Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.13, 2015

DNA Polymorphism of Gaucher Disease in Iraqi Patients

*Shayma`a J. Ahmed (B.Sc., M.Sc., Ph.D.Sc. in Biotechnology)
** Mohammad F. Ibraheem (M.B.CH.B., D.C.H., F.I.C.M., C.A.B.P)
*** Wissam Younis Al-temimi (M.B.CH.B., F.I.B.M.S)
*Iraq - University of Baghdad, College of Medicine, Professor in Department of Anatomy.
** Iraq - University of Baghdad, College of Medicine, Assistant professor in of Department Pediatric.
*** Iraq - Ibn Al Baladi Hospital.

Abstract:
Gaucher disease is the autosomally recessively inherited deficiency of the lysosomal enzyme glucocerebrosidase. Increasing storage of glucocerebrosides leads to a multisystem disease, the prevalence of which is about 1 : 40,000 in central Europe and up to 1 : 2,000 in some other countries (e. g. Israel). The acute and chronic neuronopathic forms of the disease (formerly defined as Gaucher types 2 and 3) account for only 5 to 10% of all Gaucher patients in Central Europe and Germany and are thus less frequent than the non-neuronopathic disease (formerly defined as Gaucher type 1). Gaucher's disease is usually associated with spleno- and hepatomegaly, fatigue, skeletal complications, and several corresponding hematological and laboratory abnormalities. In 5 to 10% of the patients there are also central nervous symptoms such as myoclonic seizures, oculomotoric apraxia and a slight mental retardation. Recent epidemiological data indicate that only 10 to 20% of all Gaucher patients are correctly diagnosed (and treated) in Germany. The diagnosis today can be done in all patients by noninvasive methods, i. e. determination of the glucocerebrosidase activity in peripheral leukocytes and of the genetic defect, in this research the PCR technique was used to analyze the mutation in glucocerebrosidases gene (G ---C).

Key words: Gaucher disease (GD), lysosomal storage disorder (LSD),lysosomal enzyme glucocerebrosidase, PCR, Mutation.

1- Introduction:

Gaucher disease (GD) is the most common pan-ethnic inherited lysosomal storage disorder (LSD) (Beutler & Grabowski , 2001). The accumulation of glucocerebroside in the lysosomes of macrophages results from inherited deficiency of lysosomal acid beta-glucosidase due to mutations in the GBA1 gene which is located on chromosome 1q21(Brady, 1966, Beutler & Grabowski , 2001),the disease usually seen in closed communities like in Ashkenazi Jews, Approximately 300 mutations, mostly autosomal recessive, have been described, the predominant point mutation is N370S. This mutation accounts for about75% of the mutant alleles in Jewishpatients and about 30% in non-Jewish patients. Approximately 300 mutations, mostly autosomal recessive, have been described(Koprivica *et al.* 2000, Horowitz , *et al.*(1998).
The L444P mutation is common in the Norrbottnian population, and the homozygous
state has a very high association with the neuronopathicvariants of GD(Jmoudiak & Futerman , 2005 Beutler *et al.* 1993).

There are three types of GD:

1- Type 1 (non-neuronopathic): constitutes 94% of all cases and is usually considered to be non-neuronopathic. The onset of the disease may occur at any age.
2- Type 2 (acute neuronopathic): the onset is in infancy (socalled neuronopathic infantile, cerebral, or perinatal lethal GD) and, accounts for 1% of all GD. It is characterised by a short life expectancy of 2-3 years or less due to severe neurological consequences related to the disease.
3- Type 3 (chronic neuronopathic): presents in early childhood and accounts for approximately 5% of all patients. The range of neurological involvement in this group is quite broad.

The non-neuronopathic is the most common type (93 %) in the Western world and is distinguished from the other types by the lack of primary central nervous system involvement (Grabowski *et al*. 2012,CMS 2015, Poll *et al.* 2000, Kallish & Kaplan 2013).

