machin 1989). NIHF is commonly seen with a reported incidence of around 3 per 10,000 births with much higher incidence during first and second trimester in about 85% of all fetal hydrops cases (Ismail et al. 2001). The etiology of NIHF is complex and remains unknown in 15–25% of patients even after extensive investigations (Bellini et al. 2009). Among diverse etiologies of NIHF, the most common are cardiovascular followed by chromosomal, hematologic, infection, thoracic, lymphatic dysplasia, twin-to-twin transfusion, urinary tract malformations, and an inborn error of metabolism (IEM) (Bellini et al. 2009). Inborn errors of metabolism are the heterogeneous group of autosomal recessive rare inherited disorders, with lysosomal storage disorders (LSD) as the most common subtype. To enumerate, there are 14 different LSDs: Hurler

syndrome (MPS-I; OMIM #607014), Morquio-A (MPS-IVA; OMIM #253000), Sly syndrome (MPS-VII; OMIM #253220), galactosialidosis (OMIM #256540), sialidosis (OMIM #256550), GM1 gangliosidosis (OMIM #230500), Gaucher type 2 (OMIM #230900), Niemann-Pick disease types A and C (NPD-A and NPC; OMIM #257200, #257220), Farber granulomatosis (OMIM #228000), Wolman disease (OMIM #278000), mucolipidosis II (I-cell disease; OMIM #252500), sialic acid storage disease (ISSD; OMIM #269920), and multiple sulfatase deficiency (OMIM #272200) which have been shown to be associated with NIHF and congenital ascites (Burin et al. 2004). The present study was planned to identify the commonest LSD in the Indian population and report any new cause of LSD among the growing list of various storage disorders during pregnancy. There lies an importance of enzymatic studies in the chorionic villous sample or amniotic cultured cells, once the most common conditions associated with nonimmune fetal hydrops have been ruled out.

Materials and Methods

The present prospective study was planned in 33 cases of NIHF during 2006–2016. All the procedures were followed in accordance with the ethical standards of the committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for inclusion in the study.

They were referred from different parts of the country for further investigation of hydrops. Only those cases with normal chromosome study from the placental or fetal tissue or cord blood, an absence of TORCH infection, negative Coombs test, an absence of any other infection as ruled out by normal complete blood count (CBC), and absence of any liver disease in the mother during pregnancy were included in the study. Demographic details of all patients recruited for the study are listed in Table 1. Among all, 21 cases were investigated during the prenatal period; 6 cases were investigated from placental and/or fetal tissue obtained as a product of conception (POC) and the other 6 cases from cord blood obtained from the neonate.

Prenatal sampling was done depending upon the stage of pregnancy, fetal position, and maternal health. Chorionic villous sampling (CVS) and/or amniocentesis were carried out between 10–13 and 15–18 weeks of gestation, respectively, as per the standard procedure. In the case of high-risk pregnancy, planned pregnancy termination was carried out and fetal tissue was collected. The CVS/AF (amniotic fluid) samples and/or POC material were collected in a sterile collection medium (10–15 mg fetal tissue). CVS/POC cells were checked for maternal contamination followed by washing with phosphate-buffered saline. The cells were inoculated in the growth medium and were processed under 5% CO_2 similar to AF as per standard protocol. The cells were harvested after obtaining confluence and protein activity was determined. The final concentration of protein was adjusted to 2–10 mg/ml for lysosomal enzyme study. In the case of hydropic newborn, cord blood samples were collected and enzyme activity was carried out using the synthetic 4-methylumbelliferone-fluorogenic substrate as described earlier (Sheth et al. 2004). The enzyme activity was expressed as nmol/h/mg of protein.

All lysosomal enzymes except for NPC, Farber, and Wolman disease that are known to be associated with NIHF were investigated as shown in Table 2. These include α-iduronidase (EC3.2.1.76) for MPS-I, β-galactosamine-6-sulfatase (EC3.1.6.10) for MPS-IVA, β-D-glucuronidase (EC 3.2.1.31) for MPS-VII, sphingomyelinase (EC 3.1.4.12) for NPD-A/B, β-D-galactosidase (EC 3.2.1.23) for GM1 gangliosidosis, β-glucosidase (EC 3.2.1.21) for Gaucher disease, and total and free *N*-acetylneuraminic acid for ISSD.

