RESULTS

5 RESULTS

5.1 Prevalance of LSDs- Our experience at FRIGE

From the pool of 747 cases suspected to be LSDs, affected LSDs patients were 313 and normal patients were 434. From affected LSDs patients, 50 confirmed cases of GD included in the present study. GDwas found to be the most common LSD (Sheth et al, 2013) (Figure 5.1).

Prevalance of LSDs-Total=747, Abnormal=313 (42%),
Normal=434(58%)
Data from August 2010 to March 2014

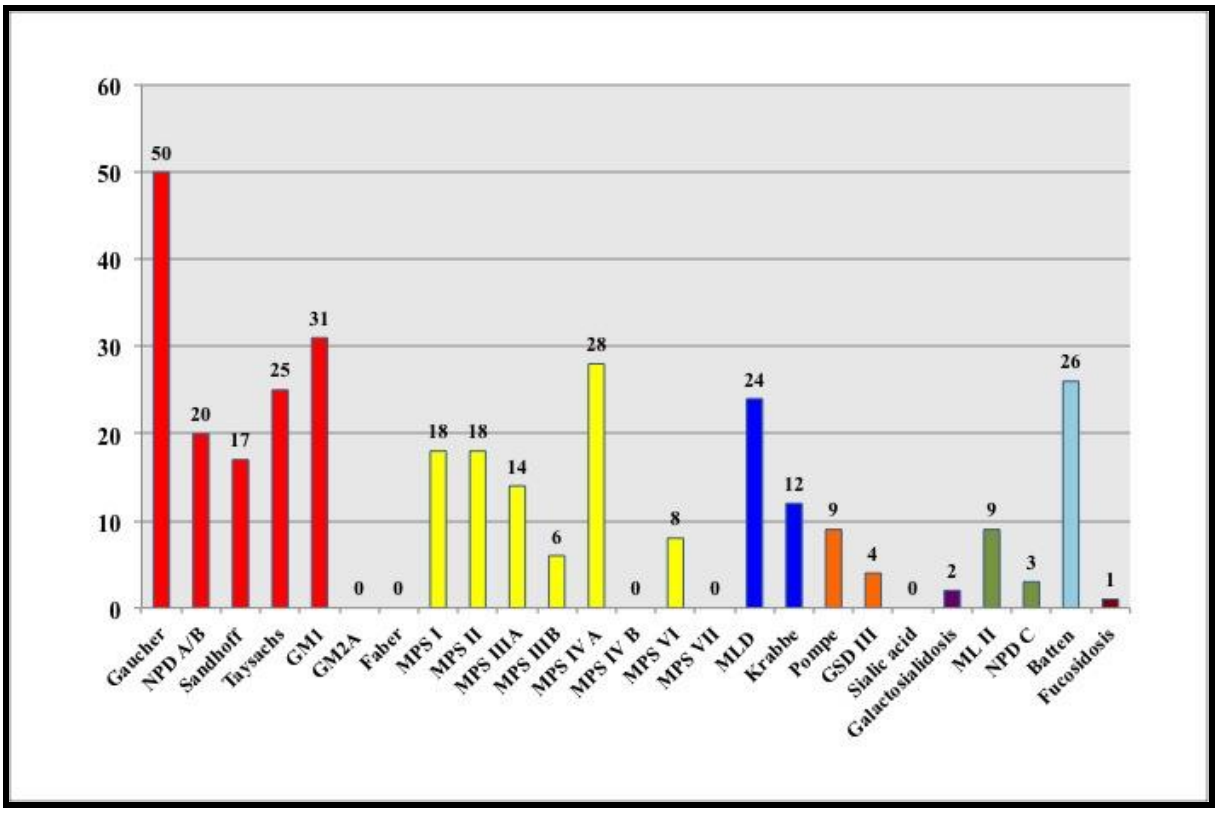


Figure 5.1: Prevalance of LSDs

5.2 Demographic data of GD

It has included cohort of 50 unrelated patients with confirmed diagnosis of GD comprising of 28 males and 22 females in the age range of 4 months to 40 years. 30% of these were born to the consanguineous parents.

From the 50 patients, 43 (86%) patients were of Type-I GD, 3 (6%) of Type-II GD and 4 (8%) of Type-III GD. Among all the three group of patients Type-I is the most commonly found (Table 5.1) and (Figure 5.2).

Table 5.1 Auxological data of GD patients with clinical symptoms

Patient ID	Age at diagnosis (Months or Years/ Sex)	Geographic origin	Cosan-guinity	Clinical features at the time of diagnosis	Type of GD
G2*	9Y/ F	Maharashtra	No	HS, bone infiltration	I
G3	2.5Y/ M	Maharashtra	No	HS, S/O. sacchadic eye movement, BM suggestive of GD	III
G5*	1.5Y/M	Gujarat	No	Massive HS, anemia, thrombocytopenia, mild regression of milestones	I
G6	19Y/ M	Maharashtra	No	HS, BM suggestive of GD, osteomyelitis of femur, splenectomy done	I
G7	2.5Y/ F	Maharashtra	No	Massive splenomegaly with mild to moderate hepatomegaly, unable to run, delayed milestones	I
G8	3Y/ M	Gujarat	No	Large HS, thrombocytopenia, thinning of limbs, scaly skin, BM suggestive of GD	I
G9*	11Mo/ F	Gujarat	No	HS, BM with lipid filled macrophages, delayed milestones	I
G10*	15 Mo/ F	Gujarat	Yes	Massive HS, unable to walk after 1 years, severe osteoporosis, anemia, thrombocytopenia, cardiomegaly with pulmonary congestion, gaucher cells occupy the sinusoids, mild hypotonia, seizures since 2 hours of birth	I
G11	16Y/ F	Maharashtra	No	Diagnosed case of GD	I
G12	21Y/ M	Maharashtra	Yes	Diagnosed case of GD	I
G13	3Y/ M	Maharashtra	Yes	HS, BM suggestive of GD, anemia, thrombocytopenia, growth retardation, deep and superficial reflexes, affected cranial nerves, power, hearing problem	II/III
G14	10Y/ M	Maharashtra	No	N/A	I
G15	1.6Y/ M	Rajasthan	No	Mild HS, growth retardation, developmental delay, o/e a febrile, family history+, neck holding +, BM suggestive of GD, anemia, thrombocytopenia	III
G16	22 Mo/ M	Gujarat	No	HS, cannot walk with support, anemia, thrombocytopenia	I
G18	1Y/ M	Gujarat	No	Massive HS, anemia, thrombocytopenia, BM suggestive GD	I
G20	3Y/ F	Maharashtra	Yes	HS, BM suggestive of GD	I
G21	1.5Y/ F	Kerela	Yes	HS, macule left back, b/I ichthyosis lower limb, family history of GD	I
G22	8Y/ M	Maharashtra	No	HS, jaundice, weakness, recurrent infections at birth, breathlessness on exertion present, GE microcephaly, short stature, pointed nose, undernourished,	III

G23	6Y/ M	Maharashtra	Yes	massive splenomegaly, anemia, thrombocytopenia, BM shows storage cells	I
G24	10 Mo/ F	Kerela	Yes	moderate HS, anemia, thrombocytopenia, material PAS stain s/o. storage disorder, pallor	I
G25	2Y/ M	Maharashtra	No	Huge HS, pancytopenia, BM shows gaucher cells	I
G26	2Y/ F	Maharashtra	No	distension of abdomen and small limbs	I
G27	1.3 Y/ F	Maharashtra	No	HS	I
G28	9Y/ F	Maharashtra	No	moderate hepatomegaly and severe splenomegaly, coryza and fever, anemia, thrombocytopenia, pallor	I
G29	2.5Y/ M	Karnataka	No	Not available	I
G30	40Y/ F	Maharashtra	No	BM showed gaucher cells	I
G31	11Y/ F	Kerela	No	severe splenomegaly	I
G32*	1Y/ F	Maharashtra	No	severe HS, anemia, thrombocytopenia, bone marrow transplantation	I
G33	6Y/ M	Maharashtra	Yes	HS, anemia, thrombocytopenia, BM showed foam cells	I
G34	2Y/ M	Maharashtra	No	NA	I
G35	2.5Y/ F	Bihar	No	HS, anemia, thrombocytopenia, BM biopsy- erythroid hyperplasia, few myeloid precursors, foamy macrophage histiocyte cells with eosinophilic, gradual paleness of body, fever, cough, cold, pallor	I
G36	4Mo/ F	Kerela	No	HS, conjugated hyperbilirubinemia	I
G37	4.5Y/ M	Srilanka	No	moderate HS, anemia, BMshows gaucher cells, growth retardation, mild coarse facial features	I
G38	11Y/ F	Srilanka	No	mild hepatomegaly with severe splenomegaly, bone marrow shows gaucher cells, anemia, thrombocytopenia, history of GD, splenectomy done	I
G39	2Y/ M	Maharashtra	No	moderate hepatomegaly with dilated portal vessels, massive splenomegaly, BM aspirates showing gaucher cells in moderate quantity	I
G40	2Y/ M	Maharashtra	No	HS	I
G41	2Y/ M	Maharashtra	Yes	HS, anemia, thrombocytopenia, BM suggestive of storage disorder, conjugative jaundice	I
G42	2Y/ M	Chhatisgarh	Yes	HS, cold, pneumonia, BM shows Niemann pick cells	I
G43	1Y/ F	Kerela	Yes	HS	I
G44	10 Mo/ M	Srilanka	Yes	HS, anemia, thrombocytopenia, non-obstructive renal calculi, growth retardation, X-ray femur : flask shape distal femur	I
G45	8Y/ M	Maharashtra	No	massive HS, BM suggestive of GD	I
G46	4.5 Mo/ F	Maharashtra	No	HS, psychomotor retardation, not gaining weight since birth, s/o. metabolic encephalopathy, pseudoedema, neonatal hepatitis is likely, yellowish discolouration of eyes, anemia, thrombocytopenia, CNS -low power UL & LL	III
G47	6 Mo/ M	Gujarat	No	HS, dystonia, bulbar palsy	II
G48*	1.3Y/ M	Maharashtra	Yes	HS, anemia, thrombocytopenia, failure to gain weight, febrile, pallor. Later symptoms thinning of legs, breathing problem, psychomotor retardation and death occur at 1.5 years	II
G49	1.4Y/ F	Andhra Pradesh	Yes	mild hepatomegaly and moderate splenomegaly with appearance are non-specific and intraabdominal mass	I

				present , anemia and thrombocytopenia failure to gain weight, reddish colour urine, o/c. conscious afebrile	
G50	22 Mo/ M	Maharashtra	No	huge HS, history of thrombocytopenia, history of blood transfusion due to pallor and not gaining weight	I
G51	20Y/ M	Gujarat	No	HS, anemia, thrombocytopenia, BM shows gaucher cells, avascular necrosis, difficulty in walking	I
G54	4Y/ F	Rajasthan	No	HS, anemia, thrombocytopenia, regression of milestones, hypotonia, younger sister died due to similar conditions	I
G55	11Mo/ F	Gujarat	Yes	HS, anemia, thrombocytopenia, Fever, cough, H/O. convulsions off and on since 1 month almost continue daily, hypertonia, deep and superficial reflexes, power, tone, aspirating pneumonia, AF: open. small, child drowsy, neck extended position, resistance to neck flexion, trismus , lack jaw , extonsor spasm , lauryngeal spasm , squint , drowsy response to painful stimuli, DTR: exagaratal, Kernitis sign	II
G56	2Y/ M	Maharashtra	No	mild hepatomegaly, moderate splenomegaly, anemia, thrombocytopenia, hepatic echoappearance s/o. of storage disorders, BM suggestive of GD	I

