ABSTRACT

Gaucher disease (GD) is the most common glycolipid storage disorder resulting from glucocerebrosidase deficiency due to pathogenic mutation in *GBA* gene. It is an autosomal recessive disorder. Due to overlapping of phenotype and limitations of carrier analysis enzymatically, combine approach of diagnosis by enzymes study followed by molecular diagnosis is the standard approach.

In the present study, 50 unrelated cases with significantly reduced activity of β-glucosidase in leukocytes have been identified. Molecular analysis of *GBA* gene for known common mutations (N370S (c.1226A>G), L444P (c.1448T>C), R463C (c.1504C>T) and Ivs2 (+1) G>A) was carried out in 50 enzymatically confirmed cases of GD followed by bi-directional sequencing to confirm the same. From this, 27 (54%) patients were identified with common mutation L444P (c.1448T>C) and R463C (c.1504C>T) in exon 10. This include presence of mutant allele L444P (c.1448T>C) in exon 10 in 25 (50%) patients from which 22 (44%) were homozygous, 1(2%) was heterozygous/ unknown and 2 (4%) patient had shown compound heterozygosityof L444P (c.1448T>C)/R496C (c.1603 C>T) in exon10/11 and L444P (c.1448T>C)/ R329C (c.1102 C>T) in exon10/8 respectively. Homozygous R463C (c.1504C>T) mutation was observed in exon 10 in 2 (4%) patients. Bidirectional exons sequencing in remaining cases had identified 13 mutations in 14 patients. From that 4 were novel missense mutationI427S (c .1397T>G), L354V (c.1177 C>G), G250A (c.866 G>C) and A100P (c.415 G>C) and remaining 9 were missense homozygous mutationsR395C (c.1300C>T), R359Q (c.1193G>A), G355D (c.1181G>A), V352M (c.1171G>A), S356F (c.1184C>T), E326K (c.1093G>A), G202R (c.721 G>A) and F213I (c.754 T>A), Y220C (c.766 A>G). Among these mutations, R395C (c.1300C>T) found in exon 9, R359Q (c.1193G>A), G355D (c.1181G>A), V352M (c.1171G>A) and S356F (c.1184C>T) in exon 8 were identified one in each patient (10%). E326K (c.1093G>A) mutation in exon 8 was observed in two Srilankan siblings (4%) in homozygous state. G202R (c.721 G>A) and F213I (c.754 T>A) found in exon 6 were identified in one (4%) patient each in homozygous state. In carrier parents where index case DNA was not available, L444P was (c.1448T>C) found in exon 10 in 4 parents and in 1

parentsY220C (c.766 A>G) mutations in exon 7 found.

Novel missense mutations were identified by *GBA* gene bidirectional sequencing covering exon-intron boundaries. Four novel missense mutations I427S (c .1397T>G), L354V (c.1177 C>G), G250A (c.866 G>C) and A100P (c.415 G>C) found in Exon 10, 8, 7 and 4 respectively in 4 (8%) patients each. From that, L354V (c.1177 C>G), G250A (c.866 G>C) and A100P (c.415 G>C) were in homozygous state. I427S (c.866 G>C) found in heterozygous state whereas another mutation could not be identified.

In 9 cases of GD, mutation could not be identified.

All novel missense variants were confirmed to be pathogenic using various bioinformatics tools like SIFT, Polyphen2 and Mutation Taster. *In Silico*Protein homology modeling study was carried out to further establish the effect of novel variants that occurred at highly evolutionarily conserved and functionally active domain residues in the protein leading to conformational changes or mRNA producing truncated protein.

Phenotype correlation with genotype demonstrated that L444P (c.1448T>C) mutant allele was observed in 19 (38%) patients of type 1, while remaining 1 (2%) patient were of type 2 and 3 (6%) with type 3 GD. Children with L444P(c.1448T>C) homozygous genotype were presented with severe phenotype of hepatomegaly 17 (77%), splenomegaly 20 (91%), anemia and thrombocytopenia 11 (50%), bone disease 5 (23%), pulmonary congestion 2 (9%). Not all cases were investigated for the presence of Gaucher cells but all those investigated have shown the presence of foamy cells resembling Gaucher like cells 8 (36%), splenectomy 2 (9%), pallor 3 (14%), ichthyosis 1 (5%) and 3 (14%) were receiving ERT while referred to us for the molecular study. Homozygous L444P (c.1448T>C) genotype was found in type 3 GD patients with phenotype of ocular involvement, affected cranial nerves, GE microcephaly in each one patients. Heterozygous L444P (c.1448T>C) mutation was found in one patient with type 2 GD presented with phenotype of hepatosplenomegaly, bulbar palsy and dystonia and other genotype was not identified.

Compound heterozygosity (L444P (c.1448T>C)/ R496C (c. 1603 C>T) was seen in 1 (2%) type I GD patient with the phenotype of hepatosplenomegaly, anemia, thrombocytopenia and bone marrow with Gaucher cells. L444P (c.1448T>C)/ R329C

(c.1102 C>T) compound heterozygosity was seen in 1 adult type I GD patient (2%) with the phenotype of avascular necrosis as the primary sign and mild hepatosplenomegaly, anemia, thrombocytopenia and bone marrow infiltrated with Gaucher cells.

R463C (c.1504C>T) homozygous mutation was seen in 2 (4%) patients with type 1 GD with hepatosplenomegaly, severe bone osteomyelitis of femur, chronic anemia, bone marrow infiltrated withGaucher cells and splenectomy was carried out because of severely enlarged spleen.

R359Q (c.1193G>A) homozygous mutation was found in one type 1 GD patient with coarse features, hepatosplenomegaly (spleen 33 cm), scaly skin, and bone marrow with severe erythroid hyperplasia, large cells with fibrilly cytoplasm and thrombocytopenia. This patient was on ERT and responded well to the therapy with reduction in spleen size to 12 cm after 2 years. G355D (c.1181G>A) and S356F (c.1184C>T) homozygous mutant allele were observed in one patient each with type 1 GD with the phenotype of hepatosplenomegaly. From that, G355D (c.1181G>A) mutation was found in 1 patient receiving ERT during study inclusion. E326K (c.1093G>A) mutation was seen in two patients with phenotype of mild to moderate hepatomegaly, severe splenomegaly, anemia, thrombocytopenia, bone marrow with Gaucher cells. Among these patients, splenectomy was done in one of this patient and another one was adult. Y220C (c.776 A>G) and V352M (c.1171G>A) mutant allele were observed in one patient each with type 1 GD with the phenotype of hepatosplenomegaly, anemia, thrombocytopenia and bone marrow infiltrated with Gaucher cells. G202R (c.721 G>A) was seen in one patient with type 2 GD presented with hepatosplenomegaly, anemia, thrombocytopenia, aspirating pneumonia, convulsions off and on since one month almost continue daily, hypertonia, deep and superficial reflexes, drowsiness with extended neck position and resistance to neck flexion, drowsy response to painful stimuli, Deep Tendon Reflex (DTR) exaggerated and kernitis sign (Corneal ulcer). F213I (c.754 T>A) homozygous mutation was seen in one patient with the phenotype of hepatosplenomegaly, anemia, thrombocytopenia and bone marrow with Gaucher cells and was considered as type I GD.

Novel mutation I466S (c .1397T>G) / with unknown another mutant allele, G289A (c.866 G>C), L393V (c.1177 C>G) and A139P (c.415 G>C) was observed in one

each patient. Among them I466S (c .1397T>G) / with unknown mutant allele was present with phenotype of type 1 GD. Homozygous mutations G289A (c.866 G>C), and L393V (c.1177 C>G) were also found in Type 1 GD patients. Homozygous A139P (c.415 G>C) mutation was found in one patient with type 2 GD at the age of 1.3 years and was found to have febrile convulsion, pallor, thinning of legs, breathing problem and psychomotor retardation. Child was admitted in the hospital and death occurs due to breathing problem.

Present study suggest that L444P (c.1448T>C) is the most common mutant allele in 50% of patient with GD in India and this can be used as the first line of molecular screening and carrier detection in the population.Study also demonstrates that exon 8 and 10 are the hotspot region of the *GBA* gene accounting 59% of the mutant allele. This is the first study from India identifying mutational spectrum for GD that can be of help in offering precise genetic counseling, treatment and prevention of the disease. It will also helps in prenatal diagnosis where index case has confirmed diagnosis proven by enzyme activity or by molecular analysis. Moreover mutation identification helps in early and accurate diagnosis in families at risk.