Journal of the Egyptian Society of Haematology & Research, Vol. 2, No. 2, September: 145-153, 2006

Genotype Phenotype Relationship in Gaucher's Disease in Egypt

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ABSTRACT

Background: Gaucher's disease is the most prevalent of the genetic lysosomal storage disorders. It is an autosomal recessive disease which was described by the French physician Philippe Gaucher in 1882. It is caused by a severe deficiency of glucocerebrosidase enzymatic activity with resultant accumulation of large quantities of glycolipid, glucocerebrosidase within the lysosomes of the phagocytic cells of the monocyte-macrophage system. Gaucher's disease is classified into three conventional types; Type I: Chronic non-neuropathic form which usually found in adults especially in Jewish population, Type II: Infantile neuropathic form which always appears by 6 months of age as a rapidly progressive neurological affection, and Type III: Juvenile sub-acute neuropathic with slowly progressive neurological disease that begins during childhood or adolescence. The aim of this study is to investigate the genotypes of Gaucher's disease, the most prevalent mutations in Egypt and to assess if a genotype-phenotype relationship could be elicited.

Patients and Methods: The present study included 20 Gaucher's disease documented patients; they were 10 males and 10 females. Their ages ranged from 3 to 43 years with of a mean of 10.15 and median of 6 years. All patients were subjected to clinical evaluation, revision of their filed clinical progress, radiological and laboratory data including full blood count, b-Glucocerebrosidase enzyme assay, liver enzymes bone marrow and splenic aspirate. In addition, we studied the most common GBA mutations using strip assay which is based on the reversed-hybridization principle The assay covered 8 of the most frequent GBA mutations: 84GG (452+G), IVS2+1 (484 G>A), N370S (1226 A>G), V394L (1297 G>T), L444p (1448 T>C), R496G (1604 G>A), and 2 recombinant alleles (RecNcil, RecTL).

Results: Neither the age of patients, age of onset of the disease nor the sex showed significant relation with homozygosity Vs double heterozygosity or the different genotype groups. The most common mutations found in this study were L444p/L444p (homozygous) and L444p/IVS2+1 (double heterozygous); 8 patients each (40%) followed by L444p/D409H (double heterozygous) in 3 patients (15%) whereas N370S/N370S (homozygous) was the least common mutation found in only 1 patient (5%). Allele frequency showed that L444p was found in 67.5% of studied chromosomes, IVS2+1 in 20%, D409H in 7.5%, whereas N370s was in only 5%. As for family history, 80% of cases had positive consanguinity, with 45% of patients parents being first cousins. All homozygous cases showed positive consanguinity (9 cases), whereas only 7 cases of 11 of the double heterozygous showed positive consanguinity. Also all patients with the genotypes L444p/L444p and N370S/N370S were from consanguineous parents; 8 of 8 and 1 of 1 respectively. On the other hand, the 3 patients with the genotype L444p/D409H had negative consanguinity, while the genotype L444p/IVS2+1 showed 7 of 8 cases with positive consanguinity and only 1 case with negative consanguinity.

Conclusion: The most common mutations found in our study were L444p/L444p homozygous and L444p/IVS2+1 double heterozygous. L444p was the most common allele. Analysis for the most common mutations was the method of choice for identification of Gaucher's disease carriers.

Both age of the patients and onset of the disease had no significant relation with either homozygosity Vs. double heterozygosity or the different genotype groups.

Gaucher's disease occured with equal frequency in males and females. Patients with homozygous gene mutations tended to have consanguineous parents.

Neurological manifestations, growth retardation and chest symptoms were the most common clinical conditions reported in studied cases. None of the previous conditions were significantly associated with certain genotype.

No correlation was detected between genotype or homozygosity Vs heterozygosity on one side and either age of patients, age of onset or clinical manifestations on the other side.

Key Words: *GD (Gaucher's disease) – GBA mutations – Reversed hybridization – Homozygous – Double heterozygous.*

INTRODUCTION AND AIM OF THE WORK

Gaucher's disease is the most prevalent of the genetic lysosomal storage disorders. It is an autosomal recessive disease which was described by the French Physician Philippe Gaucher in 1882. It is caused by a severe deficiency of glucocerebrosidase enzymatic activity with resultant accumulation of large quantities of glycolipid, glucocerebrosidase within the lysosomes of the phagocytic cells of the monocyte-macrophage system.

Gaucher's disease is classified into three conventional types in which clinical differentiation depends upon patients age and organs affected.

Type I: Chronic non-neuropathic form, usually found in adults especially in Jewish population. It is a chronic disease involving viscera and blood forming tissues. Most of these patients develop massive spleen enlargement, anemia and bleeding tendency. In addition, they may have bony pains and pathological fractures.

Type II: Infantile neuropathic form which always appears by 6 months of age. It is more rare than type I and does not have predominance in Jewish population. Neurological manifestations, spleenomegaly, pulmonary and bony affection are the most common presenting symptoms. Most patients die before 2 years of age.

Type III: Juvenile sub-acute neuropathic which includes a heterogenous group of patients with signs of chronic adult type combined with slowly progressive neurological disease that begins during childhood or adolescence **[1]** (Stone et al. 2000).

As there are limited data on the frequency of the different mutations in the Egyptian population, the aim of this study is to investigate the genotypes of Gaucher's disease, the most prevalent mutations in Egypt and to assess if a genotype-phenotype relationship could be elicited.

PATIENTS AND METHODS

Patients:

The present work included 20 Gaucher's disease patients diagnosed by enzyme assay of ß-glucosidase activity in peripheral leucocytes **[2]** (Wenger et al., 1978), who were registered and filed in hematology outpatient clinic of the New Children Pediatric Hospital (Cairo University), 10 were males and 10 were females. Their ages ranged from 3 to 43 years (with a mean of 10.15 and median of 6). All patients had been already diagnosed by clinical and laboratory investigations as Gaucher's disease patients.

Methods:

All studied cases were subjected to clinical evaluation, revision of their filed clinical progress, radiological and laboratory data as follows:

1- *Full medical history:*

Complete history was taken for age, sex, and family history including consanguinity, affected siblings and a similar condition in the family. The prenatal, past and present history of abdominal, respiratory, central nervous system, cardiovascular symptoms or any symptoms suggesting bleeding tendency, were also obtained. Assessment of both physical and mental development was performed for all patients.

2- *Full clinical examination:*

All patients were subjected to complete physical examination including assessment of weight, height and skull circumference to evaluate physical development. Vital signs including pulse, respiratory rate and temperature were determined. Systemic examination was done to every patient including abdominal examination, assessing any splenic or hepatic enlargement. Chest, cardiac and central nervous system examination was also performed.

3- *Investigations:*

A- *Preliminary investigations*:

1- CBC: to detect presence of anemia, thrombocytopenia or any other abnormalities (advia 120).
2- Liver functions which include prothrombin time, SGOT and SGPT.
3- Enzyme assay to detect levels of b-glucocerebrosidase, sphingomyelinase, as well as chitotriosidase enzyme.
4- Bone marrow aspiration to detect the presence of Gaucher's cells and/or the presence of any abnormalities of red and white series as well as megakaryocytes.

5- Splenic aspiration to detect the presence of Gaucher's cells and/or the presence of any other abnormalities.

6- Abdominal U.S to detect hepatomegally, spleenomegally and/or the presence of any other abnormalities.

B- *Specific investigations:*

Strip assay for the identification of glucocerebrosidase (GBA) gene mutations based on polymerase chain reaction (PCR) and reverse-hybridization.

Methodology:

The procedure includes three steps: **[1]** DNA isolation, **[2]** PCR amplification using biotinylated primers, **[3]** hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

The assay covers 8 common GBA mutations: 84GG [452+G], IVS2+1 [484 G>A], N370S [1226 A>G], V394L [1297 G>T], D409H [1342 G>C], L444P [1448 T>C], R463C [1504 C>T], R496H [1604 G>A], as well as 2 recombinant alleles derived from crossover between the GBA functional gene and pseudogene (rec Ncil, rec TL).

RESULTS

Family history of the studied patients revealed that 80% of cases had positive consanguinity, while 45% of patients' parents were first cousins. Eighty percent of cases had affected sibs, in 15% sibs were clinically free, while 5% did not have other sibs. History and clinical examination revealed that 11 (55%) of patients had delayed physical development, while only 2 (10%) showed mental retardation. Neurological manifestations were encountered in 6 (30%) cases; their types and frequency are shown in Table (1). Nine (45%) cases had bleeding tendency while only 1 (5%) case showed cardiac involvement.

Frequency of gene (homozygous Vs double heterozygous) among studied cases was 9 (45%) and 11 (55%) respectively.

There was no significant relation between neurological affection and the genotype, whether homozygous or double heterozygous. Also there was no relation between number of affected sibs and different genotype groups.

As regards the hematologic parameters, we did not find any significant relation between the different genotypes and the presence of anemia, leucopenia, thrombocytopenia or splenic infiltration with gaucher cells. Prothrombin concentration was low in 30% of our cases and GOT level was slightly elevated in 20% of cases. Both had no statistically significant relation with either gene homozygousity Vs double heterozygousity or the different genotype groups.

Table (1): Frequency and type of neurological affection among studied cases.

Neuro	Frequency	Percent
Free	14	70. %0
Hyperreflexia, hypotonia, +ve stepping reflex	1	5.0%
Occulomotor aprexia & squint	3	15.0%
Squint	2	10.0%
Total	20	100.0%

Table (2): Frequency of bone & chest affection among studied cases.

Bone affection	Frequency	Percent	Chest affection	Frequency	Percent
Bony pains, knock knee	1	5.0%	Dyspnea	1	5.0%
Recurrent bony crisis	1	5.0%	Recurrent chest infection	6	30.0%
Free	18	90.0%	Free	13	65.0%
Total	20	100.0%	Total	20	100.0%

Table (3): Frequency of cardiac affection and bleeding among studied cases.

Cardiac affection	Frequency	Percent	Bleeding	Frequency	Percent
Affected	1	5.0%	Positive	9	45.0%
Free	19	95.0%	Negative	11	55.0%
Total	20	100.0%	Total	20	100.0%

Table (4): Spleen and liver size among studied cases.

Spleen	Frequency	Percent	Liver	Frequency	Percent
Enlarged	5	25.0%	Enlarged	9	45.0%
Hugely enlarged	12	60.0%	Hugely enlarged	11	55.0%
Splenectomy	3	15.0%			
Total	20	100.0%	Total	20	100.0%

Table (5): Frequency of anemia, leucopenia and thrombocytopenia among studied cases.

Anemia	Frequency	Percent	Leucopenia	Frequency	Percent	Thrombocytopenia	Frequency	Percent
Absent	3	15.0%	Absent	12	60.0%	Absent	6	30.0%
present	17	85.0%	present	8	40.0%	present	14	70.0%
Total	20	100.0%	Total	20	100.0%	Total	20	100.0%

Table (6): Frequency of B.M and splenic aspirate infiltration by Gaucher's cells among studied cases.

B.M asp infiltration by gaucher's cells	Frequency	Percent	B.M asp infiltration by gaucher's cells	Frequency	Percent
No	4	20.0%	Yes	9	45.0%
Yes	11	55.0%			
Not performed	5	25.0%	Not performed	11	55.0%
Total	20	100.0%	Total	20	100.0%

Table (7): Frequency of gene mutation among studied cases.

	Frequency	Percent
L444p/D409H	3	15.0%
L444p/L444p	8	40.0%
L444p/IVSII+1	8	40.0%
N370s/N370s	1	5.0%
Total	20	100.0%

Table (8): Allele frequency among studied cases.

	Frequency	Percent
L444P	27	67.5%
D409H	3	7.5%
IVS2+1	8	20%
N370s	2	5%
Total	40	100%

Table (9): Relation of consanguinity to homozygousity Vs double heterozygousity.

GENE		Consanguinity			Total	*p* value
		Negative	Positive	Strong		
Homozygous	Count		6	3	9	
	percent		66.7%	33.3%	100.0%	<0.01
Double hetero	Count	4	1	6	11	(Highly significant)
	percent	36.4%	9.1%	54.5%	100.0%	
Total	Count	4	7	9	20	
	percent	20.0%	35.0%	45.0%	100.0%	

Table (10): Relation of consanguinity to homozygousity Vs double heterozygousity.

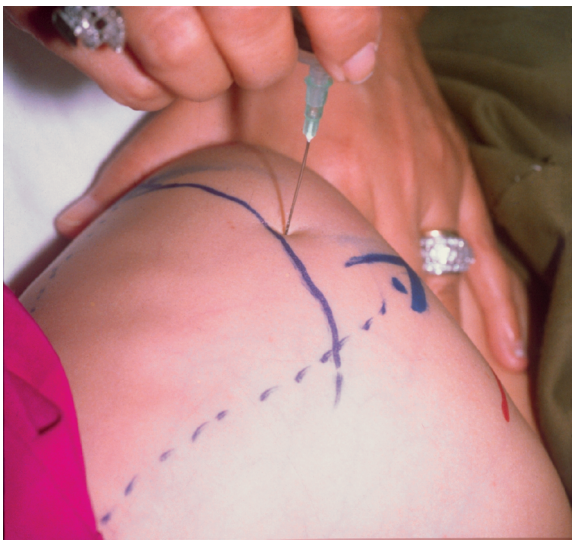
GENE		Present	First baby	No	Total	*p* value
Homozygous	Count	9			9	
	percent	100.0%			100.0%	0.061
Double Hetero	Count	7	1	3	11	(Borderline significace)
	percent	63.6%	9.1%	27.3%	100.0%	
Total	Count	16	1	3	20	
	percent	80.0%	5.0%	15.0%	100.0%	

Table (11): Relation of neurological affection to homozygous Vs double heterozygous.

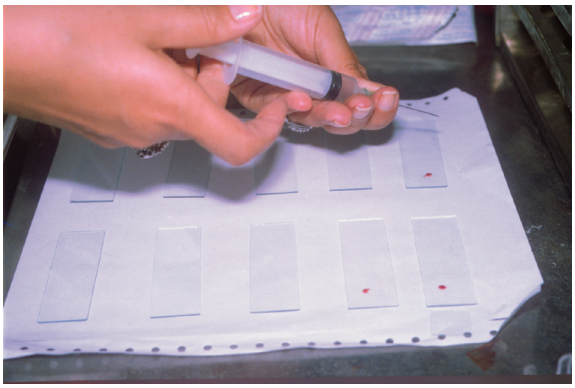
GENE		Absent	Present	Total	*p* value
Homo	Count	6	3	9	
	percent	77.7%	33.3%	100.0%	
Double hetero	Count	8	3	11	N.S
	percent	72.7%	27.3%	100.0%	
Total	Count	14	6	20	
	percent	70.0%	30.0%	100.0%	

Table (12): Relation of neurological affection to homozygous Vs double heterozygous.

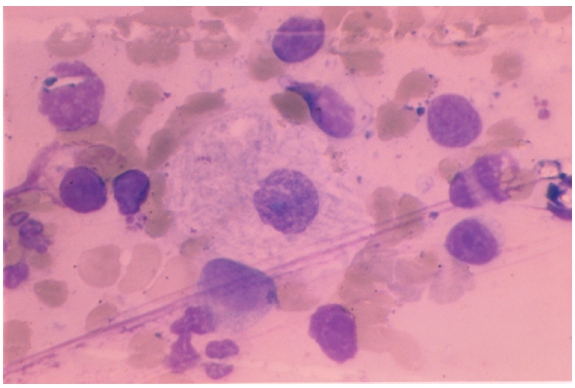
GENE		Free	Hyperreflexia, hypotonia, +ve stepping reflex	Occulomotor aprexia & squint	Squint	Total	*p* value
Homo	Count	6		1	2	9	
	percent	66.7%		11.1%	22.2%	100.0%	
Double hetero	Count	8	1	2		11	N.S
	percent	72.7%	9.1%	18.2%		100.0%	
Total	Count	14	1	3	2	20	
	percent	70.0%	5.0%	15.0%	10.0%	100.0%	



Figs. (1)



Figs. (2)



Figs. (3)

Figs. (1,2,3): Showing splenic aspirate infiltration by gaucher's cells

Fig (4): Strip showing L444P/L444P mutation.

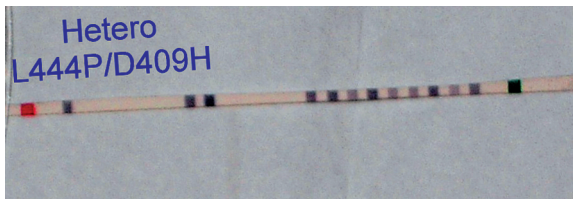


Fig 5: Strip showing L444P/D409H mutation.

Fig 6: Strip showing N370S/N370S mutation.

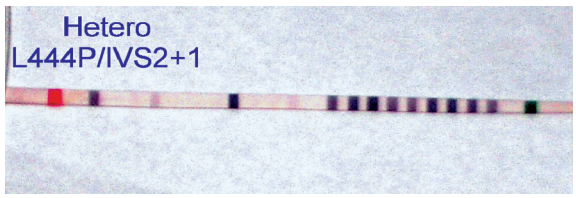


Fig 7: Strip showing L444P/IVS2+1 mutation.

DISCUSSION

In this work, 20 patients with documented Gaucher disease were investigated for their genotype. Correlation between genotype and phenotype was also evaluated.

The most common mutations encountered were L444p/L444p (homozygous) and L444p/IVS2+1 (double heterozygous), 8 patients each (40%) followed by L444p/D409H (double heterozygous) in 3 patients (15%) whereas N370S/N370S (homozygous) was found in only 1 patient (5%). Allele frequency showed that L444p was found in 67.5, IVS2+1 in 20%, D409H in 7.5%, and N370s in only 5%.

Previous molecular studies of the disease mutations revealed that although more than 50 mutations were identified in the Glucocerebrosidase gene, only four of them frequently occur. These four common mutations are (N370S, L444p, 84gg, and IVS2+1) representing 90% to 95% of the mutations associated with GD in the Ashkenazi Jewish population, and 50% to 75% of the mutations in the general population **[3]** (Beutler et al. 1991) and **[4]** (Sibille et al. 1993).

The N370S mutation is the most common in patients with GD accounting for about 75% of the mutant alleles in Ashkenazi Jewish persons and about 18% of non Ashkenazi Jewish **[5]** (Zimran et al. 1989).

The L444p mutation occurs more frequently in the non-Jewish population accounting for about 4% of the mutant alleles in Ashkenazi Jewish persons and about 32% of non Ashkenazi Jewish **[6]** (Tsujii et al. 1987).

The 84GG mutation accounts for 12% in Ashkenazi Jewish persons and 1% of non Ashkenazi Jewish. **[3]** (Beutler et al. 1991), while IVS2+1 mutation account for 3% in Ashkenazi Jewish persons and 1% of non Ashkenazi Jewish **[7]** (Sidransky et al. 1994).

(Tayebi, Stubblefield et al. 2003) **[8]** found that N370S/ N370S mutation accounts for about 29%, N370S/? mutation accounts for about 20%, N370S/L444p mutation accounts for about 16%, N370S/84GG mutation accounts for about 12%, L444p/L444p mutation accounts for about 6%, L444p/? mutation accounts for about 3%, and N370S IVS2+1 mutation accounts for 3%.

(El-Beshlawy et al. 2006) **[9]** found that 14 of 22 patients were homozygous or compound heterozygous for L444p and D409H mutations.

In our study equal sex distribution was found among patients with no significant relation with either homozygosity Vs double heterozygosity or the different genotype groups. This is in agreement with Theophilus et al. (1989), who reported that Gaucher's disease occurs with equal frequency in males and females.

The mean age of the patients was 10.15 years with a median of 6 years, the eldest was 45 years; his genotype was L444p/IVS2+1. The mean age of onset of the disease was 9 months. Both age of the patients and age of onset of the disease have no significant relation with either homozygosity Vs double heterozygosity or the different genotype groups.

As regards family history we found that 80% of cases had positive consanguinity with 45% of patientsí parents being first cousins. Relation between consanguinity and gene homozygous Vs double heterozygous was found to be highly significant p (<0.01) all 9 homozygous cases showed positive consanguinity, whereas 7 cases of 11 of the double heterozygous showed positive consanguinity. Relation between consanguinity and different genotype groups was found to be statistically significant (p<0.05). All patients with the genotypes L444p/L444p and N370S/N370S were from consanguineous parents ie: 8 cases of 8 and 1 case of 1 respectively. On the other hand all 3 patients with the genotype L444p/D409H had negative consanguinity, while the genotype L444p/IVS2+1 showed 7/8 with positive consanguinity and only 1 case with negative consanguinity.

Eighty percent of cases had affected sibs; in 15% sibs were clinically free, while 5% were a first baby. Whereas relation between number of affected sibs and gene homozygousity Vs double heterozygousity was found to be near significant (p=0.061), all sibs of homozygous patients were clinically affected. All patients of the genotype L444p/L444p had affected sibs, variable percentage of affected sibs were found in other genotypes, however, these findings were not statistically significant.

(El-Beshlawy et al. 2006) **[9]** found that Two-thirds of the patients were from consanguineous pedigrees. Another previous study revealed that 80% of cases were from consanguineous parents and 30% with affected siblings (El-Gawhary et al. 1999) **[10]**.

The high frequency of positive consanguinity and the mode of the disease inheritance drive the attention to the importance of genetic counseling and pre-natal diagnosis of the disease.

Genetic counseling prior to and following testing for Gluco-cerebrosidase mutations is essential. Obtaining an accurate family history prior to testing is necessary to identify the family members who are at risk of being affected, members who are "obligate carriers," and members who are at risk of being carriers. Information about ethnic background is relevant to the interpretation of test results. The a priori risk for individuals undergoing Gaucher's disease testing is dependent upon ethnic background and family history. For example, a negative mutation analysis (four common mutations) for an Ashkenazi Jewish individual with no family history of Gaucher's disease modifies his or her risk of being a carrier from

about 1 in 10 to about 1 in 200. Bayesian analysis should be used to calculate the modified risk of being a carrier for individuals with a family history of the disease **[4]** (Sibille et al. 1993).

Mutation analysis for the four common mutations (N370S, L444p, 84gg, and IVS2+1) detects 90% to 95% of the mutations associated with Gaucher's disease in the Ashkenazi Jewish population, and 50% to 75% of the associated mutations in the general population **[3]** (Beutler et al. 1991) and **[4]** (Sibille et al. 1993).

Mutation analysis is used in combination with Gluco-cerebrosidase enzyme assay results to diagnose Gaucher's disease and is also important for identification of carriers. Gluco-cerebrosidase enzyme assay results are frequently helpful when interpreting mutation analysis results. For example, an enzyme assay that reveals a deficiency of Gluco-cerebrosidase (<30% of normal Gluco-cerebrosidase activity) enables correct interpretation of negative mutation analyses **[4]** (Sibille et al. 1993).

Mutation analysis is the method of choice for identification of Gaucher's disease carriers. Carrier detection via enzymatic assay is unreliable because approximately 20% of obligate carriers demonstrate normal Gluco-cerebrosidase activity (false negatives) ie: An individual with normal enzyme activity but has 1 copy of N370S mutation (by mutation analysis) is an unaffected carrier. Mutation analysis for carrier detection is most informative for members of a high-risk population or for individuals with affected family members whose mutations are identified. When no mutations are detected by current methods, it is important to consider that negative results modify, but do not eliminate, the risk of being a carrier **[11]** (Beutler et al. 2004).

Three of eight of our patients with the genotype L444p/L444p had neurological affection, two of eight in the form of squint and in one of them squint was accompanied with occulomotor aprexia. In the genotype L444p/IVS2+1 three of eight patients had neurological affection one in the form of hyperreflexia, hypotonia and positive stepping reflex, while the two other patients in the form of occulomotor aprexia & squint, however this was also not statistically significant.

(Koprivica et al. 2000) **[12]** stated that patients with at least one N370S allele did not develop neurological symptoms. Patients who were homozygous for the L444p mutation tend to have severe disease, with neurological complications. This mutation results in an unstable enzyme with little or no residual activity. In a study of 31 individuals with neurological complications, L444p accounted for 25 alleles (40%) (Stone et al. 2000) **[1]**. The L444p mutation occurred alone (nine alleles), with E326K (one allele), and as part of a recombinant allele (15 alleles). In another study, homozygosity for the L444p mutation was the most common genotype among individuals with GD type 3 (10/24 individuals, or 42%) (Koprivica et al. 2000) **[12]**.

Only 5% of our cases had bone affection, 35% had chest affection, and in 5% had mild cardiac affection. No significant relation was found between these symptoms and gene homozygosity Vs double heterozygosity or different genotype groups.

Two of our patients showed bone affection, both of them had double heterozygous gene mutation, one suffered from bony pains and knock knees whose genotype was L444p/D409H; while, the other one had recurrent bony crises and his genotype was L444p/IVSII+1. X-ray evaluation of both patients revealed no recent or old pathological fracture.

(El-Beshlawy et al. 2006) **[9]** recently stated that there was no correlation between severity of bone involvement and GBA genotype.

Splenic aspiration was performed in 45% of our cases, all of them had Gaucher's cells infiltration. These findings had no statistically significant relation with either gene homozygousity Vs double heterozygousity or the different genotype groups.

(Nnaito et al. 1988) **[13]** reported that under electron microscope Gaucher's cells are filled with numerous elongated, rod shaped bodies (Gaucher bodies) that contain smooth walled tubular elements, either in bone marrow or splenic aspirate.

(Seif EI-Nasr 1993) **[14]** and (El-Gawhary et al. 1986) **[15]** observed that Gaucher cells were detected in the bone marrow of all cases with neuoropathic symptoms.

El-Gawhary et al. 1986) **[15]** reported that liver function tests were usually normal or slightly elevated, which is in agreement with our results as regards prothrombin time and serum GOT.

Conclusions:

Gaucher's disease occurs with equal frequency in males and females. The most common mutations found in our study were L444p/L444p and L444p/IVS2+1; while L444p was the most common allele. Both age of the patients and onset of the disease had no significant relation with either homozygosity Vs. double heterozygosity or the different genotype groups. Patients with homozygous gene mutations tended to have consanguineous parents.

As regards the clinical manifestations, we found that neurological involvement, growth retardation and chest symptoms were the most commonly reported in the studied cases; and less frequently bone & cardiac affection. None of the previous conditions were significantly associated with certain genotype.

The nature of Gaucher disease as a genetically inherited, debilitating disease highlights the need for preventive approaches such as genetic counseling, extended gene mutation studies for prenatal diagnosis especially in consanguineous couples with previous family history or with previously affected sibs.

Mutation analysis is the method of choice for identification of Gaucher's disease carriers; common mutation screening is the method of choice for carrier detection.

Further studies with larger number of patients will be beneficial to establish the relation between the different genotypes and the clinical manifestations of Gaucher disease, as well as the effectiveness of enzyme replacement therapy in changing its natural course.

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