Results

A study of lysosomal enzyme was carried out in 33 NIHF cases. This included 21 (63.3%) pregnancies investigated from cultured CVS or AF cells as prenatal cases, 6 (18.1%) cases with miscarriages that were investigated from cultured POC cells, and an equal number of cord blood cases ($n = 6$; 18.1%) were studied in newborn. Among these, seven cases (21.1%) of NIHF were found to be associated with different LSDs like mucopolysaccharide disorders (MPS-1 and MPS-VII) two in each, sphingolipid disorder (NPD-A/B and Gaucher's disease) one in each, and one with receptor defect (I-cell) as shown in Table 3. The study includes 11 cases with recurrence of NIHF in more than one pregnancy while remaining 22 cases presented as NIHF for the first time that includes three pregnancies with consanguineous marriages.

Two cases of MPS-I and one case each of I-cell and NPD-A/B were diagnosed prenatally from cultured amniotic fluid cells. A case of Gaucher's disease and two cases of MPS-VII were diagnosed from cultured CVS cells and POC, respectively. Those with confirmed LSDs with an etiology of NIHF were referred for prenatal diagnosis during subsequent pregnancy whereas POC cases were referred to know the cause of NIHF after finding the normal chromosomal study and ruling out other causes. None of the consanguineous families were detected to have LSDs associated with NIHF. Four out of 11 (36%) cases with a history of recurrent NIHF had LSD as its etiology. The reference range of aforementioned lysosomal enzymes was established in various tissues as shown in Table 4.

Table 1 Demographic details of patients recruited for investigation of NIHF

Sr. No.	Tissue	Gestational age (weeks)	Patient age (years)	Consanguinity	USG findings	Previous history of NIHF	Enzyme study
1	AF	18	30	No	Hydrops	No	Normal
2	AF	20		No	IUD	Yes	Normal
3	CV	11	23	No	IUD	No	Normal
4	POC	18	26	Yes	Fetal ascites	Yes	Normal
5	CV	13	25	No	Fetal ascites	No	*Gaucher*
6	CV	12	29	No	Hydrops and IUD	No	Normal
7	POC	22		No	Hydrops	Yes	Normal
8	POC	12	29	No	IUGR and hydrops	No	Normal
9	AF	16	23	No	IUD due to hydrops	No	Normal
10	AF	28	25	No	Polyhydramnios	Yes	Normal
11	Cord blood	25	35	No	Polyhydramnios	No	Normal
12	Cord blood	28	25	No	Hydrops	No	Normal
13	AF	16	26	No	Fetal edema	No	*NPD-A/B*
14	AF	14	30	No	Hydrops	Yes	Normal
15	Cord blood	28	28	No	Hydrops	No	Normal
16	AF	16	32	No	Hydrops	Yes	Normal
17	Cord blood	28	27	No	Hepatomegaly, cardiomegaly, pulmonary hypoplasia, ascites	No	Normal
18	AF	16	21	No	NIL	Yes	Normal
19	AF	20	26	No	Cystic hygroma and hydrops	Yes	*MPS-I*
20	Cord blood	19		No	Hydrops	Yes	Normal
21	Cord blood	28	31	Yes	Ascites	No	Normal
22	AF	18	30	No	Hydrops	Yes	*I-cell*
23	AF	28	29	No	Fetal edema	No	Normal
24	AF	20	30	No	Hydrops	No	Normal
25	AF	19	24	No	Hydrops	No	*MPS-I*
26	POC	18	32	No	Hydrops	No	Normal
27	AF	20	20	No	Polyhydramnios	No	Normal
28	POC	20	27	No	Hydrops with ascites	No	*MPS-VII*
29	AF	19	27	No	Hydrops	No	Normal
30	POC	18	28	No	Hydrops	Yes	*MPS-VII*
31	AF	19	28	No	Hydrops	No	Normal
32	CV	13	26	No	Hydrops	No	Normal
33	AF	20	22	Yes	Hydrops	No	Normal