F-female, M-male, Y-years, Mo-months, HS-hepatosplenomegaly, BM-bone marrow, S/o. suggestive of, H/O. history of, AP- anterioposterior, *- Death of patient occurs, NA-not available

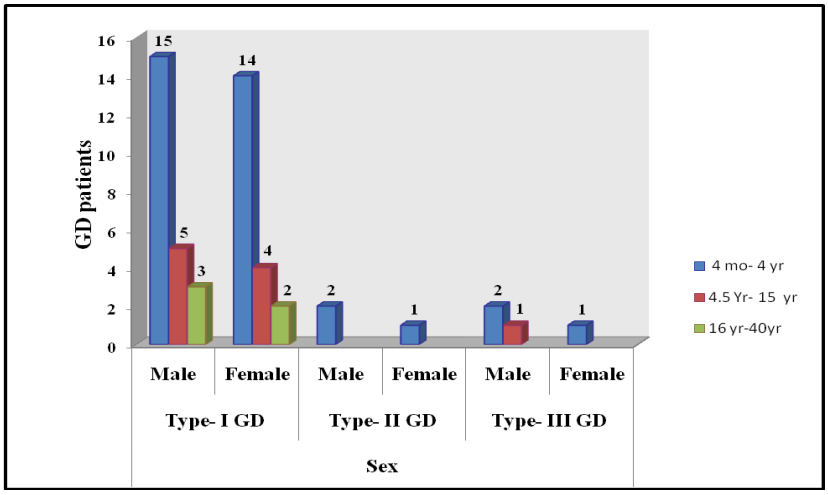


Figure 5.2: Age, Sex and Type of GD patients

5.3 Regional distribution of GD patients

GD patients are from different regions of India and three were from Srilanka. From these majority of patients were from Maharashtra (26) followed by Gujarat (9), Kerela (5), Srilanka (3), Rajasthan and Karnataka (2) each and from Bihar, Chattisgarh and Andhra Pradesh (1) each (Figure 5.3).

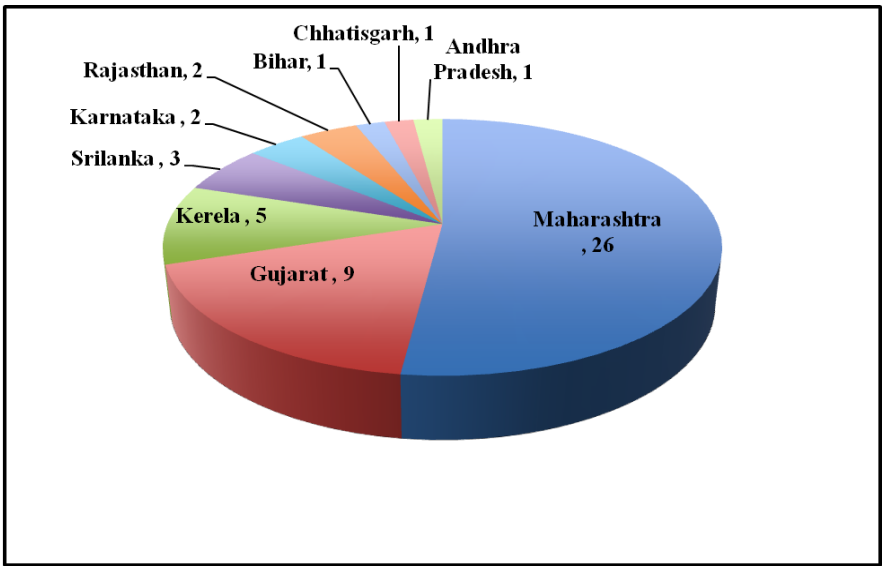


Figure 5.3: GD patients from different regions

5.4 Symptoms associated with GD patients

Hepatosplenomegaly wasthe most common symptoms found in GD patients. Hepatomegaly in 43(86%) patients, splenomegaly in 47(94%) followed by other symptoms of pancytopenia 28(56%), bone abnormalities like osteomyelitis of femur, thinning of limbs, severe osteoporosis, erlenmeyer flask deformities, thinning of legs, avascular necrosis, bone infiltration found in 10(20%), pulmonary manifestations likecardiomegaly with pulmonary congestion, breathlessness and on exertion present, aspirating pneumonia, breathing problem in 5(10%) and CNS involvement such as sacchadic eye movement, deep and superficial reflexes, affected cranial nerves, growth retardation and developmental delay, microcephaly, psychomotor retardation, encephalopathy, bulbar palsy, hypertonia in 8(16%) patients.6(12%) patients were on ERT and in 4 (8%) patients surgery like splenectomy, bone marrow transplantation was carried. Six children died at 1.3years to 7 years age and five were type I and 1 was type II. The infrequent and nonspecific skin problems likescaly skin, macule and

ichthyosis sometimes occurs and found in 2(4%) patients, (Figure 5.4) and (Table 5.1)

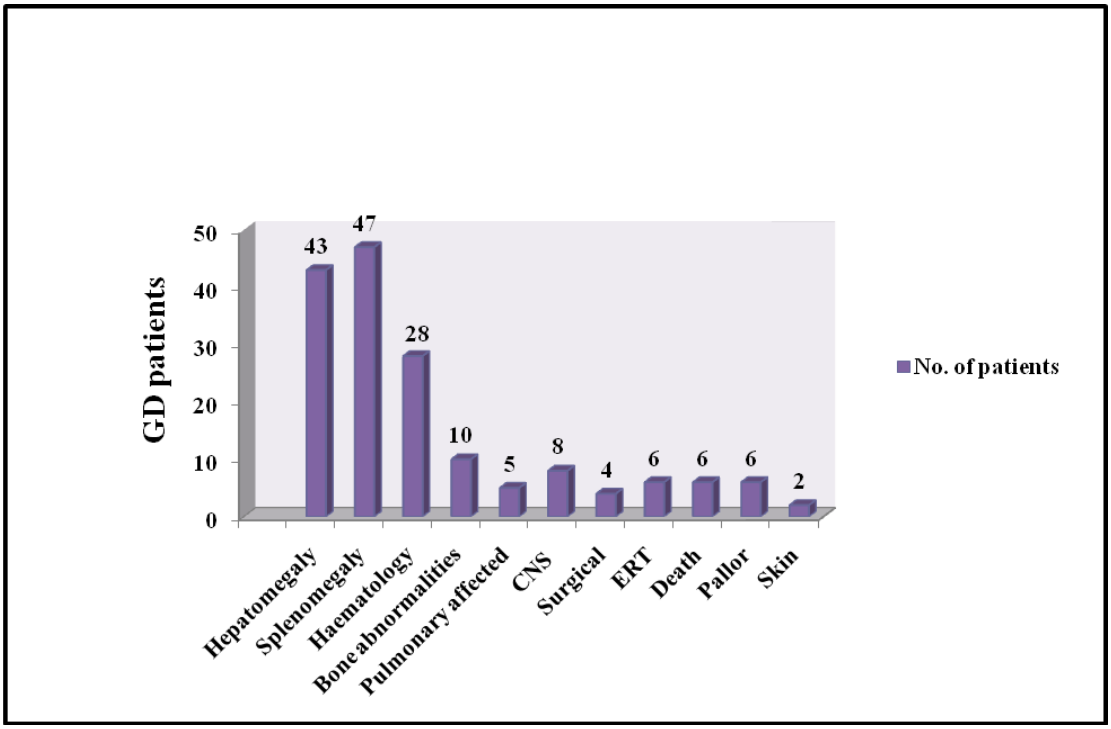


Figure 5.4: Symptoms associated with GD

5.5 Biochemical analysis of children with GD

5.5.1. Standard curve for chititriosidase and β-glucosidase

To standardize enzyme based diagnosis, we have primarily established normal range from leucocytes samples of normal healthy controls.The standard curve for measuring chitotriosidase and β-glucosidase activity was performed using 4-methylumbeliferone (4-MU) as substrate using a spectrofluorometric method. Figure 5.5 shows the standard curve for chitotriosidase and β-glucosidase, it was observed to be linear from 0.5 to 2.5 nmol with 0.9970 correlation coefficient.

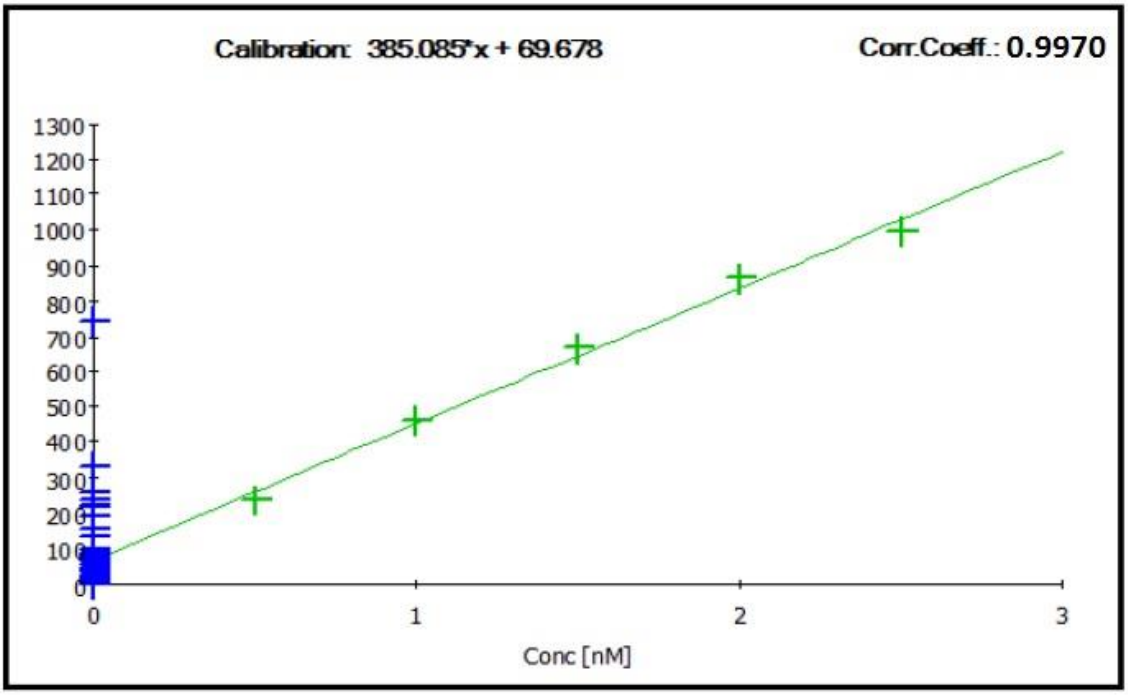


Figure 5.5 4-MU standard curve for chititriosidase and β-glucosidase

5.5.2 Screening with plasma chitotriosidase enzyme assay

Efore initiating confirmaive enzymes study all were screened with bio marker chitotriosidase This was carried out from plasma using 4-methyl umbelliferyl b-D-N, N, N', N"-triacetylchitotriosidase substrate and enzyme activity was expressed as nmol/hr/ml plasma. The normal range of plasma chitotriosidase of 28.66–62.94 nmol/hr/mL plasmawas established using normal healthy control subjects (Figure 5.5).Out of 50 patients, chitotriosidase activity was markedly elevated in 42 (84%) patients with undetectable activity in 2 (4%) and normal chitotriosidase activity in 6 (12%) patients (Figure 5.6).

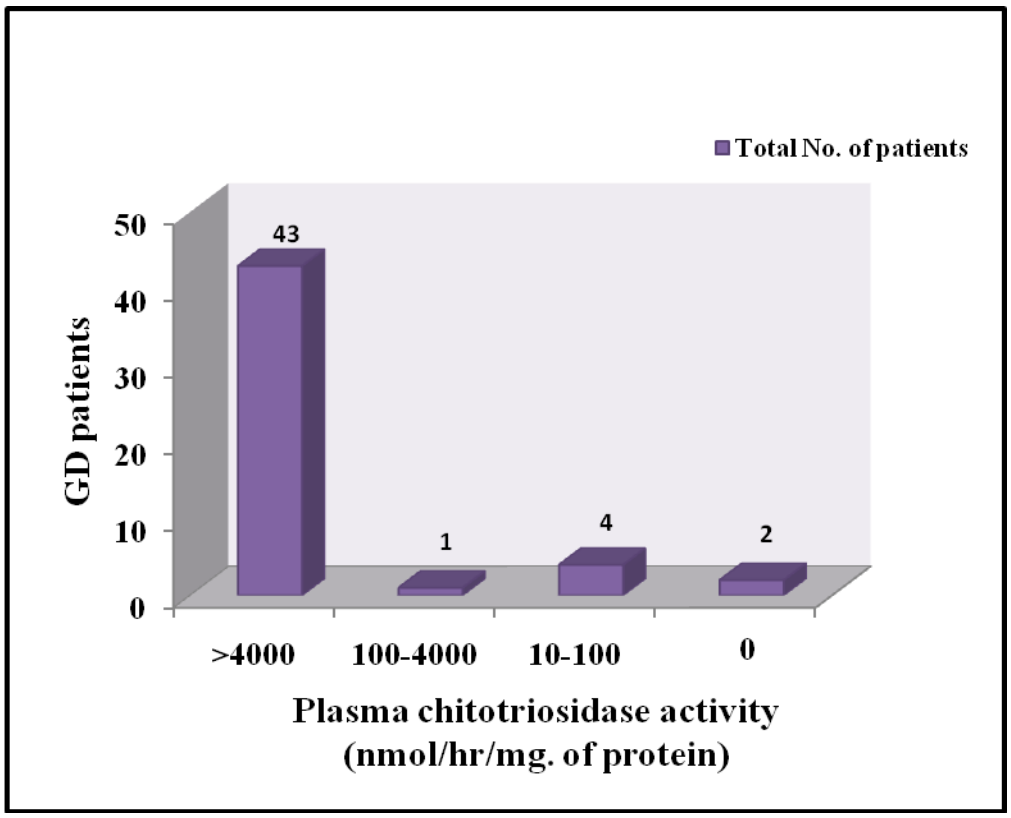


Figure 5.6: Plasma chitotriosidase activity in patients with GD

5.5.3 β-glucosidase enzyme assay

Measurement of β–glucosidaseenzyme activity in leucocytes or fibroblstsis the gold standard and was carried in all 50 patients. This assay was carried out from leukocytes using 4-methylumbelliferyl-β-D-glucopyranoside fluorescence substrate and enzyme activity was expressed as nmol/hr/mgprotein.The normal range of β-glucosidase 8.0-32.0 nmol/hr/mg.proteinwas established using normal healthy control subjects (Figure 5.5).

All patients had shown significantly reduced activity 13-29% of the enzyme β -glucosidase except one patient with high residual activity, where skin fibroblasts have shown less than 10% of normal activity 8.1 nmol/hr/mg.protein (NR: 118-401.5nmol/hr/mg protein). Two patients were on ERT when referred to us with elevated chitotriosidase activitybut β –glucosidase was not known in these patients as shown in Table.5.2.

Table 5.2: Plasma chitotriosidase and β–glucosidase activity in patients with GD

No.	Age	Sex	Plasma Chitotriosidase (nmol/hr/ml of plasma)	β-glucosidase (nmol/hr/mg protein)
1	9 Y	F	42748	8.3
2	2.5 Y	M	29923.6	2.1
3	1.5 Y	M	42748	3.6
4	19 Y	M	6412.5	3.44
5	2.5 Y	F	27786	4.4
6	3 Y	M	38473	4.8
7	11 Mo	F	72671.6	3.43
8	15 Mo	F	32	3.8
9	16 Y	F	31847.2	On ERT
10	21 Y	M	8763.3	On ERT
11	3 Y	M	85496	3
12	10 Y	M	37404.5	4.4
13	1.6 Y	M	15015.25	2.4
14	22 Mo	M	79000	1.96
15	1Y	M	73205.9	3.23
16	3Y	F	10687	0
17	1.5 Y	F	19236.6	2.6
18	8 Y	M	19770	3.3
19	6 Y	M	37618.2	2.85
20	10 Mo	F	0	3.39
21	2 Y	M	9885.4	4.4
22	2.0Y	F	9725.1	2.7
23	1.3 Y	F	4274.8	3.7
24	9 Y	F	8015.25	1
25	2.5 Y	M	9083.9	2.9

26	40 Y	F	54503.7	1.5
27	11 Y	F	1496.18	3.78
28	1 Y	F	10793.8	4
29	6 Y	M	12044 (NR: 0-200)	2.2
30	2 Y	M	24580.1	2.9
31	2.5 Y	F	13465.6	2.6
32	4 Mo	F	0	4.2
33	4.5 Y	M	12824.4	3.2
34	11 Y	F	18167.9	1.98
35	2 Y	M	1.4	2.1
36	2 Y	M	14961.8	1.8
37	2.6 Y	M	4381.65	3.4
38	2Y	M	5022.8	3.7
39	1Y	F	16030.5	2.8
40	10Mo	M	19770	3.5
41	8Y	M	10473.2	Reduced activity
42	4.5Mo	F	106.87	3.1
43	6 Mo	M	26100	2.6
44	1.3 Y	M	21.37	3.6
45	1.4 Y	F	32	3.8
46	22 Mo	M	19236	1.98
47	20Y	M	54503.7	2.5
48	4Y	F	35496	4.8
49	11 Mo	F	7308	3.1
50	2Y	M	20.8	4

Y-year, Mo- months, M-male, F-female, ERT- Enzyme replacement therapy

Reference range: Plasma chitotriosidase: 28.66–62.94nmol/hr/mlplasma;
β-glucosidase: 8.0–32.0nmol/hr/mg protein

5.6 Molecular Analysis of Gaucher Disease

5.6.1 Screening for common mutations of *GBA* gene

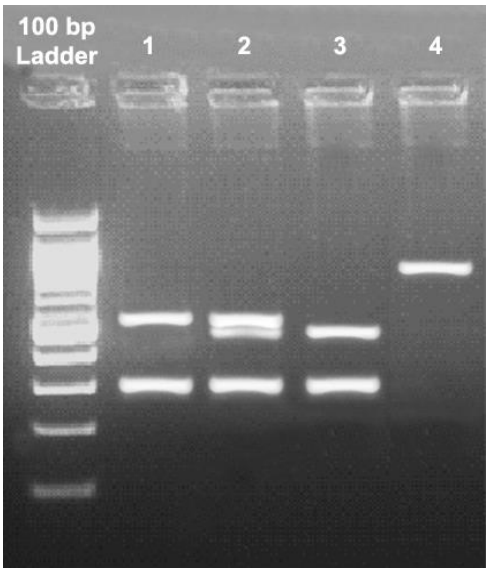
Molecular analysis of known common mutations (N370S (c.1226A>G), L444P (c.1448T>C), R463C (c.1504C>T) and Ivs2 (+1) G>A) was carried out in 50 confirmed cases of GD followed by bi-directional sequencing for common mutations.

In this study, 27 (54%) patients were identified with common mutation L444P (c.1448T>C) and R463C (c.1504C>T) in exon 10. This include mutant allele L444P (c.1448T>C) in 25 (50%) patients from which 22 (44%) were homozygous, 1(2%) was heterozygous/ unknown and 2 (4%) patient had shown compound heterozygosity for 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 2 (4%) patients (Table 5.3) (Figure 5.7, 5.8).

Table 5.3: Common mutations screening for GD patients

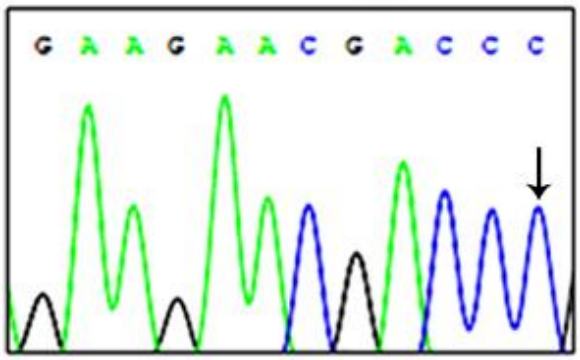
Exon/Intron	**Type**	**Nucleotide Change** *	**Amino acid change** *	**Recurrence**
Exon-10	Missense	c. 1448T>C	p. L444P	n=23 22 (Homozygous) 1 (Heterozygous)
Exon-10/11	Missense	c. 1448T>C/ c.1603 C>T	p. L444P/ p. R496C	1 (Compound heterozygous)
Exon-10/8	Missense	c. 1448T>C/ c.1102 C>T	p. L444P/ p. R329C	1(Compound heterozygous)
Exon 10	Missense	c.1504C>T/ c.1504C>T	p. R463C	2 (Homozygous)
Exon -9	Missense	c.1226A>G	p. N370S	0
Exon-2/Intron-2 boundary	Splice Junction	Ivs2(+1) G>A (c.115+1G>A)	-	0

*** = numbered according to GenBank reference sequence NM_000157.2**

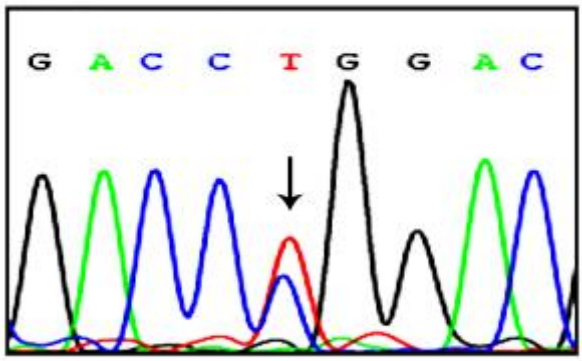


Lane 1: L444P Negative control (603bp+300bp)
Lane 2: L444P Heterozygous (603bp+547bp+300bp)
Lane 3: L444P Homozygous (603bp+300bp)
Lane 4: R463C Homozygous (903bp)

