Prenatal Screening of Sialic Acid-Storage Disease in Nonimmune Hydrops Fetalis

Original Article

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ABSTRACT

Introduction: Most lysosomal-storage disorders (LSDs) are diverse groups of autosomal-recessive inherited inborn errors of metabolism that encompass about 14 disorders linked with nonimmune hydrops fetalis (NIHF) and are responsible for 1–15% of NIHF cases, sialic acid-storage disease (SASD) is one of the most recurrent LSDs and pose a 25% estimated clinical risk of recurrence. Diagnosing or excluding SASD as an underlying cause for NIHF via quantifying sialic acid in amniotic fluid is crucial, since their recognition permits for prenatal diagnosis at an earlier stage in future gestations and enhances postnatal management.

Aim: Quantification of free sialic acid (FSA) in amniotic fluid to evaluate the association between SASD and NIHF.

Patients and Methods: The study has been conducted over 3 years and included two equally divided groups of 50 pregnant women: case group is composed of 25 pregnant females affected by NIHF and control group composed of 25 pregnant females not affected by NIHF. FSA was measured in amniotic fluid by tandem mass spectrometry.

Results: SASD was diagnosed in six out of 25 cases, which represented 24% of case group, FSA was nonsignificantly higher among hydrops fetalis cases.

The receiver-operating characteristic curve analysis has found that a cutoff value 11 μmol/l of sialic acid is the best threshold to expect SASD, with a sensitivity 24% and specificity 96%, hence, sialic acid had significant low diagnostic performance in differentiating case group from control group, where area under the curve is 0.322.

Conclusion: SASD could lead to NIHF as prognosis, however, it is still a nondominant cause, meanwhile, as increased FSA helps directing DNA sequencing to the Sialin/*SLC17A5* gene for biallelic mutations, which is a reliable diagnosis of SASD, we recommend including quantification of FSA in amniotic fluid using tandem mass spectrometry as a possible biochemical screen for LSD causes of NIHF.

Key Words: Nonimmune hydrops fetalis, prenatal screening, sialic acid-storage disease.

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INTRODUCTION

Hydrops fetalis is an abnormality characterized by extreme fluid buildup in two or more anatomical compartments, including pleural effusion, pericardial effusion, ascites, and anasarca. Nonimmune hydrops fetalis (NIHF) comprises all fetal hydrops cases that are not red cell alloimmunized (**Bellini *et al*., 2015**). The NIHF prevalence is 1/4000 births (**Steurer *et al*., 2017**).

Lysosomal-storage disorders (LSDs) may present as NIHF, most LSDs are inherited inborn errors of metabolism as autosomal-recessive traits (**Pinto *et al*., 2010**), they cause NIHF via venous blood flow obstruction due to visceromegaly and circulation issues arising secondary to storage abnormalities. Fluid accumulation may be due to anemia as an outcome of hypersplenism and drop of erythropoietic stem cells in reaction to storage cells' infiltration to bone marrow (**Staretz-Chacham *et al*., 2009**).

LSDs have been documented to be responsible for 1–15% of NIHF cases, around 14 different LSDs have been proven as being linked with NIHF develop ment (**Gimovsky *et al*., 2015**). Generally, out of the LSD disorders, sialic acid-storage disease (SASD) is one of the most recurrent ones (**Iyer *et al*., 2021**).

SASD could clinically present as hydrops fetalis and results from a defect in a protein encoded by solute carrier family 17 (acidic sugar transporter), Member 5; Sialin/*SLC17A5* gene; OMIM (#604322), that transports sialic

acid residues in the lysosome (N-acetylneuraminic acid) across the lysosomal membrane after being detached from the carbohydrate chain by sialidase enzymes (**Huizing *et al*., 2021**).

The SLC17 family of proteins is a group of structurally related membranous proteins under the superfamily of the major facilitator transporters and performs a variety of important biophysiological functions and inherited neurological/metabolic disorders have been linked to several members of this family (**Reimer, 2013**).

Lysosomal accumulation of free sialic acid (FSA) in numerous tissues is the distinctive biochemical feature of SASD, hence, high urinary excretion of FSA and detecting high FSA concentration in urine leads to diagnosis. There lies clinical significance of investigations in the amniotic fluid cells that may lead to prenatal diagnosis of SASD (**Couce *et al*., 2014**).

These single metabolic disorders represent an estimated clinical repetition risk of 25%, accordingly, it is important to identify if they are the underlying causes of NIHF, which will allow early prenatal diagnosis in future gestations and postnatal management (**Elsaba *et al*., 2019**).