Discussion

Nonimmune hydrops fetalis is a serious and life-threatening sign pointing towards a varied etiology. Though the overall incidence of NIHF is very low, it accounts for nearly 3% mortality in the perinatal period (Swain et al. 1999). This incidence may be higher, however, because intrauterine fetal death and in utero spontaneous resolution are likely to mask the true incidence. With the availability of advanced diagnostic facilities, the etiology can be determined in 60–85% of cases (Bellini et al. 2009). In our study 33 cases of NIHF, storage disorders were diagnosed in seven patients (21%). In a recent systematic review, LSD occurrence was seen in 5.2% of NIHF cases and 17.4% if all idiopathic cases were taken into consideration (Gimovsky et al. 2015). The higher percentage of 21% (7/33) in the present study shows the significance of LSDs as the underlying cause of NIHF. The current study has the largest yield compared to studies systematically reviewed by Whybra et al. (2012) and recently reported by Vianey-Saban et al. (2016). This could be attributed to the ascertainment bias as being a national referral center dedicated to LSDs and a referral may have been sent after a comprehensive workup. The recurrence of NIHF may have given a clue to the genetic

Table 2 Investigation of lysosomal enzymes

Sr. No.	Enzyme	Substrate	Disorder
1	α-Iduronidase	4-mu-α-L-iduronide	Hurler (MPS-I)
2	β-Galactosamine-6-sulfatase	4-mu-β-Galactose-6-sulfate	Morquio (MPS-IVA)
3	β-D-Glucuronidase	4-mu-β-D-glucuronide	Sly (MPS-VII)
4	Sphingomyelinase	4-mu-A-D-mannopyranoside	NPD-A/B
5	β-D-Galactosidase	4-mu-β-D-galactopyranoside	GM1 gangliosidosis
6	β-Glucosidase	4-mu-β-D-glucopyranoside	Gaucher
7	Free and total *N*-acetylneuraminic acid	*N*-acetylneuraminic acid (NANA)	Infantile sialic acid storage disorder (ISSD)

Table 3 Enzyme activity in affected LSD cases having NIHF

Disease name	Enzyme	Sample types			No. of patients ($n = 7$)
		CVS[a]	AF[a]	POC[a]	
Mucopolysaccharidosis					
Hurler disease (MPS-I)	α-Iduronidase	–	7.5 ($n = 1$)	7.9 ($n = 1$)	$n = 2$
Sly syndrome (MPS-VII)	β-Glucuronidase	–	UD ($n = 1$)	0.6 ($n = 1$)	$n = 2$
Sphingolipidosis					
Gaucher's disease	β-Glucosidase	47.46	–	–	$n = 1$
Niemann-Pick A (NPD-A)	Sphingomyelinase	–	3.0	–	$n = 1$
Mucolipidosis					
Mucolipidosis-II	β-Glucuronidase	–	11.9	–	$n = 1$
	Hexosaminidase-total	–	45.9	–	
	β-Galactosidase	–	37.5	–	

UD undetectable, *CVS* chorionic villous sample, *AF* amniotic fluid, *POC* products of conception
[a] All values are in nmol/h/mg protein

Table 4 Normal values of lysosomal enzymes in cultured CVS and cultured AF/POC

Enzymes	Enzyme activity (Sheth et al. 2014)	
	Cultured CVS[a]	Cultured AF/POC[a]
α-Iduronidase	19.27–93.4	85.5–156.3
β-Glucuronidase	26.5–149.6	50.07–197.1
β-Glucosidase	115–409	118–584.8
Sphingomyelinase	13.3–18.8	17.0–69.4
Hexosaminidase-total	4,507–15,473.2	4,487.0–16,286.2
β-Galactosidase	150–644	296–1,195

[a] All values are in nmol/h/mg protein

etiology and as a result almost one-third of the referred patients were found to have LSDs as an underlying cause.

Till date, around 14 different LSDs are known to be associated with NIHF and congenital ascites, with the highest number being associated with mucopolysaccharide disorders followed by sphingolipidosis and lysosomal transporter defects (Cheng et al. 2003). Vianey-Saban and colleagues have carried out a large study of IEM and have shown that 108 fetuses of 1,700 pregnancies with NIHF after exclusion of all causes were found to be affected by LSDs (6.3%) with Sly syndrome as the most common LSD. A present study shows that the highest number of fetuses was found to be affected with MPS-1 and MPS-VII followed by Gaucher, NPD-A, and I-cell disease. The current study has not covered Niemann-Pick disease type C, Wolman, and Farber disease, which is the major limitation of the study. Nonetheless, the overall occurrences of storage disorders in NIHF are in the same order as reported in a large series of data (Whybra et al. 2012). In a tertiary center from North India, Verma and his group (2012) had also incidentally diagnosed one case of Hurler syndrome during fetal autopsy that died in utero at 26 weeks of gestation due to NIHF. Thus, detection of Hurler syndrome in the present study and earlier reports further support the association of NIHF with Hurler syndrome.

Morquio syndrome and sialidosis are also found to be associated with NIHF (Applegarth et al. 1987) but none were detected in the present study.

The exact mechanism of NIHF and LSDs is controversial. The best explicable hypothesis is obstruction of venous blood return resulting from visceromegaly and decreased erythropoiesis leading to anemia with/without hypoproteinemia. There lies a great phenotypic variability among presentation of LSDs. Hydrops fetalis manifests a severe phenotype in Gaucher's disease with the 84GG mutation in *GBA* gene which portrayed as a collodion baby whereas NPD-A does not manifest with NIHF even with two knockout mutations. The effect in the latter could more likely be due to an epigenetic factor/s or gene modifiers (Burin et al. 2004).

There also have been cases of transient NIHF and LSDs, mostly associated with GM1 gangliosidosis, MPS-IVA, MPS-VII, and Niemann-Pick disease type C (Whybra et al. 2012). In a growing list of LSDs and NIHF, more numbers of LSDs are likely to be missed in transient NIHF. Such pregnancies should be investigated or followed up postnatally to know the exact incidence associated with this condition. Recently, Ota et al. (2016) followed up NIHF and merely 33% of the fetuses survived at 1 year pointing towards the possibility of some metabolic disorder that might have been missed in the study.

During the antenatal period, signs like hepatosplenomegaly, hypoplastic lungs, and milky ascitic fluid besides congenital ascites are a clue to LSDs and careful fetal sonography can aid the diagnosis (Daneman et al. 1983). Staretz-Chacham and colleagues (2009) have proposed that dysmorphic facies, irregularity of the epiphyses, and coarse trabeculations of the long bones associated with NIHF corroborate to the diagnosis of LSD. Placental histology in cases with hydrops or ascites at birth may be very helpful in providing the diagnosis of storage diseases (Parks 2015).

High incidence of LSDs in the current study demands more awareness regarding this subgroup of IEM among fetal medicine experts. It can also be inferred that Hurler syndrome and Sly syndrome are the first choices of investigation in a pregnancy associated with NIHF among inborn errors of metabolism in the Indian population.

Moreover, economic and accurate prenatal diagnosis of LSD is a major unmet need for low-middle-income countries like ours which would facilitate consideration of therapies before the occurrence of irreversible complications. The major limitation of the current study was the inability to perform enzymes for all known LSDs, especially in familial NIHF and the history of consanguinity which might have helped us to offer a definitive diagnosis to the patients and provide a further increase in the diagnostic yield.

Thus we conclude that correct diagnosis of NIHF aids in the management of lysosomal storage disorders by newer available enzyme replacement therapies and the genetic counseling in the subsequent pregnancy. This study emphasizes the fact that incidences of LSDs, specifically MPS-I, are likely to be higher than published in the reported studies, predominantly with a history of recurrence NIHF, with Hurler and Sly syndromes being among the common causes in Indian population. The differential diagnosis of LSDs needs to be considered for transient forms of ascites and postnatal follow-up remains obligatory for such patients.

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Synopsis

A subset of inborn errors of metabolism is a noteworthy cause for recurrent nonimmune fetal hydrops, with Hurler and Sly syndromes being among the common causes in Indian population.

Conflict of Interests

Jayesh Sheth, Mehul Mistri, Krati Shah, Mayank Chaudhary, Koumudi Godbole, and Frenny Sheth declare that they have no conflict of interests (financial or nonfinancial).

Consent

Informed written consent was obtained from all the participants for publication of their clinical details and/or clinical images. A copy of the written consent is available for review by the editor of this journal.

Ethics

- Present study under submission has been approved by the institutional ethics committee [FRIGE's Institute of Human Genetics] wide approval number FRIGE/IEC/5/2010 dated 7th March, 2010. This process is in accordance with the Helsinki Declaration.
- An informed consent was obtained from the parents before enrolling for the investigations [this was in accordance with the requirement of the institutional ethics committee].
- An informed consent for publication was also obtained from the individuals included in the submission [this was in accordance with the requirement of the institutional ethics committee].

Authors' Contributions

Planned and designed the experiments: JS, MM. Clinical analysis: KS, KG, MC. Enzyme and molecular analysis: JS, MM. Wrote the first draft of the manuscript: KS and FS. Made critical revisions and approved final version: KS, JS, and FS. All authors reviewed and approved of the final manuscript.

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