The Egyptian Journal of Medical Human Genetics (2017) 18, 249–255

HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com

ORIGINAL ARTICLE

Clinical and genetic assessment of pediatric patients with Gaucher's disease in Upper Egypt

Tahia H. Saleem [a], Mohammed H. Hassan [b,*], Ahmed El-Abd Ahmed [c], Ayat A. Sayed [a], Nahed A. Mohamed [a], Khalid I. Elsayh [d], Abdallah M.A.A. El-Ebidi [e], Norhan B.B. Mohammed [b]

[a] *Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Assiut University, P.O. Box 71515, Assiut, Egypt*
[b] *Department of Medical Biochemistry and Molecular Biology, Qena Faculty of Medicine, South Valley University, P.O. Box No. 83523, Qena, Egypt*
[c] *Department of Pediatrics, Qena Faculty of Medicine, South Valley University, P.O. Box No. 83523, Qena, Egypt*
[d] *Department of Pediatrics, Faculty of Medicine, Assiut University, P.O. Box 71515, Assiut, Egypt*
[e] *Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Aswan University, P.O. Box No. 8152, Aswan, Egypt*

Received 31 July 2016; accepted 24 August 2016
Available online 24 September 2016

KEYWORDS

Gaucher's disease;
Upper Egypt;
Clinical types;
Genotypes

Abstract *Background:* Gaucher's disease (GD) is an autosomal recessive genetic disorder that results from pathogenic mutations of GBA gene encoding the enzyme glucocerebrosidase (acid β-glucosidase). Of the approximately 300 mutations associated with GD, 4 accounts for the majority of mutations seen in GD patients: N370S, L444P, 84 GG and IVS2 + 1.

Aim: Establishing and providing, clinical and molecular backgrounds of pediatric patients with GD in Upper Egypt.

Subjects and methods: The present study is a cross sectional study, carried out on 26 pediatric patients with GD. They were recruited from the pediatric outpatient clinics and inpatients Pediatric departments of Assiut and Qena University hospitals, Upper Egypt. Clinical evaluation and mutation analysis using commercially available strip assay kit after PCR amplification of the target gene were done for all included GD patients.

Results: Consanguinity between patients' parents was present in 73.1% of the included patients. 76.9% of included patients were of type 1 GD, while 23.1% were of type 3 GD and none of our patients was classified as type 2 GD. The main frequent clinical presentations of GD in this study were hepatosplenomegaly (88.5%); pallor (76.9%); abdominal distension (61.5%) and musculoskeletal involvement (37.1%). Neurological abnormalities of type 3 GD included in this study were squint, seizures and delayed mental development. Five different genotypes were detected, homozygous for the mutation L444P, homozygous for the mutation N370S, heterozygous for the

* Corresponding author.
E-mail addresses: Mohammedhosnyhassaan@yahoo.com, mohammedhosnyhassaan@med.svu.edu.eg (M.H. Hassan).

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2016.08.005
1110-8630 © 2016 Ain Shams University. Production and hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

mutations N370S and rec Ncil, heterozygous for IVS2 +1 and rec NciI, heterozygous for L444P and IVS2 +1.

Conclusions: Non-neuropathic type 1 and type 3 GD were the only clinical types found in the present study. The most common mutant alleles found in this study were L444P and N370S.

© 2016 Ain Shams University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Gaucher's disease (GD) is one of the most common lysosomal storage diseases and one of the rare genetic diseases for which therapy is now available. GD is an autosomal recessive genetic disorder that results from pathogenic mutations of GBA gene encoding the enzyme glucocerebrosidase (acid β-glucosidase), which located on 1.q21.31. The absence or low activity of this enzyme leads to a progressive accumulation of its substrate (glucosylceramide "GlcCer") into macrophages [1]. These cells are natural phagocytes that are involved in the degradation of the membrane glycolipids from red blood cells (RBCs) and leukocytes. GlcCer-laden macrophages are not killed by the accumulation of the substrate, but tend to transform into Gaucher cells [2]. Moreover, all cells of the mononuclear phagocyte system, and especially tissue macrophages of the liver (Kupffer cells), spleen, bone marrow (osteoclasts) can be affected in GD [3]. Accumulation of glucosylceramide in these organs contributes directly to massive hepatosplenomegaly and pancytopenia [4].