Gaucher disease is characterized by varying degrees of splenomegaly and hepatomegaly, anemia, thrombocytopenia, skeletal lesions, and bone pain. Types 2 and 3 are both rare, with acute and fulminating (type 2), or heterogeneous and attenuated (type 3) neurological involvement accompanying visceral manifestations(Goker-Alpan *et al*. 2003).
the chronic stimulation of the immune system by accumulated glucocerebroside, or by hyper secretion of cytokines by Gaucher cells may leads to malignant changes (e.g. multiple myeloma, lymphomas, chronic lymphatic leukemia) are not uncommon(Brautbar *et al*. 2004, Shiarn *et al*. 1993).

The most valuable and reliable method of diagnosis is the assay of glucocerebrosidase activity in peripheral blood leukocytes or cultured fibroblasts(Beutler *et al*. 1971).
GD became the first successfully managed lipid storage disease, the cornerstone of treatment for GD, is enzyme replacement therapy with a recombinant glucocerebrosidase known as imiglucerase (Cerezyme) which is given intravenously (Brady 2006).

The aim of this study is to determine the DNA Polymorphism of Gaucher Diseases in Iraqi Patient 6 month in Children welfare teaching hospital Baghdad- Iraq by using PCR technology.

2- Patients and Methods:

1- A retrospective study conducted on twenty patients admitted to the Gastroenterology and Hepatology unit in Children Welfare Teaching Hospital/ medical city complex / Baghdad, their ages were ranged between (2-21 years), during the period from the first of May 2013- first December 2013.
All patients were diagnosed with gaucher disease based upon clinical assessment (detailed history and thorough clinical examination), laboratory investigations(complete blood count (CBC), blood film and reticulocyte count, prothrombin time (PT) and partial thromboplastin time (PTT), liver function tests, renal function tests, , bone marrow examination (only done in 7 feasible patients), imaging studies included plain X-ray of long bones and chest, abdominal ultrasonography to evaluate liver and spleen volumes, echocardiography, and MRI of femurs for selected patients. And enzymatic evaluationby direct determination of (acid beta-glucosidase) activity in peripheral blood leucocytes through metabolic laboratory /Hamburg university medical center.

2- Genetic study: 2 ml of blood sample in EDTA tubes were taken from patients, DNA was isolated by using Genomic DNA Mini Kit,(Geneaid ,USA). And PCR was
AccuPower® ProFiTaq PCR PreMix was a suitable lyophilized PCR master mix.
The primer was 5`-ATCACCAAGCACACGTTTT-3` (Cormand *et al.,*1998)

Table -1- The Conditions of PCR reaction

No.	Steps	Temperature	Time	Cycles
1	Initial denaturation	95 °C	5 minutes	1
2	Denaturation	94 °C	15seconds	30
3	Annealing	60°C	45 seconds	30
4	Extension	72 °C	1minute	30
5	Final extension	72 °C	10 seconds	1

4- Results and Discussion:

Of the 20 patients included in this series 15(75%) were males and 5(25%) were females, and the commonest age of presentation for the disease was found between 2-5 years in 11 patients, and it accounts (55%) it was found that easy tiredness affect all the patients (100%) ,splenomegaly in 80%, hepatomegaly in 75%, abdominal pain& bleeding tendency in 70% for each..
While the skeletal manifestations presented as bone pain in 55%, as shown in table -2.

Table-2 Clinic-epidemiological manifestations of the study sample

Variables			Number	Percentage
Gender		Male	15	75%
		Female	5	25%
Consanguinity		Positive	13	65%
		Negative	7	35%
Age group(years)		1 year - 2 years	4	20%
		>2years- 5 years	11	55%
		>5years- 15 years	4	20%
		>20years	1	5%
Clinical presentations	Easy tiredness	Positive	20	100%
		Negative	0	0%
	Splenomegaly	Positive	16	80%
		Negative	3(+ 1 splenectomized)=4	20%
	Hepatomegaly	Positive	15	75%
		Negative	5	5%
	Bleeding tendency	Positive	14	70%
		Negative	6	30%
	Bone pain	Positive	11	55%
		Negative	9	45%

Regarding the clinical presentations, it was found that easy tiredness affect all the patients (100%) ,splenomegaly in 80%, hepatomegaly in 75%, bleeding tendency in 70% .
The skeletal manifestations presented as bone pain & short statures in 55%.The hematological situations of the patients shows dramatic increment of the platelets count after six months of treatment in 14(70%).With hemoglobin level increment in 13(65%) as shown in table-3.