Recently, it was reported that liquid chromatography–mass spectrometry (LC–MS/MS) quantifies FSA in urine (**Van der Ham *et al*., 2007**), however, this study will quantify FSA in amniotic fluid.

In addition to quantification of FSA in amniotic fluid, this study aims at evaluating the association between SASD and NIHF.

PATIENTS AND METHODS:

A prospective case–control study was done during the period from January 2019 to December 2021.

The local ethics committee has approved this study in compliance with the ethical standards laid down in the 1964 Declaration of Helsinki (Approval code 14/128) and all patients and controls have participated in the study after a written informed consent.

The study comprised 50 pregnant women divided into two groups; case group composed of 25 pregnant females affected by NIHF and control group composed of 25 pregnant females not affected by NIHF.

The control group was subjected to amniotic fluid sampling due to medical conditions other than NIHF such as prenatal genetic diagnosis of Down syndrome, Duchenne muscular dystrophy, and thalassemia.

Amniotic fluid samples of the control group were divided for the purpose of this study after all controls signed off a written informed consent.

All participants were subjected to detailed medical history, physical examination, fetal ultrasound, and Doppler imaging at 13 weeks of gestation.

Inclusion criteria

(1) Females with singleton pregnancy.

(2) RhD-positive females.

(3) Nonimmune hydrops fetalis.

Exclusion criteria

(1) Females suffering from medical disorders.

(2) Females with multiple pregnancies.

(3) Pregnancies with fetal cardiac defects, chromosomal abnormalities, or immune hydrops fetalis, cystic hygroma cases.

(4) Fetus affected by fluid accumulation limited to one cavity.

Fetal imaging

Detailed 2D ultrasound using General Electric Voluson 730 real-time ultrasound machine equipped with a multifrequency volumetric transabdominal transducer was done to determine getational age, exclude structural anomalies, and diagnose hydrops fetalis.

IBM SPSS was the statistical software tool for all calculations. Accuracy was interpreted in terms of sensitivity, specificity, and the likelihood ratio of a positive test and the likelihood ratio of a negative test.

The optimum cutoff value for FSA in predicting the occurrence of NIHF has been determined via receiver operator characteristic curve analysis. P values less than 0.05 were assumed statistically significant.

Invasive procedures

Amniocentesis was performed on outpatient basis, free-hand technique was used under ultrasound guidance performed at 16–17 weeks of gestation, patient abdomen is cleansed with antiseptic solution, 20-G spinal needle was used, 10 ml of amniotic fluid was aspirated with a sterile syringe and labeled and frozen at −20°C, and oral antibiotics were given to all participants.

Amniotic fluid sample was prepared by centrifugation for 10 min at 15 000g. For assay of FSA, 25 µl of internal standard was added to 125 µl of amniotic fluid sample.

Determination was done using Waters Xevo TQD Triple Quadruple Mass Spectrometer LC MS/MS, set to electron-spray negative ionization mode, with masslynx 4.1 operating software. Parent-to-daughter ion transitions for sialic acid and 1,2,3-13C3-N-acetyl neuraminic acid (internal standard) were established by direct infusion of each compound into the mass spectrometer.

In multiple-reaction monitoring (MRM) mode, both collision energy and cone voltage were changed for each compound and ion intensity was maximized to optimize sensitivity.

For each compound, the parameters of cone voltage and collision energy are demonstrated in (Table 1). Also, it showed that the LC system consists of Acquity UPLC autosampler and pump flow rate 0.2 ml/min. The analytical column was Waters Atlantis dc18 (3.5 µm, 2.1x100 mm) set at 35°C. The gradient-elution program using mobile phase A (0.05 ammonium format) and mobile phase B (acetonitrile) is shown in (Table 2).

Table 1: Parent-to-daughter ion transition, cone voltage, and collision energy

Compound	Parent ion	Daughter ion	Cone voltage	Collision energy
Sialic acid	308	86.9	15	28
Internal standard	310.96	89.75	15	28

Table 2: Gradient-elution program

Time	A%	B%
Initial	100	0
4	0	100
6	0	100
12	100	0

RESULTS:

The study comprised 50 pregnant women divided into two groups: case group A composed of 25 pregnant females affected by NIHF and control group B composed of 25 pregnant females not affected by NIHF.

The mean age of case group A is 28 and 29 years for control group B, and the difference between them was statistically insignificant. Case group A and control group B cases have positive consanguinity of 76 and 68%, respectively, and the difference was also statistically insignificant.

The gestational age of both case group A and control group B cases is ranging between 16 and 17 weeks and the FSA concentration for SASD cases is above 11 µmol/l in amniotic fluid supernatant (**van den Bosch *et al*., 2011**) Table 3 demonstrates the FSA concentration of case and control groups.

Tables 3 and 4 show that FSA was nonsignificantly higher among hydrops fetalis cases, while Table 5 shows the likelihood ratio of FSA being associated with case group A versus control group B.

Figure 1 shows the receiver-operating characteristic curve analysis, which found that a cutoff value 11 µmol/l of FSA is the best threshold to expect SASD, with a sensitivity 24 and specificity 96%, hence, FSA had significant low diagnostic performance in differentiating case group from control group where area under the curve is 0.322 as demonstrated in Table 6.

Meanwhile, Youden Index is 0.2 (closer to 0), which indicates that diagnostic tests will introduce close proportion of positive results for both case and control groups.

Table 5 and Figure 2 show that FSA greater than equal to 11.0 µmol/l had low sensitivity, negative predictive value but high specificity and positive predictive value, while Figure 3 demonstrates the distribution of all false and true results among case and control groups.

Figure 4 demonstrates MRM chromatograms of sialic acid and internal standard in one of the calibration points, while Figure 5 demonstrates the MRM chromatograms of sialic acid in standard and actual sample for SASD patient of FSA greater than 23 µmol/l.

Table 3: Comparison between case and control groups with reference to free sialic acid concentration

FSA concentration (µmol/l)	Case (N=25)	Control (N=25)
FSA≥11.0 [n (%)]	6 (24)	1 (4)

FSA, free sialic acid.

Table 4: Statistical distribution of free sialic acid presentation

	Case (N=25)	Control (N=25)
Mean±SD	6.9±10.0	6.1±2.7
Range	0.8–41.6	0.8–14.6
P	0.701***	

***Independent t test.

Table 5: Diagnostic characteristics of cutoff free sialic acid greater than equal to 11.0 µmol/l in differentiating case group from control group

Characteristics	Value	95% CI
Sensitivity (%)	24.0	9.4–45.1
Specificity (%)	96.0	79.7–99.9
Diagnostic accuracy (%)	60.0	45.2–73.6
Youden's Index (%)	20.0	1.6–38.4
Positive predictive value (%)	85.7	43.8–97.9
Negative predictive value (%)	55.8	50–61.5
Positive likelihood ratio (LR+)	6.00	0.78–46.3
Negative likelihood ratio (LR−)	0.79	0.63–1
Diagnostic odds ratio (LR)	7.58	0.84–68.46

CI, confidence interval.

Table 6: Area under curve table

			Asymptotic 95% confidence interval	
Area	SE	Asymptotic significance B	Lower bound	Upper bound
0.322	0.085	0.031	0.155	0.488

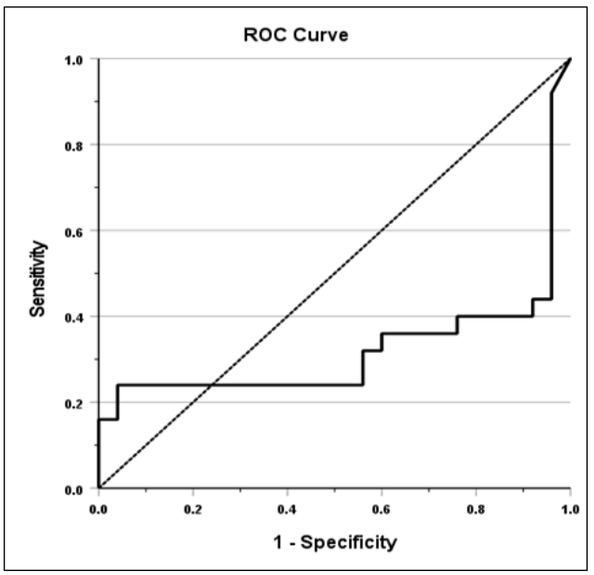

Fig. 1: ROC Curve for FSA in Differentiating Case from Control Groups (Area Under Curve is 0.322)

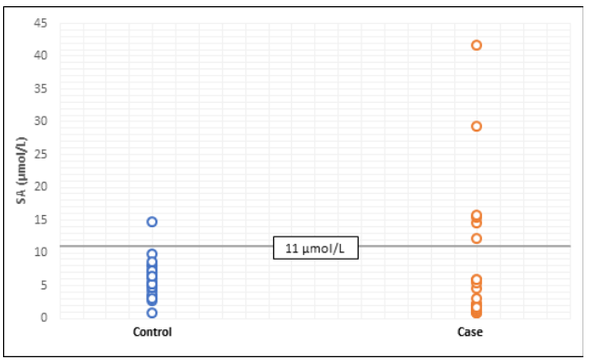

Fig. 2: Distribution of FSA among Case and Control Groups

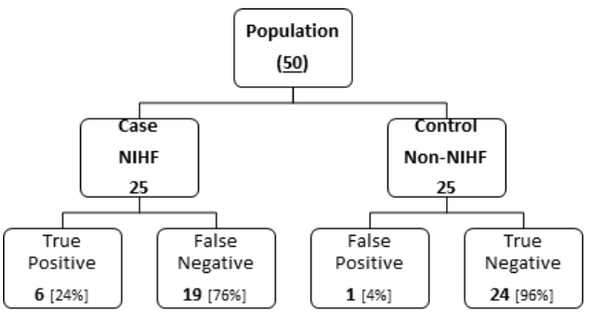

Fig. 3: Distribution of True & False Results among Case and Control Groups

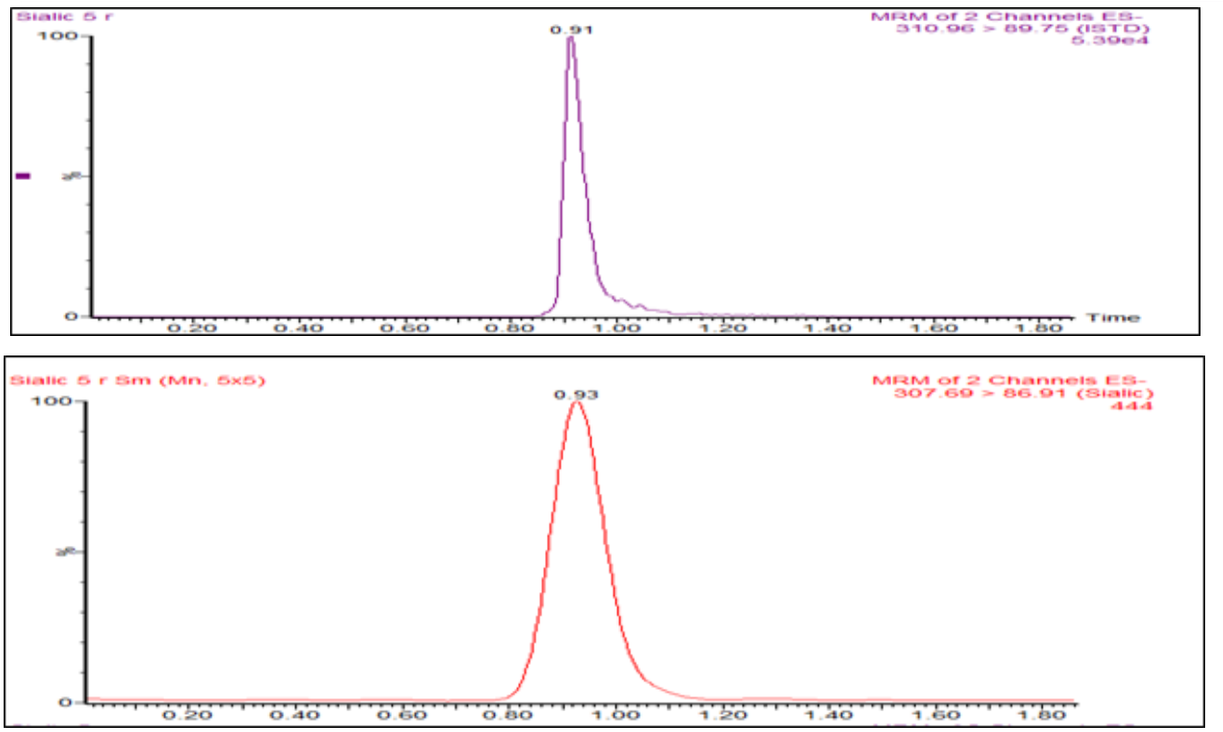

Fig. 4: MRM chromatograms of Sialic Acid and Internal standard in one of the calibration points

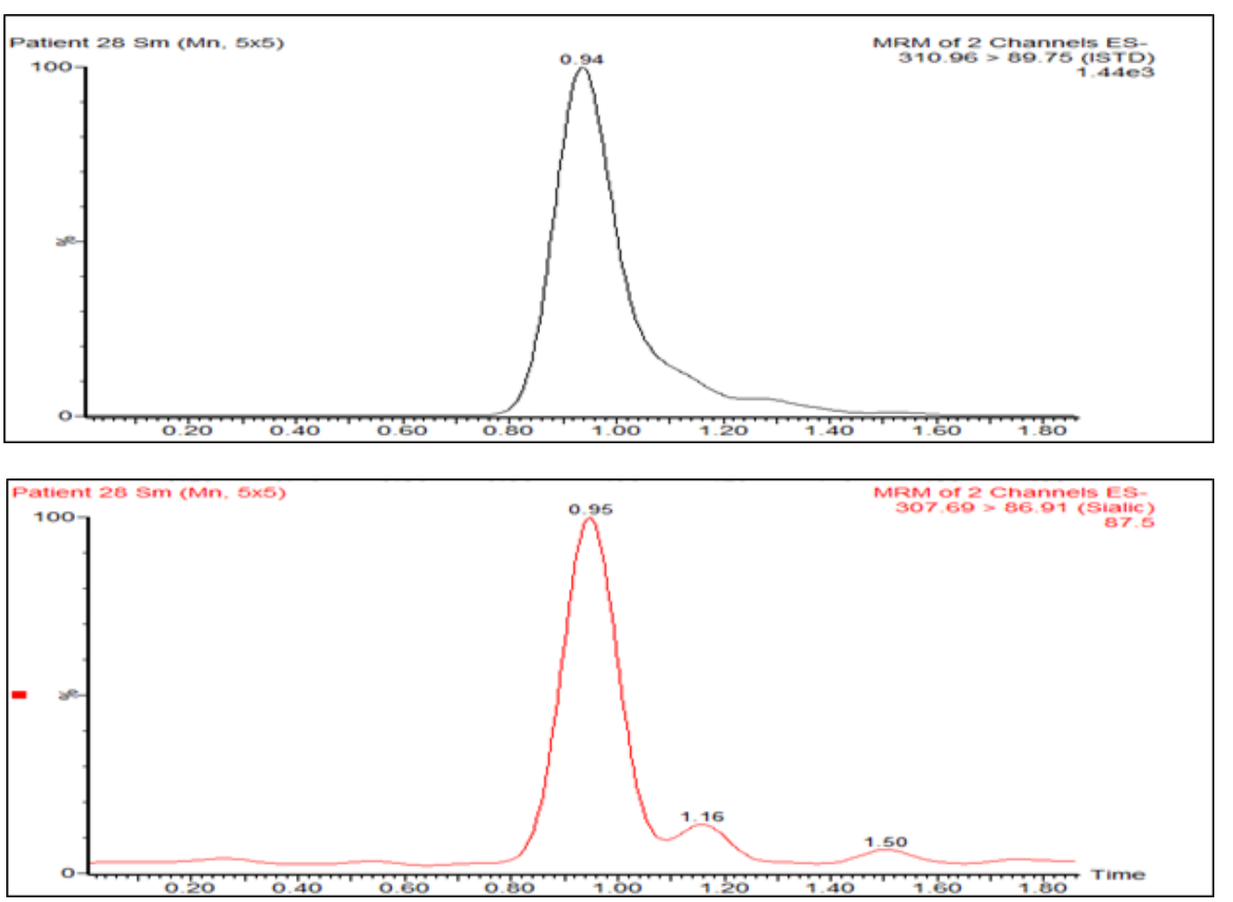

Fig. 5: MRM chromatograms of internal standard and Sialic Acid of patient with SASD (FSA >23 µmol/L)

DISCUSSION

NIHF is a life-threatening fetal condition, the intrauterine death rate in our study was 100%, meanwhile, 15.5–60.0% was the range of fetal loss rate due to NIHF according to many studies (**Heinonen *et al*., 2000; Santo *et al*., 2011**).

Many metabolic and nonmetabolic disorders lead to NIHF as prognosis, yet, the entire family of lysosomal-storage diseases are representing a nondominant cause of NIHF as in a recent study, **Al-Kouatly *et al*. (2021)** have concluded that LSDs were responsible for 15–29% of NIHF cases and 18% of those had SASD, also, **Jauniaux *et al*. (1990)** have reported 1% (LSD associated) only out of 600 NIHF cases.

In the same context, **Iyer *et al*. (2021)** have systematically reviewed literature between 1979 and January 2014 (687 cases were included) to evaluate the association between LSDs and NIHF. The incidence of NIHF with confirmed LSD was 5.2% and the most frequent disorders out of LSD family were GM1 gangliosidosis, Gaucher's disease, and mucopolysaccharidosis type VII.

Bellini *et al*. (2009) have concluded that inborn errors of metabolism incidence were responsible for 1.1% of NIHF cases after reviewing 225 articles comprising 5437 NIHF cases.

Also, **Lefebvre *et al*. (1999)** have presented a case of repeated NIHF as a rare presentation of SASD, associated with elevated concentrations of sialic acid in amniotic fluid and fetal cells.

These conclusions of previous studies support this study results that concluded SASD out of LSDs was nonsignificantly higher among hydrops fetalis cases, only 8% of case group in our study were SASD-diagnosed (considering the selected population of the sample as well as the study period).

SASD patients have undergone delayed diagnosis as an implication of disease rarity and absence of the routine urine sialic acid testing and nonspecific clinical symptoms (**Huizing *et al*., 2021**).

SASD is still a rare, neglected disease with patients experiencing misdiagnosis or delayed diagnosis. More access to routine (urinary) FSA screening may help diagnose SASD patients, increase the prevalence of SASD, and emphasize inclusion of the SLC17A5 gene in genetic screening panels for LSDs and NIHF **(Aula and Aula, 2006)**.

However, despite the incidence of LSD entire family is rare, they should not be excluded as a probable cause of NIHF, even if consanguinity is not present **(Burin *et al*., 2004).** Also, **Sheth *et al*. (2017)** recommended consideration of LSDs as a potential cause of NIHF, especially with recurrent NIHF.

Some studies have found that elevation of sialic acid as a sign of LSDs is correlated to NIHF, accordingly, quantification of sialic acid has an important role in prenatal diagnosis, **Chock *et al*. (2015)** described a fetal hydrops case with diagnosis potential for LSD, elevated levels of free and total sialic acid were present in lymphocytes as associated with SASD diagnosis.

Also, **Kang *et al*. (2018)** reported 10-times more concentration of FSA in urine in a case that is diagnosed of severe SASD with intrauterine hydrops fetalis.

In addition, **Elsaba *et al*. (2019)**, using the genetic analysis, recently showed that a genetic mutation causing SASD was present for a case that was diagnosed with prenatal hydrops fetalis at preterm 33 weeks of gestational age.

In this study, quantification of sialic acid is one of the aims, and as no index case was found, prenatal diagnosis was performed by measurement of FSA in amniotic fluid supernatant rather than mutation analysis **(Aula and Aula, 2006)**. Meanwhile, measurement of FSA in amniotic fluid supernatant as the first screening stage is more suitable to investigate SASD in prenatal studies of hydrops fetalis **(van den Bosch *et al*., 2011)**.

Many enhancements and developments to improve specificity, sensitivity, and accuracy of quantification methods of sialic acid have been introduced to get reliable diagnosis and analyze human biological fluids using advanced techniques rather than using biochemical methods **(Piraud *et al*., 2018; Van der Ham *et al*., 2010).**

Van der Ham *et al*. (2010) have quantified urinary sialic acid and validated a quantification method of sialic acid in cerebrospinal fluid by LC–tandem MS/MS method, where **van den Bosch *et al*. (2011)** and **Tebani *et al*. (2011)** have quantified sialic acid in amniotic fluid using the same method.

Along with these previous studies, our study has generated MRM chromatograms of sialic acid and internal standard in one of the calibration points, in addition to MRM chromatograms of sialic acid in standard and actual sample for SASD patient of FSA greater than 11 μmol/l.

CONCLUSION

SASD could lead to NIHF as prognosis, however, it is still a nondominant cause, meanwhile, as increased FSA helps directing DNA sequencing to the Sialin/SLC17A5 gene for biallelic mutations, which is a reliable diagnosis of SASD, we recommend including quantification of

FSA in amniotic fluid using tandem MS/MS as a possible biochemical screen for LSD causes of NIHF.

Finally, we conclude that SASD could lead to NIHF as prognosis, however, it is still a nondominant cause.

CONFLICT OF INTEREST

There are no conflicts of interest.

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