Progressive infiltration of Gaucher cells into the bone and bone marrow may lead to thinning of the cortex, pathologic fractures, bone pain and joint collapse. In about one third of patients, this infiltration leads to avascular necrosis of the bone, which can eventually result in irreversible bone destruction [5].

The phenotypes of GD are a continuum of degrees of involvement, but can be divided categorically into three major clinical types that are delineated by the absence (type 1) or presence (types 2 and 3) of primary central nervous system involvement [6]. The neuropathic involvement in types 2 and 3 GD results from accumulation of glucosylceramide and its neurotoxic derivative, glucosyl sphingosine, with subsequent neuronal loss via eliciting apoptotic signals [7].

Molecular analysis of the GBA gene is complex, as this gene is linked with an actively transcribed, highly homologous (96% identity) pseudogene (5 kb) that also harbors several mutations, however only mutations in the active gene lead to GD [8]. Of the approximately 300 mutations associated with GD, 4 accounts for the majority of mutations seen in GD patients: N370S, L444P, 84 GG and IVS2 + 1 [9].

Few studies could be traced in the literature regarding the clinical and genetic assessment of pediatric patients with GD in Egypt, especially in Upper Egypt, where a high rate of consanguineous marriage allows a higher chance for appearance of such autosomal recessive metabolic disorders, so the present study aimed to identify the main presenting manifestations, the most frequent clinical types and genotypes and correlating them to each other among pediatric patients with GD in Upper Egypt.Given the rarity of GD, we hope that this study helps to build more experience in pediatric GD in Upper Egypt.

2. Subjects and methods

2.1. Study design and setting

The present study is a cross sectional analytical study carried out on 26 pediatric patients, 14 males and 12 females. They were recruited from the pediatric outpatient clinics and inpatients Pediatric departments of Assiut and Qena University Hospital which are of the major tertiary referral pediatric hospitals in Upper Egypt after approval of the university hospital ethical committee. Prior to initiation of the study; every subject and his/her parents were informed about the aim of the study and gave a written consent. The study was carried out during the period from May 2015 to May 2016. As this is an exploratory study, only percentage and proportions are reported.

2.2. Data collections

History taking for all included pediatric patients, including: Personal history/Family History: age, sex, residence, history of consanguineous marriage among the patients' parents, history of abdominal distention, bone pain, fatigue, easy bruising, delayed growth, muscle weakness, cognitive impairment, seizures, dementia, heart and lung problems, history of blood transfusions or splenectomy. Family history of fetal deaths or miscarriages or genetically affected siblings with GD was also included. Thorough clinical examination for: vital signs; anthropometric measurements; abdominal examination (if there is splenomegaly or hepatosplenomegaly); musculoskeletal examination (if there is bone tenderness or skeletal deformities); neurological examination (if there is spasticity, opisthotonus ataxia, hypertonia, hyperreflexia); cardiac and chest examination were done for all included patients.

Diagnosis of the included patients with GD was based upon the following inclusion criteria [10]; clinically: unexplained isolated splenomegaly or hepatosplenomegaly with skeletal abnormalities (bone pain or spontaneous fractures) associated with or without delayed physical and/or mental development and/or neurologic abnormalities. Laboratory: anemia and/or thrombocytopenia to be confirmed by the presence of reduced leukocyte glucocerebrosidase enzyme activity, increased plasma chitotriosidase activity with or without typical Gaucher cells infiltrate in the bone marrow aspirate examination or liver biopsy.

2.3. Study methods

(A) Imaging tools (taken already from the file of the patients)

1. Abdominal ultrasound was done to determine liver and spleen sizes.

2. Plain X-ray for long bones and chest, Echocardiography, EEG, C.T brain for selected patients.

(B) Laboratory workup

Three cc of venous blood was drawn from the included children on EDTA tubes, stored at −20 °C till the time of mutation analysis. Strip assay method was done using a commercially available strip assay kit supplied by Vienna Lab Diagnostics GmbH, Gaudenzdorfer Guertel 43-45, A-1120 Vienna, Austria: The procedure included three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers (using VeriFlex™ 96-Well Thermal Cycler, Applied Biosystems, USA), (3) hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates. The assay covered 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 NciI, rec TL). The genotype of a sample was determined using the enclosed Collector TM sheet. The processed Test strips were placed into one of the designated fields aligned it to the schematic drawing using the red marker line (top) and the green marker line (bottom) then it was fixed with adhesive tape. The positive reaction of the uppermost Control line ensured the correct function of Conjugate Solution and Color Developer. Probes 1 to 8 (mutant) and 10 to 17 (wild type) refer to the respective alleles as present on the GBA functional gene. Rec reporter probes 9 and 18 indicate whether a recombination event has occurred due to crossover between the functional gene and the GBA pseudogene in the region of exons 9–10 (flanked by mutations V394L and L444P). The following recombinant alleles have been documented: rec TL: crossover point located between V394L and D409H and rec NciI: crossover point located between D409H and L444P. A positive signal for the mutant rec reporter probe indicates the presence of one of these rec alleles, the wild type rec reporter probe reads positive for GBA alleles without crossover in this region. In case of a positive mutant rec reporter probe, rec TL and rec NciI can be distinguished by the presence or absence of positive staining for mutant D409H.

3. Results

The present study included 29 GD pediatric patients, 14 males (53.8%) and 12 females (46.2%). The mean age of the studied patients was 8.68 ± 5.34 years with age range from 0.17 to 17 years. The males to females ratio was 1.2:1. The mean age at presentation was 3.06 ± 4.22 years with age range from 0.08 to 17 years. Most of patients were from Assiut governorate (65.4%) followed by Sohag governorate (19.2%) then Minia (7.7%) and the least number were from Qena governorate (3.8%) and Aswan governorate (3.8%). Most of patients were rural residents (92.3%) and the remaining were urban residents (7.7%). Consanguinity between patients' parents was present in 19 patients (73.1%). In addition, fourteen children (53.8%) had at least one affected sibling and two patients (7.7%) had history of sibling death in early childhood most probably due to GD. 76.9% of included patients were of type 1 GD, while 23.1% were of type 3 GD and none of our patients was classified as type 2 GD.

The frequency distribution of the main clinical and imaging findings of the studied patients at time of diagnosis are listed in Table 1, hepatosplenomegaly was present in 88.5% of cases, pallor (76.9%), and abdominal distension (61.5%). Musculoskeletal involvement was present in 73.1% in form of chronic bone pain (38.5%), bone crises (3.8%), bone fractures (19.2%), restricted mobility (7.7%) and/ or radiological bone involvement (57.7%). The major radiological bone involvement in this study were Erlenmeyer Flask Deformity (53.8%), kyphoscoliosis (11.5%), pectus carinatum (15.4%) and avascular necrosis of femoral head (3.8%). 30.8% of the studied cases complained from recurrent chest infections and 23.1% had bleeding tendency in the form of epistaxis, easy bruising, menorrhagia or prolonged bleeding after superficial wounds.

Among the 52 alleles studied, 38.5% contained the L444P mutation, 23.1% were shown to have the N370S mutation and 9.6% contained the IVS2+1 mutation. The recombinant allele recNcil was identified in 13.4%, Table 2. Five different genotypes were detected in the studied patients; homozygous for the mutation L444P (30.8%), homozygous for the mutation N370S (15.4%), heterozygous for the mutations N370S and rec Ncil (15.4%), heterozygous for IVS2 +1 and rec NciI (11.5%), heterozygous for IVS2 +1 and L444P (7.7%), heterozygous for L444P and second alleles were not detected but occurred in 7.7%, while, in 11.5% it was not possible to identify any of the alleles. Table 3 and Fig. 1.