Table-3Clinical and hematological outcomes of patients after six months of initiation the treatment

Indices		Number	Percentage
Clinical	Bleeding tendencyimprovement	20	100%
	Easy tiredness improvement	18	90%
	Splenic regression	17	85%
	Hepatic regression	15	75%
Hematological	Plateletscount increment	14	70%
	Hemoglobin level increment	13	65%

Although gaucher disease is the most common lysosomal storage disease all over the world but it still in Iraq is thought to be underestimated in part due to unavailability of diagnostic toolsas well as lack of awareness with low index of suspicion.
Out of 20 patients 15 of them were males (75%) and 5 were females (25%) with male to female ratio 3:1and this percentage similar to study done by Heitner(Heitner, 2004) in South Africa who revealed 3:1, by Nagral (Nagral *et al*, 2011) in India who showed 3.14:1 and in Albania (Shehi *et al*, 2011) who showed male to female ratio 1.75:1. But disagree with results obtained by Giraldoin Iberia (Spain& Portugal) (Giraldo *et al.*, 2012) their ratio was about 1:1, and this may be explained by different sample size taken.
It was found that consanguineous marriage present in 65% of cases which it was quite equal to study done in Egypt (Khalifa ,*et al*,2011) and 50% in India (Nagral *et al*., 2011) but this point not submit to great interest in other countries which may be due to uneventful percentage of consanguineous pedigrees.

It was found that the most common age group affected in this study is the first five years of life in three quarter of the cases which is closely related to result found in Egypt (Khalifa ,2011),71% in Japan (Eto& Ida ,1999), and in 48% in USA (Caplan *et al.*, 2006) and this may be explained by inclusion criteria of the study that involved only pediatrics age groups.

Among the clinical manifestations ;Splenomegaly was found in 80% of cases (one case was splenectomized 5%) and it is 90.9% (Lee *et al.,* 2012) in Korea and 95%in USA (Caplan *et al.*, 2006), while in Romania there was 40% with splenomegaly and 45% were splenectomized (Drugan *et al.,*2002).

The abdominal pain was present in 70% of cases while it is 81.8% in Albanian children (Shehi *et al*, 2011) and it is common presentation mostly due to big spleen with its brother some symptoms.

It was found that hepatomegaly present in 75% of patients while it is 87% in USA (Caplan et al., 2006)and 100% in Albania (Shehi *et al*, 2011) and it is 68% in Iberia (Spain& Portugal) (Giraldo *et al*.,2012).

The bleeding tendency(manifested by ecchymosis& epistaxis) was present in 70% of cases while it is 27.27% in Albania (Shehi *et al*, 2011) and this can be explained by lateness in the diagnosis and treatment of the disease until it reach advanced stage.

Bone pain manifested in 55% of patients, while in France45% of patients had skeletal symptoms (bone pain and / or bone crisis) and Korea (Lee *et al.*, 2012), and in Romania the chronic bone pain (65%)(Drugan *et al*., 2002).

The clear improvement of hematological indices(platelets count and hemoglobin percentage) were obtained after 6months of treatment and this result harmonized with other results in other countries (Weinreb et al 2002, Lee *et al* 2012, Nagral *et al* 2011).