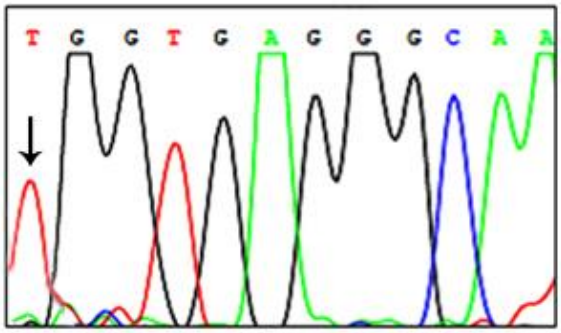
Figure 5.7: Gel profile of common mutations



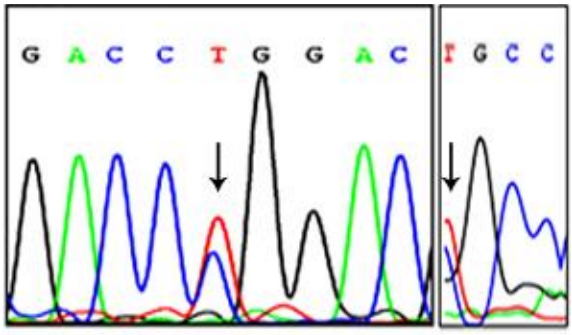
L444P (c.1448 T> C) (Homozygous)- Exon 10



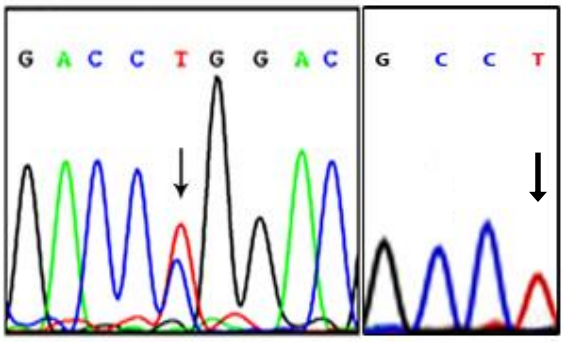
L444P (c.1448 T> C) (Heterozygous) - Exon 10



R463C (c.1504C>T) (Homozygous)-Exon 10



L444P (c. 1448T>C)/R496C (c.1603 C>T) (Compound heterozygous)- Exon 10/11



L444P (c. 1448T>C)/R329C (c.1102 C>T)
(Compound heterozygous)-Exon 10/8

Figure 5.8: DNA sequencing chromatogram of common mutation in *GBA* gene

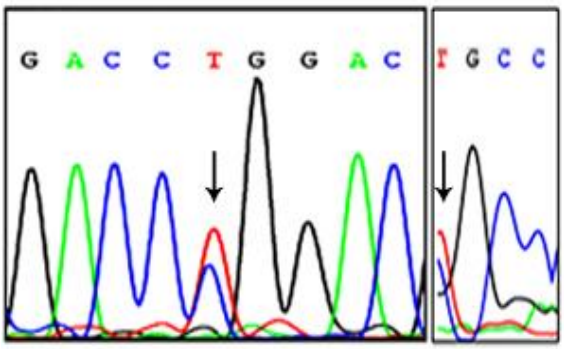
5.6.2 Bidirectional DNA sequencing of *GBA* gene exons to identify known and novel variants

Bidirectional exon sequencing was carried for exons of *GBA* gene. The sequencing analysis revealed 13 mutations in 14 patients, 4 of which were novel missense mutation, and remaining 9 were missense homozygous mutations. R395C (c.1300C>T) 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) in exon 6 were identified in one (4%) patient each in homozygous state. In 9 patients, mutations were not found in exons (Table 5.4) and (Figure 5.9).

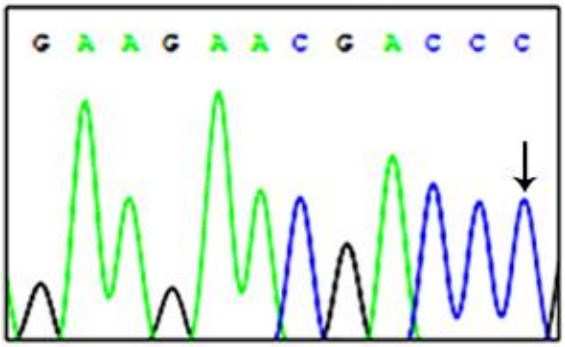
Table 5.4: List of *GBA* gene mutations in GD patients

HGMD Accession Number	Allele Name	Exons	c. DNA	Protein	Recurrence	References
CM920302	R496C	11	c.1603C>T	p. Arg535Cys	1	Kawame et al., 1992
CM870010	L444P	10	c.1448T>C	p. Leu483Pro	18	Tsuji et al., 1987
CM870010	L444P	10	c.1448T>C/?	p. Leu483Pro	1	Tsuji et al., 1987
CM900108	R463C	10	c.1504C>T	p. Arg502Cys	2	Hong et al., 1990
CM065218	R395C	9	c.1300C>T	p. Arg434Cys	1	Rozenberg et al., 2006

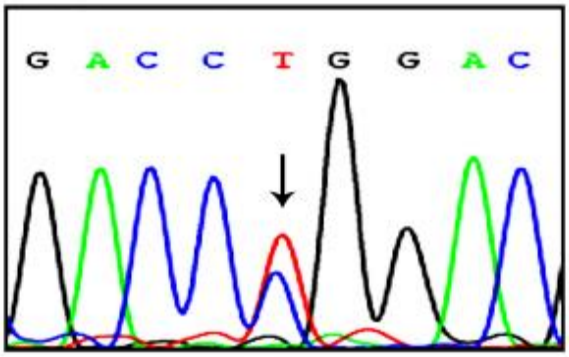
CM920300	R359Q	8	c.1193G>A	p. Arg398Gln	1	Kawame et al., 1992
HM971755	G355D	8	c.1181G>A	p. Gly394Asp	1	Cooper et al., 2001
CM077548	V352M	8	c. 1171G>A	p. Val391Met	1	Bukina and Tsvetkova, 2007
HM971756	S356F	8	c.1184C>T	p. Ser395Phe	1	Cooper et al., 2001
CM043285	R329C	8	c.1102 C>T	p. Arg368Cys	1	Rozenberg et al., 2006
CM910176	E326K	8	c.1093G>A	p. Glu365Lys	2	Eyal et al., 1991
CM910173	F213I	6	c.754 T>A	p. Phe252Ile	1	Kawame and Eto, 1991
CM940807	G202R	6	c.721 G>A	p. Gly241Arg	1	Beutler et al., 1994



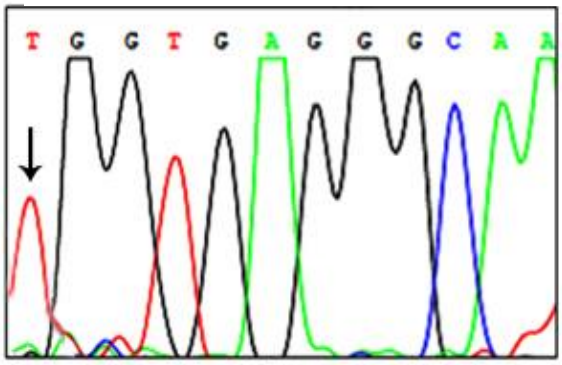
L444P (c. 1448T>C)/R496C (c.1603 C>T) (Compound heterozygous)- Exon 10/11



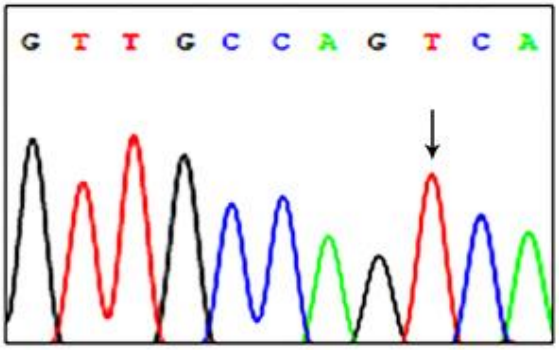
L444P (c.148 T> C) (Homozygous)- Exon 10



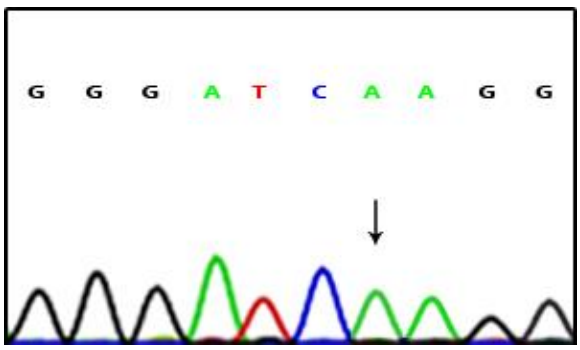
L444P (c.1448 T> C) (Heterozygous)- Exon 10



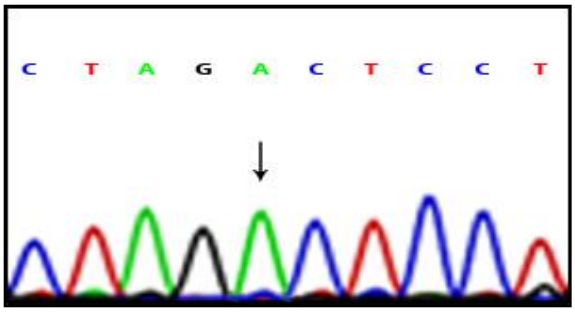
R463C (c.1504C>T)(Homozygous)-Exon 10



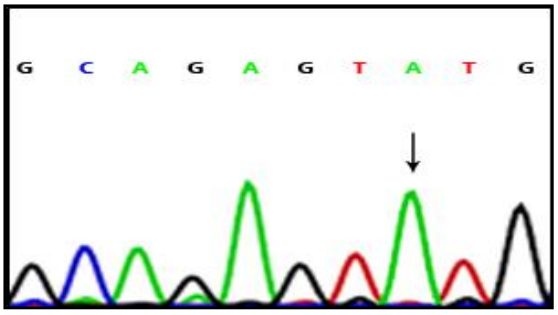
R395C (c.1300C>T)(Homozygous)-Exon 9



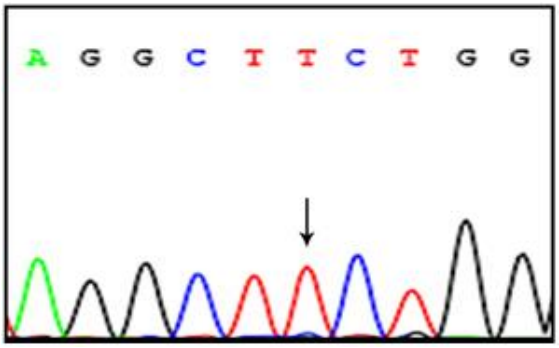
R359Q (c.1193G>A)(Homozygous)-Exon 8



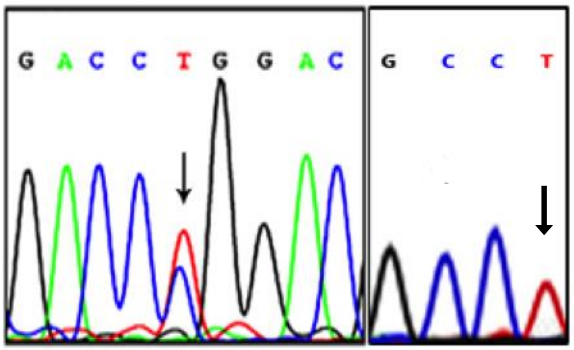
G355D (c.1181G>A) (Homozygous)- Exon 8



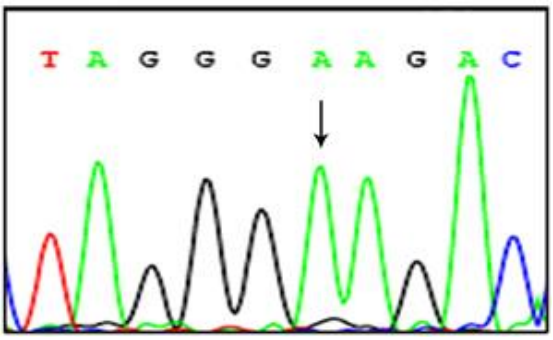
V352M (c. 1171G>A) (Homozygous)- Exon 8



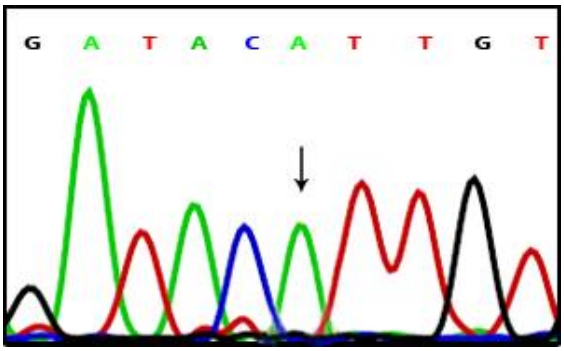
S356F (c.1184C>T) (Homozygous)- Exon 8



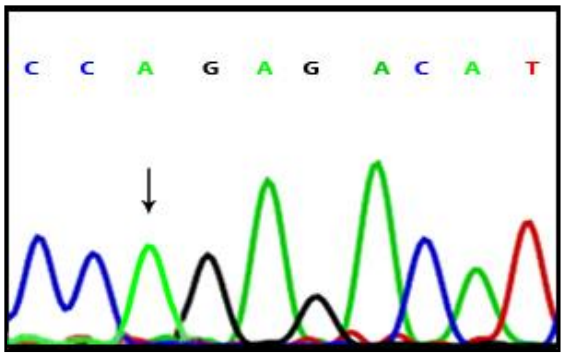
L444P (c. 1448T>C)/R329C (c.1102 C>T) (Compound heterozygous)- Exon 10/8



E326K (c.1093G>A)(Homozygous)- Exon 8



F213I (c.754 T>A) (Homozygous)- Exon 6



G202R (c.721 G>A) (Homozygous)- Exon 6

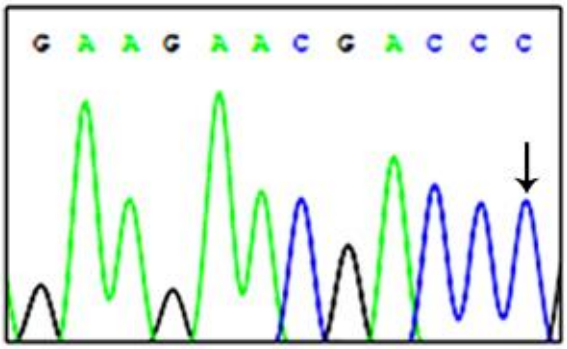
Figure 5.9: DNA sequencing chromatogram of mutations in exons of *GBA* gene

5.6.3 Molecular analysis in carrier parents

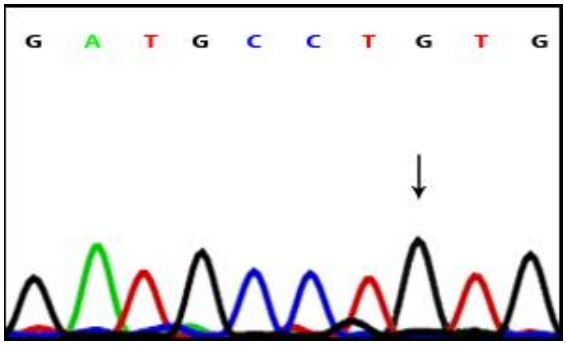
Molecular analysis was also carried out in 6 carrier parents. In these carrier parents analysis was carried as child DNA was not available. The common mutations screening analysis was carried out and four of them had shown presence of common missense mutations L444P (c.1448 T>C). Furthermore, confirmation of L444P(c.1448 T>C) was carried by bi-directional exon sequencing study in all of them. In one carrier parents, missense Y220C (c.766 A>G) mutations in exon 7 as shown in Table 5.5. For all, the presence of one mutant and one normal copy of this allele in both the parents suggest that the child would have had the mutation in the homozygous state, thus being responsible for causing of the disease. In one patient carrier parents mutation was not found (Table 5.5).

Table 5.5: List of *GBA* gene mutations in carrier parents

HGMD Accession Number	Allele Name	Exons	c.DNA	Protein	Reccurence	References
CM870010	L444P	10	c.1448T>C	p. Leu483Pro	4	Tsuji et al., 1987
CM065219	Y220C	7	c.776 A>G	p. Tyr259Cys	1	Rozenberg et al., 2006



L444P (c.1448 T> C) (Homozygous) - Exon 10



Y220C (c.776 A>G) (Homozygous)- Exon 7

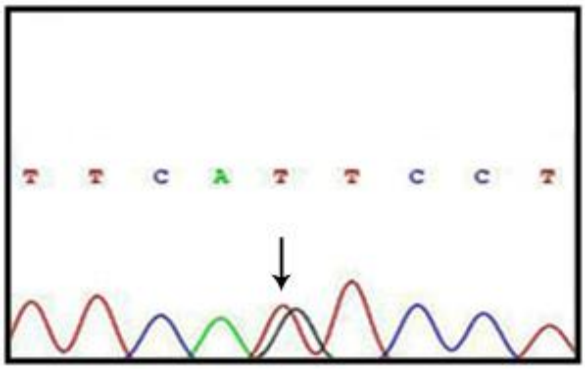
Figure 5.10: DNA sequencing chromatogram of carrier parents

5.6.4 Identification of Novel mutations in *GBA* gene

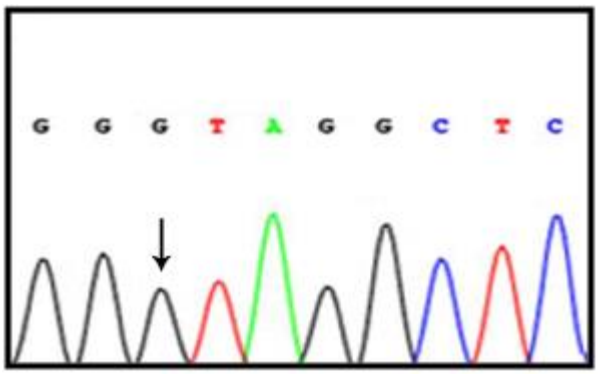
Novel missense mutations were identified by *GBA* gene bidirectional sequencing. Four novel missense mutations I427S (c .1397T>G), L354V (c.1177 C>G), G250A (c.866 G>C) and A100P (c.415 G>C) found in Exon 10, 8, 7 and 4 respectively in 4 (8%) patients each. From that, L354V (c.1177 C>G), G250A (c.866 G>C) and A100P (c.415 G>C) were in homozygous state. I427S (c.866 G>C) found in heterozygous state whereas other mutations were not found in exons(Table 5.6).

Table 5.6: Novel mutations identified in *GBA* gene in GD patients

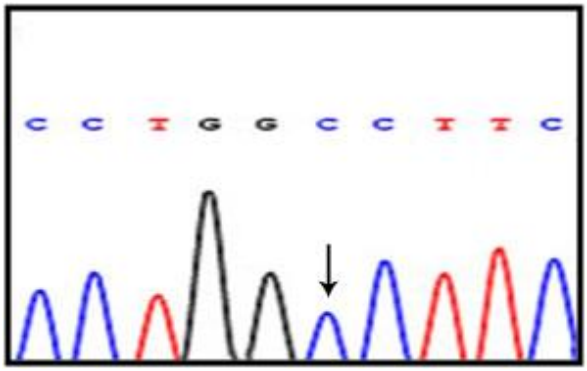
Allele Name	Exons	c.DNA	Protein	Reccurence	References
I427S	10	c .1397T>G	p. Ile466Ser	1	Ankleshwaria et al., 2014
L354V	8	c.1177 C>G	p. Leu393Val	1	Present study
G250A	7	c.866 G>C	p. Gly289Ala	1	Ankleshwaria et al., 2014
A100P	4	c.415 G>C	p. Ala139pro	1	Present study



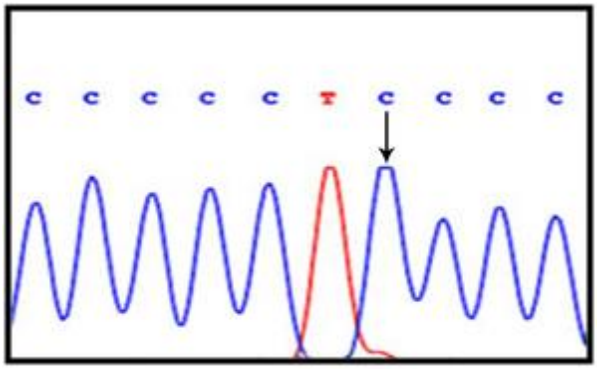
I427S (c .1397T>G) (Heterozygous) – Exon 10



L354V (c.1177C>G)(Homozygous) – Exon 8



G250A (c.866G>C)(Homozygous) – Exon 7



A100P (c.415G>C)(Homozygous) – Exon 4

Figure 5.11: DNA sequencing chromatogram showing novel mutations. a) I427S (c.1397T>G) in heterozygous state in exon 10 b) L354V in homozygous state in exon 8 c) G250A (c.866G>C) in homozygous state in exon 7 d) A100P in homozygous state in exon 4

5.6.4.1 *In silico* analysis of novel mutations in *GBA* gene

The program Polyphen 2, SIFT/PROVEAN and Mutation T@sting are used for identification of the non-synonymous single nucleotide substitutions as given in Table 5.7. This program identified four novel missense mutations I427S (c .1397T>G), L354V (c.1177 C>G), G250A (c.866 G>C) and A100P (c.415 G>C) and in Exon 10, 8, 7 and 4 respectively as probably damaging, deleterious and disease causing that are affecting proteins (Table 5.7).

Table 5.7: *In silico* analysis of novel mutations in *GBA* gene

Allele Name	Exons	Mutation T@ster score	SIFT Score	Polyphen2 Score (sensitivity, specificity)	Amino acid change
I427S	Exon 10	3.87 (DC)	-5.045 (D)	1.000 (0.00,1.00) (PD)	Non- polar to polar
L354V	Exon 8	0.87 (DC)	-2.808 (D)	1.000 (0.00,1.00) (PD)	Non-polar to non-polar
G250A	Exon 7	1.64 (DC)	-3.233 (D)	0.963 (0.78,0.95) (PD)	Hydrophobic to non-polar
A100P	Exon 4	0.74 (DC)	-2.598 (D)	0.977 (0.76,0.96) (PD)	Non-polar to non-polar

DC = Disease causing, D= Deleterious, PD= Probably damaging

The Mutations T@ster score is taken from an amino acid substitution matrix (Grantham Matrix) which takes into account the physico-chemical characteristics of amino acids and scores substitutions according to the degree of difference between the original and the new amino acid scores may range from 0.0 to 6.0.

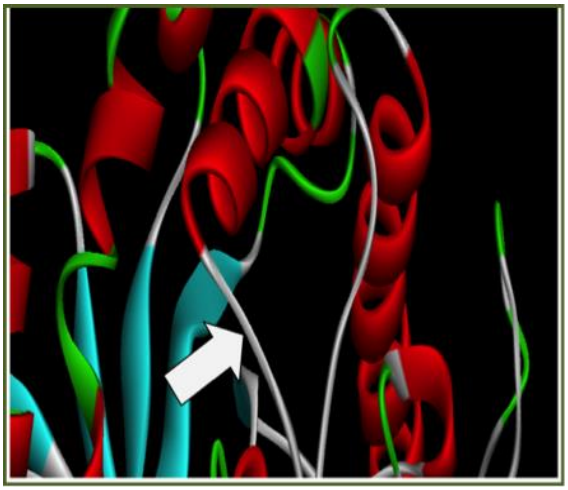
Variants with a score equal to or below -2.5 are considered deleterious.

The Polyphen2 score is the naïve Bayes posterior to probability that this mutation is damaging

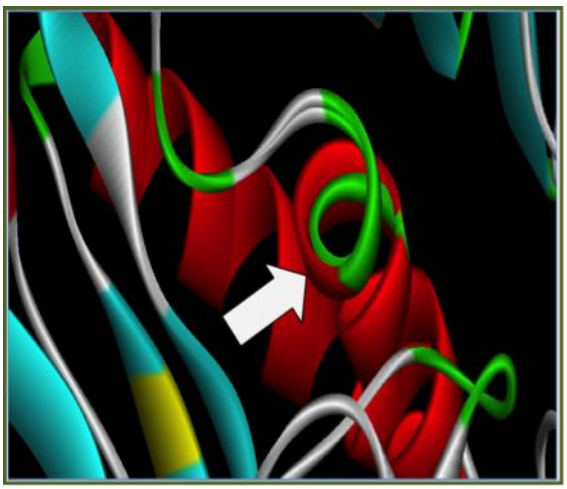
and thus ranges from 0 to 1.

5.6.4.2 Protein structure of novel mutations

Accelrys Discovery Studio software generated super- imposed 10GS PDB native structures and 10GS PDB mutant structure of the acid-β -glucosidase (GlcCerase) for the novel mutant allele. It showed that G250A (c.866 G>C) mutant allele located in β4 strand and root-mean-square deviation (RMSD) value for superimposition was very small (0.009 Angstrom), which suggests that this mutation has little effect on the structure and mutant allele I427S (c .1397T>G) has created extra turn in α8 helices. Both these mutation were found to destabilize the protein structure. In remaining two novel mutations, its effect on protein structure is under progress (Figure5.12).



(a) Mutation p. G250A located in β_4 strand and destabilize the protein structure



(b) Mutation p.I427S has extra turn in α_8 helices and destabilize the protein structure

Figure 5.12:Protein structure of novel mutations. Superimposed native structures (green) and mutant structure (red) of the of acid-β -glucosidase (GlcCerase) produced using Accelrys Discovery Studio software: (Using 10GS PDB)

5.6.5 Identification of carrier frequency for common mutations by mass screening

In present study, L444P (c.1448T>C) and R463C (c.1504C>T) are the most common mutations identified in 27/50 (54%) cases. Considering these data, we developed a simple cost effective PCR based diagnosis to identify the affected as well as carrier individuals. In this study, 200 unrelated subjects were studied for the carrier frequency

of L444P (c.1448T>C) and R463C (c.1504C>T) in general population by PCR-RFLP (Figure 5.13). It was found that none of the patients studied are carrier for these mutations.

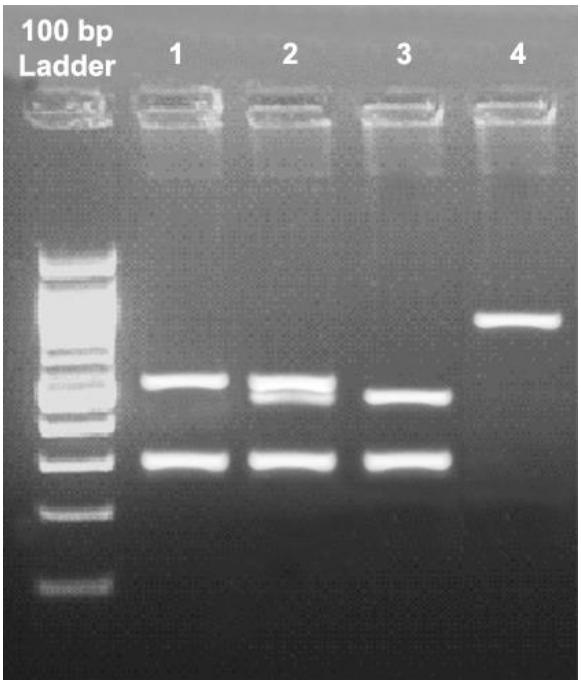


Figure 5.13: Gel profile of common mutations

Lane 1: L444P Negative control (603bp+300bp)
Lane 2: L444P Heterozygous (603bp+ 547bp+300bp)
Lane 3: L444P Homozygous (547bp+300bp)
Lane 4: R463C Homozygous (903 bp)

5.6.6 Genotype correlation with phenotype and use this information for differential diagnosis of the disease and prognostication

L444P (c.1448T>C) in exon 10 in 25 of 50 (50%) patients from which 22 (44%) were homozygous, 1(2%) was heterozygous/ unknown and 2 (4%) patient had shown compound heterozygosity for 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.

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. 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 (Table 5.8,5.9).

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) were 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.

Table 5.8: Clinical, Biochemical and Molecular data of Indian GD patients

Patient ID	Age at diagnosis (Months or Years/ Sex)	Geographic origin	Cosan - guinity	Clinical features at the time of diagnosis	Plasma chitotriosidase (nmol/hr. /ml. plasma)	β-glucosidase (nmol/ hr./ mg. protein) from leucocytes	Type of GD	Genotype
G2*	9Y/ F	Maharashtra	No	HS, bone infiltration	42748	9.3	I	L444P (c.1448T>C / L444P c.1448T>C)
G3	2.5Y/ M	Maharashtra	No	HS, S/O. sacchadic eye	29923.6	2.1	III	L444P (c.1448T>C / L444P (c.1448T>C)

				movement, BM suggestive of GD				
G5*	1.5Y/M	Gujarat	No	Massive HS, anemia, thrombocytopenia, mild regression of milestones	42748	3.6	I	Y220C (c.776 A>G)/ Y220C (c.776 A>G)
G6	19Y/ M	Maharashtra	No	HS, BM suggestive of GD, osteomyelitis of femur, splenectomy done	6412.5	3.44	I	R463C (c.1504C>T)/ R463C (c.1504C>T)
G7	2.5Y/ F	Maharashtra	No	Massive splenomegaly with mild to moderate hepatomegaly, unable to run, delayed milestones	27786	4.4	I	L444P (c.1448T>C / L444P (c.1448T>C)
G8	3Y/ M	Gujarat	No	Large HS, thrombocytopenia, thinning of limbs, scaly skin, BM suggestive of GD	38473	4.8	I	R359Q (c.1193G>A)/ R359Q (c.1193G>A)
G9*	11Mo/ F	Gujarat	No	HS, BM with lipid filled macrophages, delayed milestones	72671.6	3.43	I	Not found
G10*	15 Mo/ F	Gujarat	Yes	Massive HS, unable to walk after 1 years, severe osteoporosis, anemia, thrombocytopenia, cardiomegaly with pulmonary congestion, gaucher cells occupy the	32	3.8	I	L444P (c.1448T>C / L444P (c.1448T>C)

				sinusoids, mild hypotonia, seizures since 2 hours of birth				
G11	16Y/ F	Maharashtra	No	Diagnosed case of GD	31847.2	On ERT	I	R463C (c.1504C>T)/ R463C (c.1504C>T)
G12	21Y/ M	Maharashtra	Yes	Diagnosed case of GD	8763.3	On ERT	I	G355D (c.1181G>A)/ G355D (c.1181G>A)
G13	3Y/ M	Maharashtra	Yes	HS, BM suggestive of GD, anemia, thrombocytopenia, growth retardation, deep and superficial reflexes, affected cranial nerves, power, hearing problem	85496	3	II/III	L444P (c.1448T>C / L444P (c.1448T>C)
G14	10Y/ M	Maharashtra	No	N/A	37404.5	4.4	I	L444P (c.1448T>C / L444P (c.1448T>C)
G15	1.6Y/ M	Rajasthan	No	Mild HS, growth retardation, developmental delay, o/e a febrile, family history+, neck holding +, BM suggestive of GD, anemia, thrombocytopenia	15015.25	2.4	III	Not found
G16	22 Mo/ M	Gujarat	No	HS, cannot walk with support, anemia, thrombocytopenia	79000	1.96	I	L444P (c.1448T>C / L444P (c.1448T>C)
G18	1Y/ M	Gujarat	No	Massive HS, anemia, thrombocytopenia, BM suggestive	73205.9	3.23	I	L444P (c.1448T>C / L444P (c.1448T>C)

				GD				
G20	3Y/ F	Maharashtra	Yes	HS, BM suggestive of GD	10687	0	I	L444P (c.1448T>C / L444P (c.1448T>C)
G21	1.5Y/ F	Kerela	Yes	HS, macule left back, b/I ichthyosis lower limb, family history of GD	19236.6	2.6	I	L444P (c.1448T>C / L444P (c.1448T>C)
G22	8Y/ M	Maharashtra	No	HS, jaundice, weakness, recurrent infections at birth, breathlessnes s on exertion present, GE microcephaly , short stature, pointed nose, undernourish ed,	19770	3.3	III	L444P (c.1448T>C / L444P (c.1448T>C)
G23	6Y/ M	Maharashtra	Yes	massive splenomegaly , anemia, thrombocytop enia, BM shows storage cells	37618.2	2.85	I	L444P (c.1448T>C / L444P (c.1448T>C)
G24	10 Mo/ F	Kerela	Yes	moderate HS, anemia, thrombocytop enia, material PAS stain s/o. storage disorder, pallor	0	3.39	I	L444P (c.1448T>C / L444P (c.1448T>C)
G25	2Y/ M	Maharashtra	No	Huge HS, pancytopenia, BM shows gaucher cells	9885.4	4.4	I	Not found
G26	2Y/ F	Maharashtra	No	distension of abdomen and small limbs	9725.1	2.7	I	L444P (c.1448T>C / L444P (c.1448T>C)
G27	1.3 Y/ F	Maharashtra	No	HS	4274.8	3.7	I	Not found
G28	9Y/ F	Maharashtra	No	moderate hepatomegaly and severe splenomegaly , coryza and	8015.25	1	I	I427S (c.1397 T>G) / ?

				fever, anemia, thrombocytopenia, pallor				
G29	2.5Y/ M	Karnataka	No	Not available	9083.9	2.9	I	L444P (c.1448T>C / L444P (c.1448T>C)
G30	40Y/ F	Maharashtra	No	BM showed gaucher cells	54503.7	1.5	I	R395C (c.1300C>T) / R395C (c.1300C>T)
G31	11Y/ F	Kerela	No	severe splenomegaly	1496.18	3.78	I	Not found
G32*	1Y/ F	Maharashtra	No	severe HS, anemia, thrombocytopenia, bone marrow transplantation	10793.8	4	I	L444P (c.1448T>C / L444P (c.1448T>C)
G33	6Y/ M	Maharashtra	Yes	HS, anemia, thrombocytopenia, BM showed foam cells	12044 (NR: 0-200)	2.2	I	V352M(c.1171G>A)/ V352M (c.1171G>A)
G34	2Y/ M	Maharashtra	No	NA	24580.1	2.9	I	L444P (c.1448T>C) / L444P (c.1448T>C)
G35	2.5Y/ F	Bihar	No	HS, anemia, thrombocytopenia, BM biopsy- erythroid hyperplasia, few myeloid precursors, foamy macrophage histiocyte cells with eosinophilic, gradual paleness of body, fever, cough, cold, pallor	13465.6	2.6	I	L444P (c.1448T>C) / R496C (c.1603C>T)
G36	4Mo/ F	Kerela	No	HS, conjugated hyperbilirubinemia	0	4.2	I	Not found
G37	4.5Y/ M	Srilanka	No	moderate HS, anemia, BMshows gaucher cells, growth retardation,	12824.4	3.2	I	E326K (c.1093G>A)/ E326K (c.1093 G>A)

				mild coarse facial features				
G38	11Y/ F	Srilanka	No	mild hepatomegaly with severe splenomegaly, bone marrow shows gaucher cells, anemia, thrombocytopenia, history of GD, splenectomy done	18167.9	1.98	I	E326K (c.1093G>A)/ E326K (c.1093 G>A)
G39	2Y/ M	Maharashtra	No	moderate hepatomegaly with dilated portal vessels, massive splenomegaly, BM aspirates showing gaucher cells in moderate quantity	1.4 (S)	2.1(S)	I	L444P (c.1448T>C) / L444P (c.1448T>C)
G40	2Y/ M	Maharashtra	No	HS	14961.8	1.8	I	L444P (c.1448T>C) / L444P (c.1448T>C)
G41	2Y/ M	Maharashtra	Yes	HS, anemia, thrombocytopenia, BM suggestive of storage disorder, conjugative jaundice	4381.65	3.4	I	Not found
G42	2Y/ M	Chhatisgarh	Yes	HS, cold, pneumonia, BM shows Niemann pick cells	5022.8	3.7	I	Not found
G43	1Y/ F	Kerela	Yes	HS	16030.5	2.8	I	G250A (c. 866G>C) / G250A (c.866G>C)
G44	10 Mo/ M	Srilanka	Yes	HS, anemia, thrombocytopenia, non-obstructive renal calculi,	19770	3.5	I	L444P (c.1448T>C) / L444P (c.1448T>C)

				growth retardation, X-ray femur : flask shape distal femur				
G45	8Y/ M	Maharashtra	No	massive HS, BM suggestive of GD	10473.2	Reduced activity	I	S356F (c.1184C>T) / S356F (c.1184C>T)
G46	4.5 Mo/ F	Maharashtra	No	HS, psychomotor retardation, not gaining weight since birth, s/o. metabolic encephalopathy, pseudoedema, neonatal hepatitis is likely, yellowish discolouration of eyes, anemia, thrombocytopenia, CNS - low power UL & LL	106.87	3.1	III	Not found
G47	6 Mo/ M	Gujarat	No	HS, dystonia, bulbar palsy	26100	2.6	II	L444P (c.1448T>C) / ?
G48*	1.3Y/ M	Maharashtra	Yes	HS, anemia, thrombocytopenia, failure to gain weight, febrile, pallor. Later symptoms thinning of legs, breathing problem, psychomotor retardation and death occur at 1.5 years	21.37	3.6	II	A100P (c.415 G>C)/ A100P (c.415 G>C)
G49	1.4Y/ F	Andhra Pradesh	Yes	mild hepatomegaly	32	3.8	I	L354V (c.1177C>G)/ L354V (c.1177 C>G)

				and moderate splenomegaly with appearance are non-specific and intraabdominal mass present , anemia and thrombocytopenia failure to gain weight, reddish colour urine, o/c. conscious afebrile				
G50	22 Mo/ M	Maharashtra	No	huge HS, history of thrombocytopenia, history of blood transfusion due to pallor and not gaining weight	19236	1.98	I	L444P (c.1448T>C) / L444P (c.1448T>C)
G51	20Y/ M	Gujarat	No	HS, anemia, thrombocytopenia, BM shows gaucher cells, avascular necrosis, difficulty in walking	54503.7	2.5	I	L444P (c.1448T>C) / R329C (c.1102 C>T)
G54	4Y/ F	Rajasthan	No	HS, anemia, thrombocytopenia, regression of milestones, hypotonia, younger sister died due to similar conditions	35496	4.8*	I	L444P (c.1448T>C) / L444P (c.1448T>C)
G55	11Mo/ F	Gujarat	Yes	HS, anemia, thrombocytopenia, Fever, cough, H/O. convulsions off and on	7308	3.1*	II	G202R (c.721 G>A) / G202R (c.721 G>A)

				since 1 month almost continue daily, hypertonia, deep and superficial reflexes, power, tone, aspirating pneumonia, AF: open. small, child drowsy, neck extended position, resistance to neck flexion, trismus , lack jaw , extonsor spasm , lauryngeal spasm , squint , drowsy response to painful stimuli, DTR: exagaratal, Kernitis sign				
G56	2Y/ M	Maharashtra	No	mild hepatomegaly , moderate splenomegaly , anemia, thrombocytopenia, hepatic echoappearance s/o. of storage disorders, BM suggestive of GD	20.8	4	I	F213I (c.754 T>A) / F213I (c.754 T>A)

Y-year, Mo- months, M-male, F-female, ERT- Enzyme replacement therapy

Reference range: Plasma chitotriosidase: 28.66–62.94nmol/hr/mlplasma; β-glucosidase: 8.0–32.0nmol/hr/mg protein *-Death of patient occur, F-female, M-male, Y-years, Mo-months, HS-hepatosplenomegaly, BM-bone marrow, S/o. suggestive of , H/O. history of, AP-anterioposterior, NA-not available.

Table 5.9: Genotype-phenotype correlation in Indian GD patients

Phenotypes / Genotypes	No of Pts. (n)	Hepatomegaly	Splenomegaly	Pancytopenia	Bone abnormalities	Pulmonary affected	CNS affected	BM	Surgical procedur	ERT	Skin	Death
L444P /L444P	22	17 (77%)	20 (91%)	11 (50%)	5 (23 %)	2 (9%)	3 (14 %)	8 (36%)	2 (9%)	3 (14 %)	1 (5%)	3 (14%)
L444P/?	1	1 (100%)	1 (100%)	1 (100%)	1 (100 %)	-	-	1 (100%)	-	-	-	-
L444P/R496C	1	1 (100%)	1 (100%)	1 (100%)	-	-	-	1 (100%)	-	-	-	-
L444P/R329C	1	1 (100%)	1 (100%)	1 (100%)	1 (100 %)	-	-	1 (100%)	-	-	-	-
R463C/R463C	2	2 (100%)	2 (100%)	1 (50%)	1 (50 %)	-	-	-	1 (50%)	1 (50 %)	-	-
I427S/?	1	1 (100%)	1 (100%)	1 (100%)	-	-	-	-	-	-	-	-
R395C/R395C	1	1 (100%)	1 (100%)	-	-	-	-	1 (100%)	-	-	-	-
R359Q/R359Q	1	1 (100%)	1 (100%)	1 (100%)	1 (100 %)	-	-	1 (100%)	-	1 (100 %)	1 (100 %)	-
S356F/S356F	1	1 (100%)	1 (100%)	-	-	-	-	1 (100%)	-	-	-	-
G355D/G355D	1	1 (100%)	1 (100%)	-	-	-	-	-	-	1 (100 %)	-	
L354V/L354V	1	1	1	1	-	-	-	-	-	-	-	-

		(100%)	(100%)	(100%)								
V352M/V352M	1	1 (100%)	1 (100%)	1 (100%)	-	-	-	1 (100%)	-	-	-	-
E326K/E326K	2	2 (100%)	2 (100%)	2 (100%)	-	-	-	2 (100%)	1 (50%)	-	-	-
G250A/G250A	1	1 (100%)	1 (100%)	-	-	-	-	-	-	-	-	-
Y220C/Y220C	1	1 (100%)	1 (100%)	1 (100%)	-	-	-	-	-	-	-	1 (100 %)
F213I/F213I	1	1 (100%)	1 (100%)	1 (100%)	-	-	-	1 (100%)	-	-	-	-
G202R/G202R	1	1 (100%)	1 (100%)	1 (100%)	-	1 (100 %)	1 (100 %)	-	-	-	-	-
A100P/A100P	1	1 (100%)	1 (100%)	1 (100%)	1 (100 %)	1 (100 %)	1 (100 %)	-	-	-	-	1 (100 %)
Correlation	**41**	**36 (88%)**	**39 (95%)**	**25 (61%)**	**10 (24 %)**	**4 (10 %)**	**5 (12 %)**	**18 (44%)**	**4 (10%)**	**6 (15 %)**	**2 (5%)**	**5 (12%)**

Mutations was not identified in nine patients hence correlation wascarried out in 41/50 patients

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Novel mutations in the glucocerebrosidase gene of Indian patients with Gaucher disease

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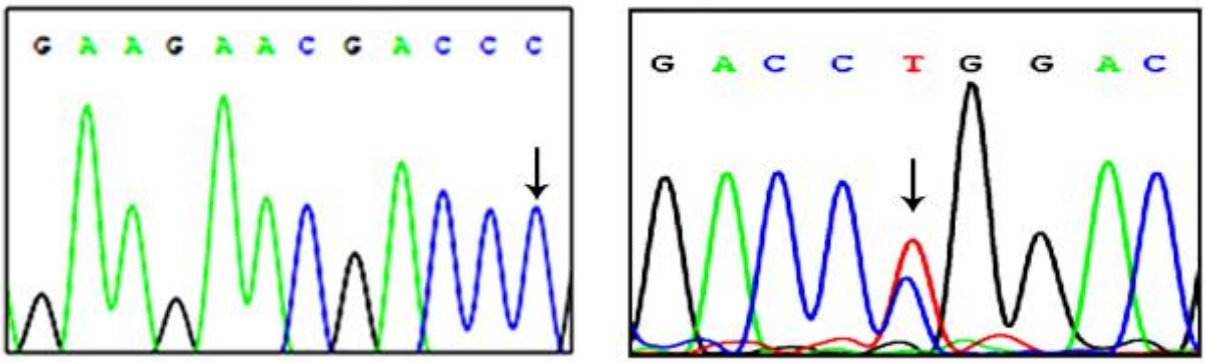
Gaucher disease (GD) is the most common glycolipid storage disorder resulting from glucocerebrosidase deficiency due to mutations in the GBA gene. Study was performed in 33 unrelated patients with low β-glucosidase activity in leukocytes and/or fibroblasts. The exons and exon–intron boundaries of the GBA gene were bidirectionally sequenced using an automated sequencer. Mutations were confirmed in parents and were looked up in public databases, and in silico analysis was carried for novel mutations. We identified two novel missense mutations G289A (c.866G>C) and I466S (c.1397T>G) in exons 7 and 10, respectively, in two (6.06%) patients that destabilize the protein structure. L444P (c.1448T>C) was the most common mutation identified in 20/33 (60.60%) non-neuronopathic and 1/33 (3.03%) sub-acute neuronopathic form based on clinical presentation at the time of investigation. Other nine rare mutations were: R463C (c.1504C>T), R395C (c.1300C>T), R359Q (c.1193G>A), G355D (c.1181G>A), V352M (c.1171G>A) and S356F (c.1184C>T) found in each patient (18.18%). Compound heterozygous mutation L444P (c.1448T>C)/R496C (c.1603C>T) in exon 10/11 and L444P (c.1448T>C)/R329C (c.1102C>T) were observed in exon 10/8 in one each patient (6.06%). Two patients (6.06%) from Sri Lanka showed E326K (c.1093G>A) mutation in exon 8. We conclude that L444P is the most common mutant allele with exons 8 and 10 as the hot spot region of GBA gene observed in Indian GD patients.

5.7 Prenatal Diagnosis

Prenatal diagnosis was provided only to one family where index case was confirmed with GD both by enzyme and molecular study. In index case, plasma chitotriosidase was found normal 32.00 nmol/hr/mL plasma, and β-glucosidase activity from leucocytes was low 3.8nmol/hr/mg protein (normal range: 8.0–32.0 nmol/hr/mg protein) suggesting GD.Further confirmation of the disease was carried out by molecular analysis of *GBA* gene. This has shown homozygous status for L444P (c.1448T>C), confirming GD.

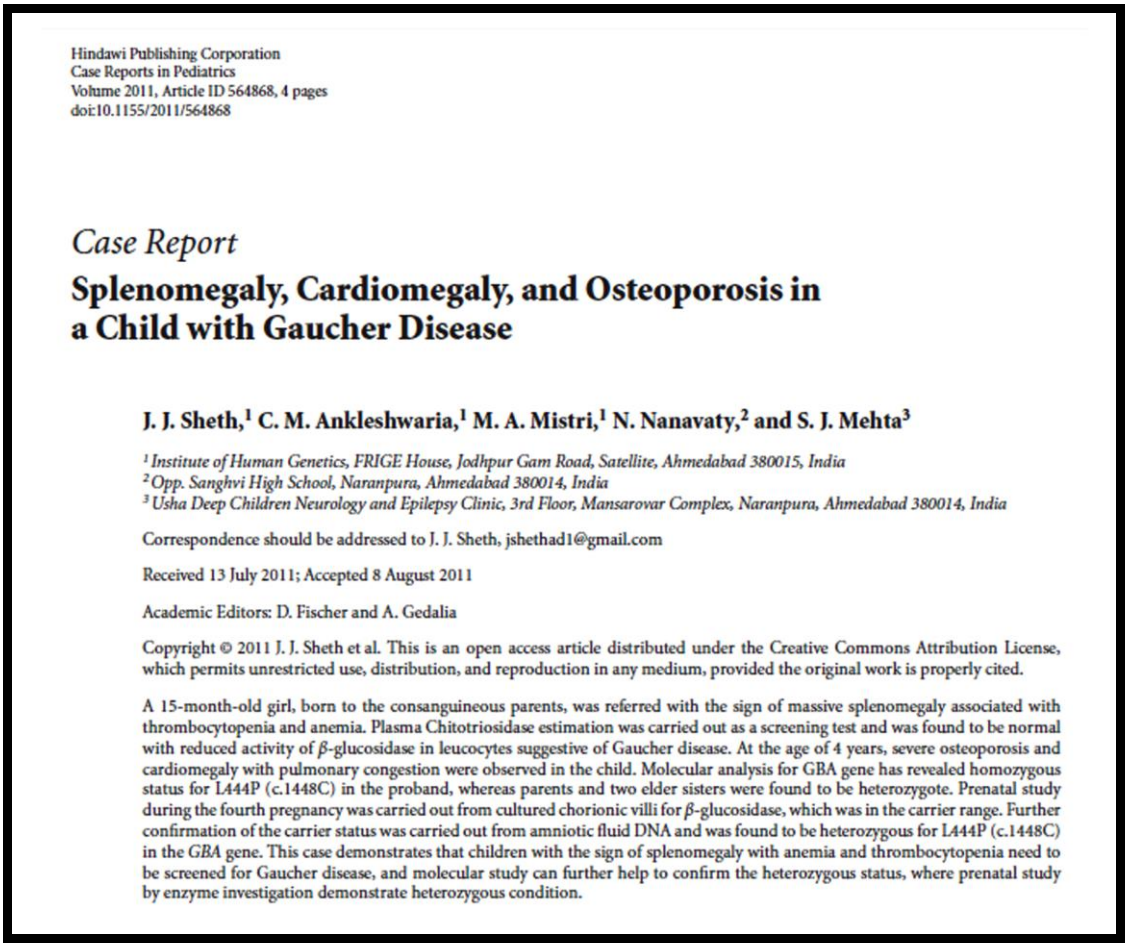
Parents and two unaffected elder sisters revealed heterozygous status for L444P (c.1448T>C)mutation. During the fourth pregnancy cultured chorionic villus study was carried out for β-gluosidase, which was in the carrier range 139.58nmol/hr/ mg protein with control value of 328.3 nmol/hr/mg protein. Further confirmation was carried out from amniotic fluid DNA, which has confirmed heterozygous status of the fetus for the L444P (c.1448T>C)mutation (Figure 5.14).



L444P (c.1448 T> C) (Homozygous)- Exon 10

L444P (c.1448 T> C) (Heterozygous) - Exon 10

Figure 5.14: DNA sequencing chromatogram of prenatal diagnosis. a) Index case showing homozygous L444P(c.1448T>C) b) Fetus showing heterogzygous L444P(c.1448T>C)



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Case Report

Splenomegaly, Cardiomegaly, and Osteoporosis in a Child with Gaucher Disease

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A 15-month-old girl, born to the consanguineous parents, was referred with the sign of massive splenomegaly associated with thrombocytopenia and anemia. Plasma Chitotriosidase estimation was carried out as a screening test and was found to be normal with reduced activity of β-glucosidase in leucocytes suggestive of Gaucher disease. At the age of 4 years, severe osteoporosis and cardiomegaly with pulmonary congestion were observed in the child. Molecular analysis for GBA gene has revealed homozygous status for L444P (c.1448C) in the proband, whereas parents and two elder sisters were found to be heterozygote. Prenatal study during the fourth pregnancy was carried out from cultured chorionic villi for β-glucosidase, which was in the carrier range. Further confirmation of the carrier status was carried out from amniotic fluid DNA and was found to be heterozygous for L444P (c.1448C) in the *GBA* gene. This case demonstrates that children with the sign of splenomegaly with anemia and thrombocytopenia need to be screened for Gaucher disease, and molecular study can further help to confirm the heterozygous status, where prenatal study by enzyme investigation demonstrate heterozygous condition.

5.8 Effect of genotype on patients receiving ERT

Six type I GD patients were on Enzyme Replacement Therapy (ERT) when referred to us. From these, 2 patients were on ERT with the genotype of L444P (c.1448T>C) and R359Q (c.1193G>A). At present, these two patients are responding well to ERT & are phenotypically normal as determined by reduction in spleen size. This was also determined by plasma chitotriosidase activity as a marker of decreased macrophage activation.

One patient on ERT had genotype of L444P (c.1448T>C) with phenotype of hepatosplenomegaly, bone infiltration with Gaucher cells with type I GD. This patient died after 7 years of receiving ERT due to non-availability of enzyme.

Two other patients receiving ERT were having genotype of R463C (c.1504C>T) and G355D (c.1181G>A) whereas in one patient genotype was not identified in exonic sequencing (Table 5.10).

Table 5.10: Genotype and phenotype of GD patients on ERT

Patient Id	Age/ sex	Region	chitotriosidase	β-glucosidase	Mutation	Type of GD	Receiving ERT or not	Phenotypes at time of diagnosis	Phenotypes after ERT treatment and follow up
G2*	9 Y/ F	Maharashtra	42748	8.3	L444P (c.1448T>C) / L444P (c.1448T>C)	I	Yes	hepatosplenomegaly, Bone infiltration, history of GD	Death occur after 8 yrs due to non-availability of enzyme and only after treatment blood transfusion was not needed
G7	2.5Y/ F	Maharashtra	27786	4.4	L444P (c.1448T>C) / L444P (c.1448T>C)	I	Yes	massivesplenomegaly with mild to moderate hepatomegaly, delayed milestones,unable to run	normal, academic is good, nutrition supplementation is given in additional
G8	3Y/ M	Gujarat	38473	4.8	R359Q (c.1193G>A)/R359Q (c.1193G>A)	I	Yes	large hepatosplenomegaly, BM shows storage cells of GD, thrombocytopenia, thinning of limbs, scaly skin, thrombocytopenia	normal
G11	16Y/ F	Maharashtra	31847.2	On ERT	R463C (c.1504C>T) / R463C (c.1504C>T)	I	Yes	Diagnosed case of GD	N/A
G12	21Y/	Maharashtra	8763.3	On ERT	G355D (c.1181G>A	I	Yes	Diagnosed case of GD	N/A

	M				)/ G355D (c.1181G>A)				
G16 *	22Mo /M	Gujarat	79000	1.96	L444P (c.1448T>C) / L444P (c.1448T>C)	I	Yes	hepatosplenomegaly, anemia, thrombocytopenia, cannot walk without support,	N/A

- Index case DNA not available, BM- bone marrow, Y-year, Mo- months, M-male, F-female, ERT- Enzyme replacement therapy