amino acid change p.G566D and baby presented with typical manifestations of HLH. Early genetic testing is needed to confirm FHL as allogenic HCT is the only curative therapy. It further helps in testing of at risk relatives, carrier testing, genetic counseling and prenatal testing for pregnancies at risk if disease causing mutation in family are known. As in our case we did prenatal diagnosis for the second child which was negative for mutation in *STXBP2* gene.

Acknowledgement: Dr Maurizio Arico, Director and Dr. Valentina Cetica, Department Pediatric Hematology Oncology, Azienda Ospedaliero-Universitaria Meyer, Florence, Italy for carrying out HLH mutation analysis.
Contributors: RJ: prepared the manuscript. MP: involved in case management and sending mutation analysis. PDM: edited the paper. ES: involved in genetic analysis. RJ and PDM: revised the paper for important intellectual content. All authors approved the final paper to be published.
Funding: None;
Competing interests: None stated.

REFERENCES

1. Henter JI, Horne A, Arico M, Egeler RM , Filipovich AH , Imashuku S, *et al*. HLH-2004 : Diagnostic and therapeutic guidelines for HLH. Pediatr Blood Cancer. 2007;48:124-131.
2. Cote M, Menager MM, Burgess A ,Mahlaoui N, Picard C, Schaffner C, *et al*. Munc 18-2 deficiency causes FHL 5 and impaires cytotoxic granules exocytosis in patient NK cells. J Clin Invest. 2009;119:3765-73.
3. Cetica V, Santoro A Glimou r KC, Seini E, Beutel K, Arico M, *et al*. STX BP 2 mutation in children with FHL 5. J Med Genet. 2010;47:595-600.
4. Sudhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. Science. 2009;323:474-7.
5. ZurStadt U, Rohr J, Seifert W, Florian K, Grieve S, Pagel J, *et al*. Familial Hemophagocytic Lymphohistiocytosis type 5 is caused by mutations in Munc 18-2 and impaired binding to syntaxin 11. Am J Hum Genet. 2009;85:482-92.
6. Meeths M, Entesarian M, Al-herz W, Nordenskjold M , Bryceson Y, Henter JI, *et al*. Spectrum of clinical presentation in familial hemophagocytic lymphohistiocytosis type 5 patients with mutation in STXBP2. Blood. 2010;116:2635-43.

Infant with Type A Niemann Pick Disease and Undetectable Niemann Pick Cells in Bone Marrow

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Received: August 24, 2011;
Initial review: September 13, 2011;
Accepted: October 19, 2011.

Bone marrow aspiration is the preliminary investigation in Niemann Pick disease type A when enzyme assays and mutation studies are unavailable. We report an infant with typical phenotype and enzyme deficiency, but undetectable Niemann Pick cells in the bone marrow. A new mutation R542X in *SMPD* gene was also detected.

Key words: *Bone marrow, Diagnosis, Niemann pick disease type A, Storage cells.*

Niemann Pick Disease (NPD) is a lysosomal storage disorder caused by absence or deficiency of Acid Sphingomyelinase (ASM), leading to pathological accumulation of sphingomyelin and cholesterol in the monocyte-macrophage system. This is characterized by large lipid laden macrophages or Niemann Pick cells (NP cells) in various tissues. According to clinical presentation NPD is phenotypically classified as Type A (Classical infantile neuronopathic form), Type B (Non-neuronopathic visceral form) and Type C (Juvenile form). We report an infant with NPA, who despite having typical phenotype and enzyme deficiency, failed to display NP cells in the bone marrow.

CASE REPORT

A six month old boy presented with gradually progressive abdominal distension since late neonatal period. There was no history of persistent fever, vomiting, abnormal bowel movements, pallor, jaundice, bleeding, rash or additional swelling. Acquisition of all developmental milestones was delayed. Seizures and altered consciousness were absent. Antenatal and perinatal periods were normal. Birth was at

term with a weight of 2.3 kg. He was the second issue of non-consanguineous, healthy, hindu parents. A male sibling had expired at 14 months of age with similar illness.

Anthropometry was within normal range for age, with weight 6.6 Kg (83.5 % of 50th percentile of WHO child growth standards), length 65.4 cm (96.75% of 50th percentile of WHO child growth standards) and head circumference 42.5 cm (between 10th and 25th percentile). The facies appeared coarse with a broad forehead, depressed nasal bridge, thick lips and anteverted nostrils. There was no icterus or lymphadenopathy. The abdomen was protuberant with firm hepatosplenomegaly (liver span was 11 cm in mid clavicular line and spleen size was 6 cm in splenic axis below costal margin) and no free fluid. Salient neurological findings were a conscious but apathetic infant with cherry red spots, normal cranial nerves and power, generalized hypotonia and hyporeflexia with extensor plantar responses. Structured developmental assessment demonstrated a Development Quotient of 62, suggestive of mild global developmental delay. Differential diagnoses of Niemann-Pick disease, Sandhoff disease and GM1 gangliosidosis were considered in order of suspicion.

Investigations revealed mild normocytic normochromic anemia (Hb 9.5 gm/dL) with normal total and differential leukocyte, platelet and reticulocyte counts. Liver function tests were deranged; total bilirubin 1.4 mg/dL, direct bilirubin 0.4 mg/dL, Aspartate amniotransferase 790 IU. Alanine amniotransferase 907 IU, alkaline phosphatase 1481 IU). Serum cholesterol level was 180 mg/dl (normal for age 65-175 mg/dL). Abdominal ultrasonography confirmed liver and spleen enlargement with normal echotextures and normal portal vein diameter. Hearing and visual evaluation (by BERAphone screening and Visual Evoked Response) and skeletal survey were normal. Bone marrow aspiration revealed cellular bone marrow with normoblastic erythroid series, myeloid series and megakaryocytes. Storage cells were not detected even on bone marrow biopsy. Thyroid Function tests and MRI cranium were normal. Sequential enzyme assays were planned. Serum levels of ASM were undetectable which was diagnostic of Niemann-Pick disease, the phenotypes suggestive of type A (Classical infantile neuronapathic form).

During follow up, neuro-developmental status remained static. Hepatosplenomegaly progressively increased but without further enzyme derangement. At 11 months, he contracted severe pneumonia and succumbed enroute to hospital. Permission could be obtained only for a post mortem liver biopsy, the histopathology of which revealed NP cells. Gene sequencing of the *Spingomyelin phosphodiesterase 1 (SMPD1)* gene revealed compound heterozygosity for stop codon mutations R443X and R542X in exon 3 and 5, respectively (***Fig.* 1**). Origin was maternal in the former and paternal in the latter.

DISCUSSION

In type A and B NPD, the affected enzyme is encoded by the *SMPD1* gene located on chromosome bands 11p15.1-p15.4, resulting in primary ASM deficiency with activity 1-10% of normal. Pathological sphingomyelin deposition results in infiltration of bone marrow, spleen, liver and lymph nodes with NP cells. In developed countries, diagnosis is established by enzyme assay and mutation analysis, rather than more invasive alternatives like BMA. However, in developing countries, these modalities are expensive and not easily available. Common practice in such circumstances is demonstration of NP cells by BMA.

Organomegaly has been reported as the commonest presentation of NPD-A, with a median age of diagnosis 6 months [1]. Elevated cholesterol was considered an early marker of the disease [1]. Since reticuloendothelial organs are completely infiltrated with NP cells originating from lipid-accumulating bone marrow progenitor cells, non-demonstration of NP cells in symptomatic patients is unusual. Extensive literature revealed no prior studies reporting absence of NP cells in BMA. Suboptimal sensitivity of BMA has been previously reported in children with type C NPD presenting with cholestatic jaundice. The overall sensitivity of 64% decreased to 57% when BMA was performed during the first year [2]. This can be explained by the later age of onset in type C. The clinical profile of our case (static neuro-developmental status, hepatosplenomegaly without increasing enzyme deterioration and normal cholesterol) reflects early illness with probably less extensive infiltration of the bone marrow. This questions the reliability of BMA in early

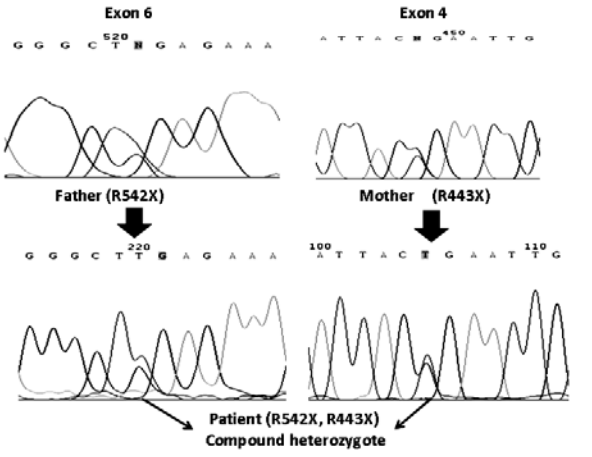

FIG. 1 *Gene sequencing of patient demonstrating paternal origin of mutation R542X and maternal origin of R443X.*

illness or young infants with NPD-A.

Mutational analysis has revealed many disease associated alleles in NPD-A. Most are sporadic with only a few having ethnic predilection like p.R496L, p.L302P and p.P330fs in Ashkenazi population and c.677delT in Israeli Arabs [3]. Most mutations are single base substitutions and small deletions with or without a frameshift [4]. Small deletions or nonsense mutations result in truncated ASM polypeptide and missense mutations render the enzyme non-catalytic in NPD-A, whereas enzyme is defective with residual catalytic activity and milder phenotype in NPD-B [5]. In this case, mutation R542X (arginine to stop codon at amino acid 542) is novel whereas mutation R443X (arginine to stop codon at amino acid 443) has been reported earlier in a homoallelic patient, also of Indian origin [6]. Only further studies will be able to confirm a possible Indian predilection.

In resource limited settings, demonstration of storage cells on BMA is the preliminary investigation despite its invasiveness. Confirmatory enzyme assays are performed subsequently. Absence of NP cells in the bone marrow usually leads to considering alternative diagnoses. When strong clinical suspicion of NPD-A exists, a normal BMA should not exclude the diagnosis without an enzyme assay. If services are unavailable locally, blood can be collected as 'spots' on 903S&S filter paper (GE) which remain sufficiently stable to be transported to the testing laboratory for enzyme analysis [7].

Acknowledgements: Dr Ashwin Dalal, Director, Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics, Hyderabad for providing his valuable expertise.

Contributors: SBM, MP and SK were involved in clinical diagnosis and manuscript writing. SBM and SK will stand as guarantors. TPP was involved in the genetic tests and their interpretation. All authors approved the final manuscript.

Funding: None; *Competing interests:* None stated.

REFERENCES

1. McGovern MM, Aron A, Brodie SE, Desnick RJ, Wasserstein MP. Natural history of Type A Niemann-Pick disease: Possible endpoints for therapeutic trials. Neurology. *2*006; 66:228-32.
2. Rodrigues AF, Gray RG, Preece MA, Brown R, Hill FG, Baumann U, *et al*. The usefulness of bone marrow aspiration in the diagnosis of Niemann-Pick disease type C in infantile liver disease. Arch Dis Child. 2006;91:841-4.
3. Ricci V, Stroppiano M, Corsolini F, Di Rocco M, Parenti G, Regis S, *et al*. Screening of 25 Italian patients with Niemann Pick A reveals fourteen new mutations, one common and three private in SMPD1. Hum Mutat.2004;24:105.
4. Sikora J, Pavlu-Pereira H, Elleder M, Roelof H, Wever RA. Seven novel acid sphingomyelinase gene mutations in Niemann Pick type A and B patients. Ann Hum Genet. 2003;67:63-70.
5. Takahashi T, Suchi M, Desnick RJ, Takada G, Schuchman EH. Identification and expression of five mutations in the human acid sphingomyelinase gene causing types A and B Niemann-Pick disease: molecular evidence for genetic heterogeneity in the neuronopathic and non-neuronopathic forms. J. Biol. Chem. 1992;267:12552-8.
6. Schuchman E. Two new mutations in the Acid sphingomyelinase gene causing type A niemann-Pick Disease: N389T and R441X. Hum Mutat. 1995;6:352-54.
7. Chamoles NA, Blanco M, Gaggioli D, Casentini C. Gaucher and Niemann-Pick diseases—enzymatic diagnosis in dried blood spots on filter paper: retrospective diagnoses in newborn-screening cards. Clin Chim Acta. 2002;317:191-7.