The dramatic regressionachievement of hepatosplenomegaly wasagreed with other studies done in other countries (Shehi *et al*, 2011, Nagral *et al*., 2011)**.**

The DNA electrophoresis results were shown in the figure (1). In lane1 DNA marker (Lambda DNA\ EcoR+ Hind 111) which had 4 bands (21.226, 5.148, 4.268, 2.027bp), lane 2 was normal (control group) had no band compare with DNA marker, Lane3-9 were cases with Gaucher disease had one band (21.226) compare with DNA marker. The bands were in 7 of 20 patients this mean there is a mutation in glucocerebrosidases gene (G C).

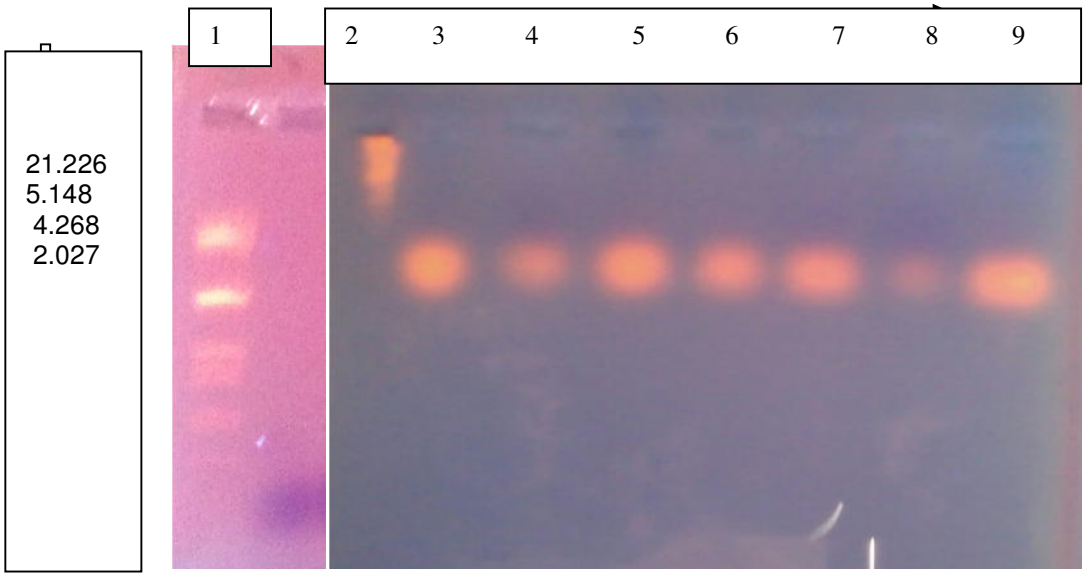

Figure (1)The DNA electrophoresis of PCR products

The DNA electrophoresis results were shown in the:

1- Lane-1- DNA marker (Lambda DNA\ EcoR+ Hind 111) which had 4 bands (21.226, 5.148, 4.268, 2.027bp).
2- Lane- 2- was normal (control group) had no band compare with DNA marker.
3- Lane3-9 were cases with Gaucher disease.

Acknowledgements

The authors thank all the members of the Gaucher Disease treatment team in the Children Welfare Teaching Hospital who worked diligently and dutifully, and also grateful to the patients and their families whose participation made this work possible. Finally, the authors are extremely grateful to Genzyme Corporation for their priceless kind assistance in reaching the diagnosis by sending blood samples to metabolic laboratory in Hamburg/Germany.

References:

1- Beutler E, Kuhl W, Trinidad F, *et al.*(1971) Beta-glucosidase activity in fibroblasts from homozygotes and heterozygotes for Gaucher's disease. Am J Hum Genet; 23(1):62-6.
2- Beutler E, Nguyen NJ, Henneberger MW, *et al.*(1993) Gaucher disease: gene frequencies in the Ashkenazi Jewish population. Am J Hum Genet.;52:85– 88.
3- Beutler E,& Grabowski GA.(2001) Gaucher disease. In: Scriver C, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York: McGraw-Hill:3635-3668.
4- Brady RO, Kanfer JN, Bradley RM & Shapiro D.(1966). Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gauchers disease. J Clin Invest.; 45(7): 1112–1115.
5- Brady RO.(2006) Enzyme replacement for lysosomal diseases. Ann Rev Med ; 57:283–296.
6- Brautbar A, Elstein D, Pines G, *et al.*(2004) Effect of enzyme replacement therapy on gammopathies in Gaucher disease. Blood Cells Mol Dis; 32(1):214-7.
7- CMScript Council for Medical Schemes. (2015), Issue 4 (http://www.medicalschemes.com/files/CMScript/CMScript4Of2015.pdf).
8- Cormand B.,Harboe TL,Gort L, *et al.*(1998). Mutation analysis of Gaucher disease patients from Argentina: high prevalence of the RecNciI mutation. Am J Med Genet. Dec 4;80(4):343-51.
9- Drugan C, Procopciuc L, Jebeleanu G*, et al.* (2002).Gaucher disease in Romanian patients: incidence of the most common mutations and phenotypic manifestations, European Journal of Human Genetics; 10: 511 – 515.
10- Eto Y.& Ida H (1999). Clinical and Molecular Characteristics of Japanese Gaucher Disease. Neurochemical Research; 24(2): 207-211.
11- GiraldoP., Alfonso P., Irún P., *et al*.(2012) Mapping the genetic and clinical characteristics of Gaucher disease in the Iberian Peninsula.Orphanet Journal of Rare Diseases; 7:17.
12- Goker-Alpan O, Schiffmann R, Park JK, *et al.*(2003) Phenotypic continuum inneuronopathic Gaucher disease: an intermediate phenol type between type 2 and type 3. J Pediatrics; 143:273-6.
13- Grabowski GA, Kolodny EH, WeinrebNJ,et al . Mistry PK (2012) Gaucherdisease: phenotypic and genetic variation, Chapter 146.1. Online Mendelian Inheritance in Man.At www.ncbi.nlm.nih.gov/Omim.
14- Horowitz M, Pasmanik-Chor M, Borochowitz Z, *et al.*(1998) Prevalence of glucocerebrosidase mutations in the Israeli Ashkenazi Jewish population. Hum Mutat;12:240–244.
15- Jmoudiak M, Futerman AH.(2005) Gaucher disease: pathological mechanisms and modern management. *Br J Haemtol.*;129:178–188.
16- Kallish S , Kaplan P(2013). A disease severity scoring system for children with type 1 Gaucher disease. Eur J Pediatr; 172:39–43.
17- Khalifa A S. ,TantawyAA. , ShawkyR M, *et al*(2011). Outcome of enzyme replacement therapy in children with Gaucher disease: The Egyptian experience. The Egyptian Journal of Medical Human Genetics. 12: 9–14.
18- Koprivica VV, Stone DL, Park JK ,*et al.*(2000)Analysis and classificationof 304 mutant alleles in patients with Type 1 and Type3 Gaucher disease. Am J Hum Genet;66:1777-86.
19- Lee,JY, Lee B H, Kim, GH *et al.*(2012)Clinical and genetic characteristics of Gaucher diseaseaccording to phenotypic subgroups.Korean J Pediatr .55(2):48-53.
20- Nagral A, Mewawalla P , Jagadeesh S *, et al*.(2011) Recombinant Macrophage Targeted Enzyme Replacement Therapy for Gaucher Disease in India, Indian pediatrics ;48:779-784.
21- Poll, L.W., Koch, J.-A. Dahl, S. V *et al.*(2000) Type I Gaucher disease: extraosseous extension of skeletal disease. Skeletal Radiol; 29:15–21.
22- Shehi B., BoçariG., VyshkaG(2011).Gaucher's Disease in Albanian Children: Casuistics and Treatment. Iran J Pediatr; 21(1):1-7.
23- Shiran A, Brenner B, Laor A, *et al.*(1993) Increased risk of cancer in patients with Gaucher disease. Cancer;72(1):219-24.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar