




Recurrent Nonimmune Fetal Hydrops Due to a Novel Pathogenic Variant in *PIEZO1* Gene: A Case Report from South India

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J Fetal Med 2023;10:69–72.

Abstract

Keywords

- ▶ lymphatic malformation-6
- ▶ generalized lymphatic dysplasia
- ▶ lymphangiectasia
- ▶ nonimmune hydrops fetalis
- ▶ *PIEZO1* gene

Nonimmune fetal hydrops (NIFH) has underlying diverse etiology with generalized lymphatic dysplasia being one such cause. Lymphatic malformation-6 is a type of lymphatic dysplasia that is due to homozygous or compound heterozygous variants in the *PIEZO1* gene. The clinical features associated with this condition during fetal life are nonimmune fetal hydrops that manifests with widespread lymphatic edema, with other systemic manifestations like pericardial/pleural effusions, chylothorax along with lymphangiectasia seen primarily in lungs and intestines. We present a case of recurrent NIFH in a family due to a novel pathogenic mutation in *PIEZO1* gene. This variant was identified in homozygous state in all the three affected fetuses and in heterozygous state in both the parents. The couple were counseled regarding recurrence of this condition and given reproductive options for future pregnancies.

Introduction

Fetal hydrops occurs in 1/1700 to 1/3000 pregnancies.¹ Fetal hydrops can be broadly classified into immune and nonimmune based on the etiology. Nonimmune fetal hydrops (NIFH) has a wide spectrum of genetic causes. Immune causes need to be ruled out prior to evaluation of genetic causes.¹ Further evaluation with prenatal ultrasound and fetal autopsy after the pregnancy gets terminated followed by strategic genetic evaluation in the extracted fetal DNA becomes indispensable. Of the various established causes of NIFH, generalized lymphatic dysplasia (GLD)² is a fairly common entity with a few known associated genes like

CCBE1, *FAT4*, *PIEZO1*, *ITGA9*, *VEGFR3*, *SOX18*, *FOXC2*, and *GATA2*. The variations in these genes can cause syndromic and/or nonsyndromic GLD.^{1,2}

Lymphatic malformation-6 is a type of GLD caused by homozygous or compound heterozygous mutations in the *PIEZO1* gene.³ It is characterized by a uniform and widespread lymphedema in the fetus, and affects all segments of the body. There can be systemic involvement such as pericardial and/or pleural effusion, chylothorax as well as pulmonary and intestinal lymphangiectasia. Facial dysmorphism due to edema could also be seen. Occasionally there could be complete resolution of the fetal hydrops with a later onset of severe facial and limb edema during childhood. *PIEZO1* gene codes for

article published online
May 12, 2023

DOI <https://doi.org/10.1055/s-0043-57037>.
ISSN 2348-1153.

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the component of an ion channel that is essential for the proper formation of lymphatic vessels well as membrane stability of red blood cells. As affected individuals and sometimes the heterozygous carriers could show features of hemolysis (dehydrated hereditary stomatocytosis) and anemia with the peripheral smear showing macrocytosis and stomatocytes. The lymphatic dysplasia can cause impaired drainage of lymphatic fluid that results in seepage of fluid into interstitial tissue causing massive fetal edema. In utero diagnosis of NIFH is very critical to identify the etiology and prognostication of the current pregnancy as well as to ensure better care in future pregnancies.⁴ Using invasive prenatal testing and molecular genetic testing, we report a case of recurring NIFH associated with lymphatic malformation-6 in three consecutive pregnancies of a young couple with pertinent details of molecular genetic testing.

Case Report

A South Indian woman aged 23 years, primigravida, in a non-consanguineous marriage, was referred to our fetal medicine unit with an ultrasound diagnosis suggestive of fetal hydrops at 26 weeks of pregnancy. This was her first pregnancy. Targeted ultrasound examination revealed no structural anomalies. Immune causes of fetal hydrops were ruled out by appropriate investigations. The parents were counseled regarding the probable etiologies, and the pregnancy outcomes. Amniocentesis was performed and DNA was extracted and stored. Chromosomal microarray was done on fetal DNA that revealed no pathogenic or likely pathogenic copy number variants. The couple were not willing to do further molecular genetic evaluation due to economic constraints. The fetus died in utero at 30 weeks, but the parents were not willing for autopsy evaluation at that time.

During the second pregnancy, fetal hydrops was detected at around 21 weeks gestation. The couple were given genetic counseling and amniocentesis was done immediately, fetal DNA was extracted and stored. ►**Fig. 1A** shows the ultrasound image of the subcutaneous edema of the scalp of the fetus, while ►**Fig. 1B** shows the abdominal wall

edema. The pregnancy was medically terminated after obtaining parental consent. Exome sequencing of fetal DNA showed a homozygous novel variant with two base pair deletion in exon 39 of *PIEZO1* gene (chr16: g.88787786_88787787delTT; c.5455_5456delAA) that results in a frame-shift and premature truncation of the protein 46 amino acids downstream to codon 1819 (p. Lys1819GlufsTer46). Maternal contamination was ruled out. Familial segregation study was then. The same variant was observed in heterozygous state in both the parents by Sanger sequencing. Targeted sequencing was done in the DNA of the first affected fetus, which showed the presence of this variant in homozygous state, thereby proving the disease etiology in the family.⁵ Autopsy was performed in the second fetus that showed the evidence of hydrops in the form of skin and scalp edema with prominent eyebrows and puffy lower eyelids. The pleural, pericardial, and peritoneal spaces were filled with light straw-colored serous fluid. The umbilical cord as well as placenta showed edema. The fetus had mild intrauterine growth retardation. Other systems were within normal limits. Histopathological examination showed the characteristic dilatation of subcutaneous lymphatic vessels suggesting malformation of the lymphatic system. Similar findings were seen in intestinal and lung tissues as well. Both the clinical phenotype and the histopathological findings in the fetus were similar to those described with this gene. Hence, this novel mutation in *PIEZO1* gene was found to cause NIFH with lymphatic malformation-6 in both the pregnancies. Since the couple were heterozygous carriers of the variant, they were counseled regarding the 25% risk of recurrence risk and the need for prenatal evaluation in every future pregnancy.

They conceived for the third time. Chorionic villus sampling was planned at 13 weeks. But with coronavirus disease 2019d restrictions, amniocentesis was done at 20 weeks and DNA extraction done followed by targeted variant analysis which showed that this fetus also had the same variant in homozygous state. Targeted anomaly scan done showed evidence of hydrops in the form of generalized skin edema. The couple were counseled and they decided to terminate the pregnancy. Thus, the mutation in *PIEZO1* gene was found

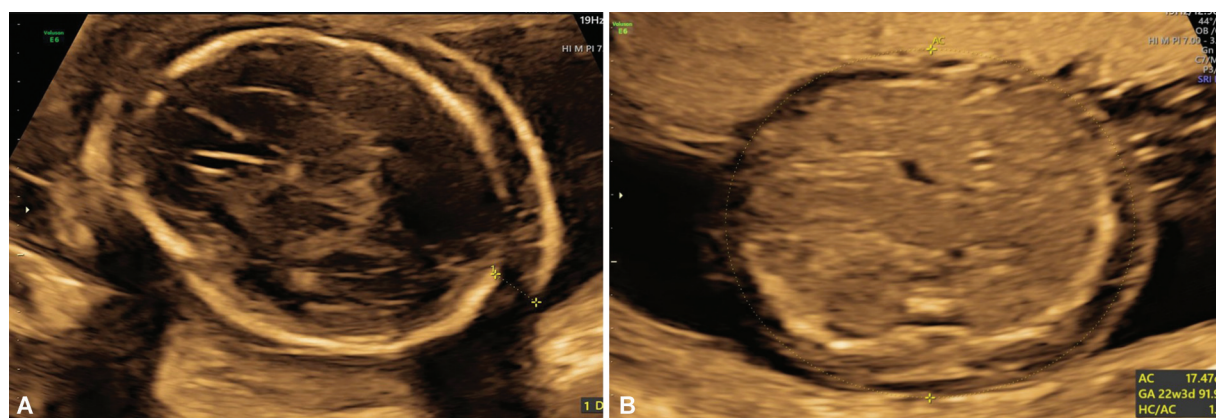


Fig. 1 Images of fetus with nonimmune fetal hydrops due *PIEZO1* gene variant. (A) Axial image of fetal skull showing subcutaneous edema beneath the scalp. (B) Axial image of fetal abdomen showing subcutaneous edema. No ascites was noted.

to cause autosomal recessive lymphatic malformation-6 with NIFH in the three pregnancies. The couple were advised regarding the recurrence risk as well as reproductive options available.

Discussion

Hydrops fetalis is defined as excessive accumulation of fluid within the fetal extravascular compartments and body cavities.⁶ NIFH accounts for 76 to 87% of all cases of hydrops.^{7,8} It affects 1 in 1,700⁹ to 1 in 3,750 deliveries.¹⁰ The reported prenatal mortality rate ranges from 55 to 98% with this condition.¹¹ It is an end-stage process for a heterogeneous group of disorders and by itself constitutes a poor prognostic factor for any particular disorder.¹²

There have been many etiologies associated with NIFH.¹ We should look into the clinical features, especially systemic involvement in detail by ultrasound scanning to discern the etiology. When chromosomal etiology is ruled out, we have to evaluate for the monogenic causes.¹³ The monogenic causes are classified again, in relation to the system that is primarily affected in the fetus, few of the cases of NIFH are amenable to antenatal management like fetal intravenous/intra peritoneal fresh frozen plasma infusion for Smith-Lemli-Opitz syndrome,^{14,15} in utero meningomyelocele repair,¹⁶ and repair of structural cardiac defects.¹⁷ When it comes to certain disorders like congenital diaphragmatic hernia (CDH), it is known that the in utero repair gives a better prognosis if it is nonsyndromic CDH than a syndromic CDH due to the obvious reasons of associated anomalies is seen in syndromic CDH. Hence, knowing the etiology behind each case of NIFH becomes necessary.¹⁸ And also, the overall mortality and morbidity are linked to the underlying genetic etiology in each case.¹⁹

The median gestational age at the diagnosis of fetal hydrops is 24 weeks.⁵ The diagnostic evaluation of NIHF can be done by obstetric ultrasound, fetal echocardiogram, and fetal MRI followed by invasive procedure to extract fetal DNA to do chromosomal microarray analysis,^{20,21} viral polymerase chain reaction, and more specialized testing like exome sequencing.²¹ As previously discussed, knowing the etiology of the NIFH is essential for prognosticating the pregnancy. With the knowledge of the etiology, further management of the pregnancy, as well as counseling about the prognosis, recurrence risk, and management of the baby after birth will become precise.

Using invasive prenatal testing and molecular genetic testing, a homozygous novel variant was found in the *PIEZO1* gene. This variant is not found in the population databases or the mutation databases. The in silico prediction of the variant is damaging. The reference codon is conserved across primates. Literature search showed a recent study of NIFH in Indian population by Correa et al,²² in which one of the fetuses had NIFH due to two novel variants in *PIEZO1* gene in compound heterozygous state, but those two variants were not in or around the codon affected in our case. Additionally, the clinical phenotype found in the fetus was similar to those described with this gene. The familial segregation study was

also suggesting the novel variant as the probable etiology. Hence, this novel mutation in *PIEZO1* gene was found to cause NIHF with lymphatic malformation-6 in both the pregnancies. The fact that they were nonconsanguineous and carrying the same rare variant suggests inbreeding in certain communities in the Northern parts of Kerala.

Disorders of lymphatic system have an important role in the cause of NIHF and 15% of NIHF cases are reported due to lymphatic dysplasia.²³ *PIEZO1* gene is associated with lymphatic malformation type 6.^{3,23} It encodes a protein that induces mechanically activated currents in various cell types.²⁴ Mechanotransduction is important for many physiological processes in the body and is also vital for regulation of embryonic development.²⁴ Andolfo et al reported that *PIEZO1* plays an important role during the development of lymphatic vasculature after observing it in lymphatic vessels of the peritoneum in human fetal tissue at 17 weeks of gestation.²⁵ Homozygous or compound heterozygous mutation in the *PIEZO1* gene on chromosome 16q24 can cause lymphatic malformation-6, an autosomal recessive form of GLD associated with NIHF and chronic peripheral primary lymphoedema in later childhood, with 25% chance of recurrence in future pregnancies.^{3,26,27}

Conclusion

NIHF is not a diagnosis in itself but a symptom and end-stage result of a wide variety of disorders. The overall mortality rate in NIHF is 75.5%,²⁸ making identification of its etiology crucial for prenatal counseling and management of current and future pregnancies. Whole exome sequencing must be routinely considered when chromosomal microarray fails to yield any result while evaluating NIHF.²⁹ Familial segregation study and fetal autopsy helped in confirming the pathogenicity of the novel variant detected in this family. With all the evidence, we confirmed lymphatic malformation-6 to be the underlying cause of NIHF in the three consecutive pregnancies. After proving the etiology, genetic counseling becomes important to ensure better care in future pregnancies. Larger studies need to be done to know the specific etiologies on monogenic causes of NIFH in the Indian population.

Conflict of Interest

None declared.

Acknowledgments

The authors are thankful to the couple who participated in this study. The fund for this study was met solely by the couple. We also acknowledge the Medgenome Laboratory for doing the Exome sequencing and the data analysis of the study.

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