DOI: 10.7860/NJLM/2021/50512.2553

Case Report

Pathology Section

Gaucher's Disease with Rare Genotype- A Case Report

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ABSTRACT

Gaucher's Disease (GD) is a rare inherited Lysosomal Storage Disorder (LSD) caused by autosomal recessive inheritance of homozygous mutations in the Glucocerebrosidase (GBA) gene encoding the lysosomal enzyme acid β-glucosidase. GD is usually under-diagnosed in developing countries due to limitations in diagnostic resources for genetic evaluation. However, under such circumstances a meticulous Bone Marrow Examination (BME) and clinical correlation gives clue to the diagnosis of GD. Hence, the authors here report a case of 2-years-old male child who presented with abdominal distension and fullness with refractory anaemia for which he was transfused blood in the past. The ultrasound sonography revealed disproportionate hepatosplenomegaly. The clinical differential diagnoses were haematolymphoid malignancy and haemolytic anaemia. Haemoglobin (Hb) electrophoresis revealed no structurally abnormal Hb while the BME revealed hallmark Gaucher's cells. To confirm the diagnosis of GD, enzymatic assay was done which revealed reduced β (beta) glucosidase and elevated plasma chitotriosidase levels followed by genetic work up which revealed a rare genotype with compound heterozygous mutation in GBA gene having uncommon variants.

Keywords: Bone marrow, Gaucher's cells, Lysosomal storage disorder

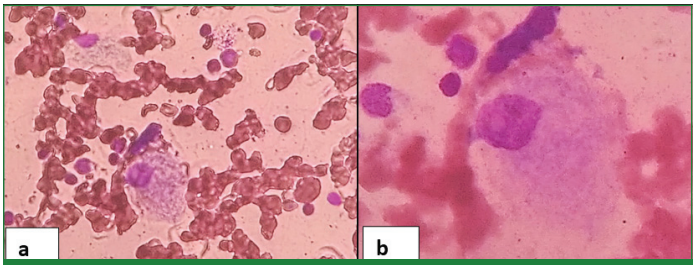
CASE REPORT

A two years old male child, born of a non consanguineous marriage, was brought to paediatric Outpatient Department (OPD) by mother who noticed abdominal distension and fullness in the child since last three months. It was insidious in onset and gradually progressive, primarily noticed on left side of abdomen. The child had been immunised up-to-date and developmental history was satisfactory. Past history revealed hospitalisation in private hospital where he was transfused one packed cell volume for low Hb level, the details of which were not available.

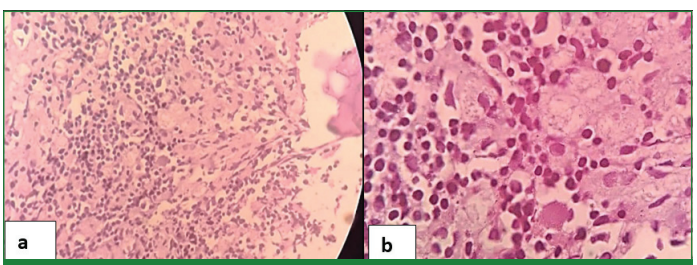
On examination, the child had severe pallor with hepatosplenomegaly. On palpation, there was moderate hepatomegaly with massive splenomegaly, with spleen reaching up to left iliac fossa. Rest of the systemic examination was within normal limits. Ultrasound sonography of abdomen revealed liver size of 12.4 cm and spleen size of 15.1 cm thus confirming palpatory findings of disproportionate hepatosplenomegaly. The laboratory findings revealed Hb 7.16 g/dL, total leukocyte count 6500/cu mm, platelets 45000/cu mm with Red Blood Cell (RBC) indices being less than lower limit. Renal and liver function tests were within normal limits.

Considering the clinical, radiological and laboratory findings, the differential diagnosis of haematolymphoid malignancy and haemolytic anaemia were thought of. Hence, further investigations advised were Hb electrophoresis to rule out haemolytic anaemia and BME to rule out haematolymphoid malignancy.

High Performance Liquid Chromatography (HPLC) revealed no structurally abnormal Hb. The peripheral blood smear revealed microcytic hypochromic anaemia with thrombocytopenia with absence of blasts or abnormal cells. The bone marrow aspirate was diluted showing scanty cellularity with very few scattered large cells (40-50 µm) with abundant metachromatic cytoplasm having fine and wavy like crumpled tissue paper appearance and single central to eccentrically placed nucleus with distinct nucleoli suggestive of Gaucher's cells [Table/Fig-1a,b]. The bone marrow biopsy also revealed similar cells in clusters and singly scattered amidst the trabeculae [Table/Fig-2a,b]. Thus, a diagnosis of LSD suggestive of GD was made. Further investigations to confirm the diagnosis were done which included enzyme assay and genetic work-up. As these tests were not available in this institute, the samples for the same were sent to higher centre. Beta glucosidase enzyme assay on dried blood spot sample revealed level of 1.02 nmol/hr/mL (normal range 2.3-18.4 nmol/hr/mL) and chitotriosidase level of 1216.7 nmol/hr/mL (normal range 0-90 nmol/hr/mL) favouring GD. The mutational testing by target gene sequencing revealed compound heterozygous variant of GBA gene and the variation was seen in exon 11 and exon 8 of GBA gene [Table/Fig-3]. Thus final diagnosis of GD was made.The patient was offered Enzyme Replacement Therapy (ERT). However, the patient was non affording and unable to bear the expenses of ERT, the parents were advised to take help and guidance from Non Government Organisations (NGO).



[Table/Fig-1]: a) Bone marrow aspirates showing Gaucher's cells (Leishman's stain, 40x) and b) showing 'crumpled tissue paper chromatin' (Leishman's stain, 100x).



[Table/Fig-2]: a) Bone marrow biopsy showing intertrabecular space with marrow elements and Gaucher's cells in clusters (H&E stain, 10x) and b) showing hallmark Gaucher's cells (H&E stain, 40x).

Gene (Transcript)	Location	Variant	Zygosity	Disease	Inheritance	Classification
GBA (-) (ENST00000327247.5)	Exon 11	c.1504C>T (p.Arg502Cys)	Heterozygous	Gaucher disease type I/ II/ III/ IIIC	Autosomal recessive	Pathogenic
	Exon 8	c.970C>T (p.Arg324Cys)	Heterozygous			Likely pathogenic

[Table/Fig-3]: Target gene sequencing report.

DISCUSSION

The LSD is a group of diseases which occur due to accumulation of glucosylceramide/glucocerebroside and some related compounds within the lysosomes resulting from defects in lysosomal hydrolytic enzymes. GD is the most common amongst various LSD [1]. It is caused by autosomal recessive inheritance of mutations in the GBA gene encoding the enzyme acid β glucosidase [2]. Its global prevalence is approximately 1/57,000 to 1/75,000 births but more incidence of 1/800 births is seen in Ashkenazi Jews [1]. However, the prevalence in the Indian subcontinent is unknown due to paucity of cases attributed to its rarity [2]. Also, in resource-poor countries, the confirmatory diagnosis and treatment by ERT still remains a distant dream.

In 1882, Phillippe Gaucher first described the cells in GD which was two decades prior to the dictum of "Inborn errors of metabolism" given by Sir Archibald Garrod. He observed large cells in a splenic aspirate while evaluation of a large spleen and thought that it was evidence of a primary neoplasm of the spleen [1]. In 1924, Epstein first recognised the storage of glucocerebroside [1] while in 1934 a study by Aghion revealed that the distorted macrophages, also called Gaucher cells, resulted due to an accumulation of the lipid glucocerebroside [3]. In 1965, Brady and Patrick discovered that the metabolic defect in GD was due to deficiency of the enzyme β glucocerebrosidase [3]. In 1968, Nalysnyk L et al., established the lysosomal localisation of β glucocerebrosidase following which GD was classified as LSD [2].

The metabolic defect in GD is due to deficiency of acid β glucosidase (lysosomal glucocerebrosidase) caused by bi-allelic mutations in GBA gene which results in the accumulation of glucocerebroside in lysosomes, classically in tissue macrophages. It has three phenotypic variants depending on the age and presence of the neurological deficit [Table/Fig-4] [1].

Glycolipid glucosylceramide is derived mainly from leukocyte cell membranes and red cell membranes and is broken down by acid β glucosidase. Absence of this enzyme results in accumulation of cerebroside in the macrophages converting them into Gaucher's cells. Hence peripheral leukocyte assay or enzyme activity in cultured skin fibroblasts or other nucleated cell is used to diagnose GD. An artificial substrate, 4-methylumbilliferyl-beta-glucoside, is required for the peripheral leukocyte assay and a residual enzymatic activity (10-15% of the control enzyme activity) is considered to be deficient [1]. Chronically, activated phagocytes, especially glucocerebroside-laden macrophages express chitotriosidase, a human analog from non vertebrate chitinase [4]. Hence, plasma chitotriosidase activity has been suggested to indicate total body Gaucher cell load. Patients with active GD have elevated values of chitotriosidase which is found to be 1000-fold above normal values [4]. Its level correlates with liver and spleen volume, haemoglobin concentration, platelet count, and bone manifestations [5]. It decreases dramatically after initiation of ERT and rises when treatment is stopped and hence is used to monitor the disease control [4]. Patients are diagnosed according to the criterias- Disproportionate hepatosplenomegaly and anaemia on clinical examination. Pathological testing reveals anaemia with thrombocytopenia in haemogram and presence of Gaucher's cells in bone marrow aspiration cytology and biopsy. Biochemical examinations reveal increased levels of serum angiotensin converting enzyme, plasma chitotriosidase, serum acid phosphatase and serum ferritin levels while reduced enzyme β-glucosidase assay. Further the diagnosis is confirmed by hypergammaglobulinemia and genetic mutation analysis.

The GBA gene is located on chromosome 1q21 [1]. More than 300 distinct mutations of the GBA gene have been described in which 80% are single nucleotide substitutions while rare or unknown alleles account for the remaining 20% [1]. Among the pathogenic variants, the Ashkenazi Jewish population shows the following four most common variants accounting to approximately 90%:

- c.84dupG (formerly known as 84GG)
- c.115+1G>A (formerly known as IVS2+1)
- p.Asn409Ser (formerly known as p.N370S)
- p.Leu483Pro (formerly known as p.L444P).

While in non Jewish populations, the same four alleles account for approximately 50%-60% and they tend to be compound heterozygotes with one common and one rare pathogenic variant or a unique pathogenic variant [6]. The mutation p.Leu483Pro remains as the most commonly observed mutation in Indian GD patients [4]. Also, homozygous mutation is more common than the compound heterozygous mutation in GBA gene [4]. However, in the present case, there was compound heterozygous mutation in GBA gene with p.Arg502Cys on exon 11 and p.Arg324Cys on exon 8 variant, both being rare genotype of GD.

Since early 1990s, the advent of ERT has changed the management and survival of these patients. In addition to this, the advancement in management which has broadened the horizon includes substrate reduction, pharmacological chaperone, and gene therapies [1]. Thus, GD is considered as a model for applications of molecular medicine to clinical delineation, diagnosis, and treatment [1]. The survival of these patients is increased because of ERT.

Comparative analysis	Type I (Adult subtype-non neuronopathic)	Type II (Infantile subtype-acute neuronopathic)	Type III (Juvenile subtype-chronic neuronopathic)
Ethnicity	Panethnic and Ashkenazi Jews	Panethnic	Panethnic and Norrbottnian type from Sweden
Onset of disease	Childhood/ adulthood	Infant childhood or adolescence	Childhood/ adulthood
Hepatosplenomegaly	Present	Present	Present
Bone involvement	Present	Absent	Present
Neurodegeneration	Absent	Present	Present
Other systems	Hepatic fibrosis, pulmonary hypertension, lymphoma	Congenital ichthyosis	Cardiac and vascular calcifications
Mutation association	N370S	Diverse	L444P
Life expectancy	Normal with enzyme replacement therapy	Death ensues by three years of age (rapid neurodegenerative course)	Death occurs in third to fourth decade)

[Table/Fig-4]: Phenotypic variants of Gaucher's disease [1].

As GD is an autosomal recessive disorder, it has 25% risk of recurrence in each pregnancy. Hence, pretest and post-test counselling must be done so that the family understands the risk of disease in each pregnancy, the reproductive options to prevent recurrence, implications of giving birth to a child with GD, and the options available to them if the foetus is identified to be affected.

A prerequisite to prenatal testing is confirmation of the diagnosis in the affected child by enzyme testing and preferably also mutation identification, done most commonly by chorionic villus sampling which is performed at 11-13 weeks of gestation. Prenatal foetal testing is conventionally performed by measurement of enzyme activity in uncultured chorionic villi tissue. Cultured amniotic fluid cells (15-18 weeks) or cord blood (19-20 weeks) can also be used if the family comes in late gestation. Ideally biochemical results should be correlated with mutation studies if the latter are available. Molecular testing is the technique of choice for carrier detection too. It should be offered to extended family members, especially in communities where consanguinity is common. The siblings of patient diagnosed with GD should be screened and if found to be affected, they should be monitored to assess rate of progression, though being presymptomatic. As there is wide phenotypic variation even among siblings; the pre-symptomatic diagnosis should not in itself indicate the initiation of therapy. Such children should be monitored for development of cytopenia, splenomegaly and skeletal disease, at 6-monthly intervals [7].

The genotype information helps in prognostication, carrier-testing and prenatal diagnosis. Hence, GBA mutation analysis is recommended before initiation of therapy. However, there are >300 known GBA mutations catalogued in GD and full gene sequencing is required when one or both alleles are other than the most common mutation [7]. In the advent of diagnostic tools, BME still remains a prerequisite to diagnose GD. Also simultaneous occurrence of GD with Myelodysplastic Syndrome (MDS) has been reported wherein the BME was found to be an important diagnostic tool. Hence BME must be done in all suspected cases [8].

CONCLUSION(S)

The GD is usually under diagnosed especially in developing countries due to limitations in diagnostic resources for genetic testing and evaluation. However, under such circumstances, a meticulous BME and clinical correlation gives clue to the diagnosis of GD. Though enzymatic assay or genetic studies remain gold standard for confirmation of GD, BME revealing hallmark Gaucher's cells and disproportionate hepatosplenomegaly can be used to make the diagnosis. Hence, through this case report, we have tried to highlight the significance of initial BME as a pointer for GD which is an invasive yet affordable technique, done easily at any set up with only some expertise needed.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 22, 2021
- Manual Googling: Jul 30, 2021
- iThenticate Software: Aug 18, 2021 (13%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **May 27, 2021**
Date of Peer Review: **Jun 22, 2021**
Date of Acceptance: **Aug 06, 2021**
Date of Publishing: **Oct 01, 2021**