4. Discussion

Regarding the demographic data of the pediatric patients with Gaucher disease involved in this study; males to females ratio was 1.2:1, which indicates that the inheritance of GD is not linked to the sex chromosomes and reflects the autosomal recessive nature of such metabolic disorders that affect males and females in an equal proportion. Most of patients were rural residents and few of them were urban residents. In agreement with this finding, Giraldo et al. [11] and Tantawy et al. [12], both reported no sex differences between patients having Gaucher disease involved in their studies. Shawky et al. [13] reported that the overall frequency of consanguinity in Egypt is high and varies by region as it was higher in rural areas than in semi-urban and urban areas and attributed this to the fact that many Egyptian families in the rural communities prefer marriage to the first cousins to preserve family structure, links and provide social, economic and cultural benefits. This could explain why most of the GD patients involved in the present study were of rural residents. Consanguinity between patients' parents in this study was present in 73.1% of patients. In agreement with our findings, El-Beshlawy et al. [14], Khalifa et al. [15] and Elgawhary et al. [16], all these studies reported a higher rate of consanguineous marriage among the parents of GD patients.

The international Collaborative Gaucher Group (ICGG) Gaucher Registry revealed that non-neuropathic type 1 GD is the most prevalent form worldwide [17]. Patients in the present study were classified according to presence and nature of neurological symptoms and signs, which were evaluated by a

Table 1 Frequency distribution of the main clinical and imaging findings of the studied patients according to their clinical types at time of diagnosis.

Variables[*]	GD type I (n = 20)		GD type III (n = 6)		Total (n = 26)	
	No.	%	No.	%	No.	%
Developmental delay						
Physical delay	1	5.0	3	50.0	4	15.4
Physical and mental delay	0	0.0	3	50.0	3	11.5
Abdominal distension	12	60.0	4	66.7	16	61.5
Bleeding tendency	5	25.0	1	16.7	6	23.1
Recurrent chest infection	5	25.0	3	50.0	8	30.8
Pallor	14	70.0	6	100	20	76.9
Hepatosplenomegaly[**]	17	85.0	6	100	23	88.5
Isolated Splenomegaly[**]	2	10	0	0.0	2	7.7
Neurological involvement			6	100	6	23.1
Squint	–	–	5	83.3	5	19.2
Seizures	–	–	3	50.0	3	11.5
Musculoskeletal involvement	13	65.0	6	100	19	73.1
Bone pain	8	40.0	2	33.3	10	38.5
Bone crises	1	5.0	0	0.0	1	3.8
Bone fracture	5	83.3	0	0.0	5	19.2
Restricted mobility	0	0.0	2	33.3	2	7.7
Radiological evidence of bone involvement[***]	9	45.0	6	100	15	57.7
Blood transfusion	10	50.0	4	66.7	14	53.8
Splenectomy	1	5.0	0	0.0	1	3.8
Joint replacement	1	5.0	0	0.0	1	3.8
Joint replacement	1	5.0	0	0.0	1	3.8

[*] Many cases presented with more than one clinical finding.
[**] Organ volumes were obtained by means of magnetic resonance imaging (MRI), computed tomography (CT) or ultrasound.
[***] Radiological bone findings in this study were Erlenmeyer Flask Deformity, kyphoscoliosis, pectuscarinatum and avascular necrosis of femoral head.

Table 2 Frequency distribution of the mutant alleles among the studied patients according to their clinical types.

Mutant allele	GD type I (n = 20)		GD type III (n = 6)		Total (n = 26)	
	No.	%	No.	%	No.	%
L444P	8	20.0	12	100	20	38.5
N370S	12	30.0	0	0.0	12	23.1
Rec NciI	7	17.5	0	0.0	7	13.4
IVS2 + 1	5	12.5	0	0.0	5	9.6
Unknown	8	20.0	0	0.0	8	15.4
Total number of mutant alleles	40		12		52	

Table 3 Frequency distribution of the genotypes among the studied patients according to their clinical types.

Genotype	GD type I (n = 20)		GD type III (n = 6)		Total (n = 26)	
	No.	%	No.	%	No.	%
L444P/L444P	2	7.7	6	100	8	30.8
N370S/N370S	4	15.4	0	0.0	4	15.4
IVS2 + 1/L444P	2	7.7	0	0.0	2	7.7
N370S/rec NciI	4	15.4	0	0.0	4	15.4
IVS2 + 1/rec NciI	3	11.5	0	0.0	3	11.5
*?/L444P	2	7.7	0	0.0	2	7.7
*?/?	3	11.5	0	0.0	3	11.5

*? means that the allele couldn't be identified.

pediatric neurologist. Most of patients were of type 1 GD and few of them were of type 3 GD, while, none of our patients was classified as type 2 GD, however in this study, two children with homozygous L444P mutation (7.7%) exhibited type 1 phenotype and they need careful observation as they are at risk for development of neuronopathic disease (type 3) in later

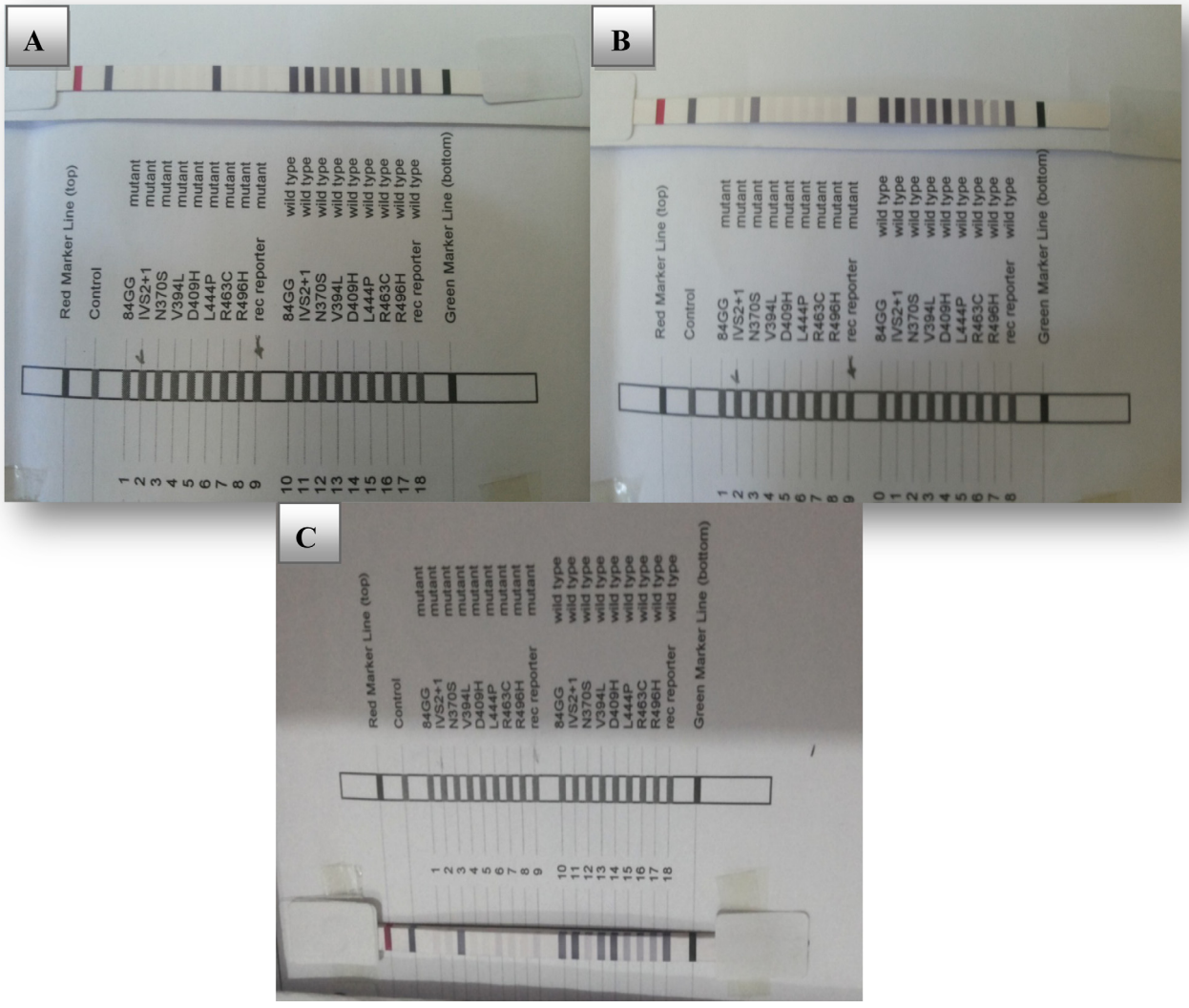

Figure 1 Mutation analysis strip assay showing; (A) L444P/L444P mutation; (B) N370S/rec NciI compound heterozygous mutation; (C) N370S/N370S mutation.

childhood and should undergo regular evaluation for central nervous system manifestations of GD. In agreement with these findings, a study done by Stirnemann et al. [18] on GD patients living in France concluded that type 1GD had the highest frequency followed by types 2 and 3, both have the least frequency. Also, a study done by El-Morsy et al. [8] on Egyptian pediatric patients with GD found that about 2/3 of patients have with type 1 GD and about 1/3 of patients have type 3 GD with no type 2 GD in their study. On the contrary, a study done by Khalifa et al. [15] on Egyptian patients with GD revealed that type 3 GD has the highest frequency followed by type1 while type2 has the lowest frequency. This could be explained by the fact that N370S homozygotes often have mild disease and may have existed undiagnosed in the catchment area of the Khalifa study.

Regarding the main clinical characteristics of GD patients involved in this study, hepatosplenomegaly, pallor and abdominal distension were among the most frequent encountered symptoms of GD, followed by musculoskeletal involvement in the form of chronic bone pain, bone crises, bone fractures, restricted mobility and/or radiological bone disease in the form of Erlenmeyer Flask deformity, kyphoscoliosis, pectus carinatum and avascular necrosis of femoral head. Recurrent chest infections and bleeding tendency (in the form of epistaxis, easy bruising, menorrhagia or prolonged bleeding after superficial wounds), were among the presenting manifestations .Neurological abnormalities of type 3 GD included in this study were squint, seizures and developmental delay with delayed both physical and mental development. In agreement with these findings what was found in a study done by El-Morsy et al. [8] on Egyptian pediatric patients with type1 and type3 GD.

In the present study, five different genotypes were detected among GD patients, homozygous for the mutation (L444P) has the highest frequency which was the only encountered mutation in all pediatric patients with the neuronopathic type 3 GD, followed by homozygous for the mutation (N370S) and heterozygous for the mutations (N370S and rec Ncil), which were the most frequent genotypes in type 1 GD, then,

heterozygous for the mutations (IVS2 + 1 and rec NciI), while the least frequent mutations were heterozygous for (L444P and IVS2 + 1).

In agreement with these findings: a study done by Elmonem et al. [19] on Egyptian pediatric patients with GD found that the most frequent genotype in these patients were homozygous for the mutation L444P, with lower frequency of N370S homozygous and N370S and IVS2 + 1 heterozygous and few of these patients were heterozygous for L444P and second alleles were not detected or non-mutant alleles were discovered. Another study done by El-Beshlawy et al. [14] on Egyptian children with GD revealed that the most frequent genotype of these patients were homozygous for the mutation L444P, with lower frequency of D409H homozygous mutation, compound heterozygous for L444Pand D409H, R359Q homozygous and compound heterozygous for N370S and rec allele.

Another study done by Khalifa et al. [15] showed that N370S/N370S was the most frequent genotype in type 1 GD patients, while, all of type 3 GD patients tested in their study were homozygous for the L444P mutation. Also another study done by Koprivica et al. [20] demonstrated that homozygosity for mutation L444P was present in large percent of patients with type 3 GD in their study. Another study done by Stein et al. [21] on patients with GD1 found that the most common genotype was N370S/N370S. Also, a study done by Giraldo et al. [22] on patients with GD in the Iberian Peninsula, N370S/L444P was the most frequent genotype in type 1 GD patients, followed by N370S/N370S, while the most frequent genotype in type 3 GD were L444P/L444P and D409H/D409H.

Regarding the association between genotype, age at diagnosis and clinical manifestations in the present study, the mean age at diagnosis was older than 5 years for the patients with N370S/N370S; while mean age at diagnosis was younger than 5 years for the remaining genotypes. Hepatosplenomegaly was present in all patients with L444P/L444P, IVS2 + 1/L444P and N370S/rec NciI genotypes, in 75% of patients with N370S/N370S and in 33.3% of patients with IVS2 + 1/rec NciI Musculoskeletal manifestations in the form of clinical and/or radiological bone involvement were present in all patients with at least one N370S allele and in 87.5% of patients homozygous for L444P. On the contrary, a study done by El-Morsy et al. [8] concluded that no significant association between GBA alleles or genotype frequencies and different phenotypes of GD in their study.

5. Conclusion

Non-neuronopathic type 1 and type 3 GD were the only clinical types found in the present study with homozygous N370S and N370S/ rec NciI mutations were the most frequent genotypes in type 1 GD patients, while homozygous L444P mutation was the only encountered genotype in patients with type 3 GD.

Recommendations

Additional more comprehensive large-scale studies to strongly correlate genotype and phenotype among pediatric patients with GD in Upper Egypt are recommended.

Conflict of interest

None.

Ethical approval

The Research Committee at Qena Faculty of Medicine, South Valley University approved this study (R. Nr. 10/02/015).

Funding

This research was funded by South Valley University – Qena faculty of Medicine – Qena – Egypt.

Acknowledgments

We would like to acknowledge the team work of the Metabolic and Genetic Disorders Unit – Faculty of Medicine – Assiut University, where the laboratory work of this study has been done. Special thanks to Prof. Abbas Mansour, the president of South Valley University and Prof. Hamdy M. Hussien, the Dean of Qena faculty of Medicine for their support and facilitating the funding process.

References

[1] Essabar L, Meskini T, Lamalmi N, Ettair S, Erreimi N, Mouane N. Gaucher's disease: report of 11 cases with review of literature. Pan Afr. Med. J. 2015;20:18. http://dx.doi.org/10.11604/pamj.2015.20.18.4112.

[2] Franco M, Collec E, Connes P, van den Akker E, de Villemeur TB, Belmatoug N, et al. Abnormal properties of red blood cells suggest a role in the pathophysiology of Gaucher disease. Blood 2013;121:546–55.

[3] Machaczka M, Markuszewska-Kuczyńska A, Regenthal S, Jurczyszyn A, Gałązka K, Wahlin BE, et al. Clinical utility of different bone marrow examination methods in the diagnosis of adults with sporadic Gaucher disease type 1. Pol Arch Med Wewn 2014;124(11):587–92.

[4] Sheth JJ, Ankleshwaria CM, Mistri MA, Nanavaty N, Mehta SJ. Splenomegaly, cardiomegaly, and osteoporosis in a child with gaucher disease. Case Rep. Pediatrics 2011;2011:564868. http://dx.doi.org/10.1155/2011/564868.

[5] Weinreb NJ, Goldblatt J, Villalobos J, Charrow J, Cole JA, Kerstenetzky M, vom Dahl S, Hollak C. Long-term clinical outcomes in type 1 Gaucher disease following 10 years of imiglucerase treatment. J. Inherited Metab. Dis. 2013;36(3):543–53.

[6] Burrow TA, Barnes S, Grabowski GA. Prevalence and management of Gaucher disease. Pediatric Health Med. Ther. 2011;2:59–73.

[7] Grabowski GA. Gaucher disease and other storage disorders. Hematol Am Soc Hematol Educ Program 2012;2012:13–8.

[8] El-Morsy Z, Khashaba MT, Soliman OES, Yahia S, El-Hady DA. Glucosidase acid beta gene mutations in Egyptian children with Gaucher disease and relation to disease phenotypes. World J. Pediatrics 2011;7(4):326–30.

[9] Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). Hum. Mutat. 2008;29:567–83.

[10] Thomas AS, Mehta AB, Hughes DA. Diagnosing Gaucher disease: an on-going need for increased awareness amongst haematologists. Blood Cells Mol. Dis. 2013;50(3):212–7.

[11] Giraldo P, Pérez-López J, Núñez R, de la Puebla RF, Luño E, Saura-Grau S, et al. Patients with type 1 Gaucher disease in Spain: a cross-sectional evaluation of health status. Blood Cells Mol. Dis. 2016;56(1):23–30.
[12] Tantawy AAG, Sherif EM, Adly AAM, Hassanine S, Awad AH. Evoked potentials and neurocognitive functions in pediatric Egyptian Gaucher patients on enzyme replacement therapy: a single center experience. J. Inherit. Metab. Dis. 2013;36 (6):1025–37.
[13] Shawky RM, El-Awady MY, Elsayed SM, Hamadan GE. Consanguineous matings among Egyptian population. Egypt. J. Med. Human Genet. 2011;12(2):157–63.
[14] El-Beshlawy A, Ragab L, Youssry I, Yakout K, El-Kiki H, Eid K, et al. Enzyme replacement therapy and bony changes in Egyptian paediatric Gaucher disease patients. J. Inherit. Metab. Dis. 2006;29:92–8.
[15] Khalifa A, Tantawy A, Shawky R, Monir E, Elsayed SM, Fateen E, et al. Outcome of enzyme replacement therapy in children with Gaucher disease: the Egyptian experience. Egypt J Med Hum Genet 2011;12(1):9–14.
[16] Elgawhary S, Ghaffar HA, Eid K, Wahab MA, Ragab WS, Saleh WF. Prenatal genotyping of Gaucher disease in Egypt. Egypt J Med Human Genet 2013;14(4):361–5.
[17] Kaplan P, Andersson HC, Kacena KA, Yee JD. The clinical and demographic characteristics of nonneuronopathic gaucher disease in 887 children at diagnosis. Arch. Pediatr. Adolesc. Med. 2006;160:603–8.
[18] Stirnemann J, Vigan M, Hamroun D, et al. The French Gaucher's disease registry: clinical characteristics, complications and treatment of 562 patients. Orphanet J Rare Dis 2012;7(1):77.
[19] Elmonem MA, Mahmoud IG, Mehaney DA, et al. Lysosomal storage disorders in Egyptian children. Indian J Pediatrics 2016;83 (8):805–13.
[20] Koprivica V, Stone DL, Park JK, et al. Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. Am J Human Genet 2000;66(6):1777–86, 2016.
[21] Stein P, Yu H, Jain D, Mistry PK. Hyperferritinemia and iron overload in type 1 Gaucher disease. Am. J. Hematol. 2010;85 (7):472–6.
[22] Giraldo P, Alfonso P, Irún P, et al. Mapping the genetic and clinical characteristics of Gaucher disease in the Iberian Peninsula. Orphanet J Rare Dis 2012;7(17):1–10.