MUTATION ANALYSIS FOR SOME TYPES OF GLYCOGEN STORAGE DISEASES FOR PREVENTIVE STRATEGY

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ABSTRACT

Background: All GSDs are due to a failure to use or store glycogen. Many of the enzymes and transporters for these processes are key to the etiology of GSDs. An increasing number of GSDs are being identified, but some are very rare.

Aim and objectives: to diagnose molecularly some types of GSDs screened clinically by the basic symptoms and signs and confirmed by metabolic screen.

Patients and Methods: this prospective study was conducted at Department of of Pediatrics, Faculty of Medicine - Mansoura University children's hospital (MUCH) in period of (September,2021 -2023).

Results: The median age of initial diagnosis as awhole is around 90 days with statistically significant difference (p=0.017*) among subtype of GSDs being early in GSDII (10 days), late in GSDs III, XI (120,135 days respectively).

Conclusion: Molecular diagnosis in our study showed that, six patients are type Ia, three patients of them had Japanese mutation, [727G-T] two patients had [arg83-to-cys (R83C)] another one patient had [G6PC, IVS4, G-T, +86] mutation.

Key words: Glycogen Storage Diseases, Preventive Strategy, Molecular diagnosis.

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INTRODUCTION

Glycogen storage diseases (GSDs) are inherited inborn errors of metabolism (IEM) involving carbohydrate metabolism. IEMs are often caused by single gene mutations that encode specific proteins, these diseases may first manifest themselves in neonates or early childhood. GSDs, depending on the specific type, can result from a failure to convert glycogen into energy and/or a toxic glycogen accumulation. All GSDs are due to a failure to use or store glycogen **(1)**.

GSD type 0 is rare abnormality of glycogen metabolism **(2)**. Begin in infancy or in early childhood and may include drowsiness, fasting hypoglycemia associated with hyperketonemia, seizures, and other findings **(3)**. It is caused by a deficiency of the enzyme glycogen synthetase in the liver, due to mutations in the GYS2 gene. Treatment involves a specific diet that includes frequent meals with high protein intake during the day, and uncooked starch in the evening **(2)**. Another form of GSD 0 which chiefly affects the muscles and heart and is thought to be caused by mutations in the GYS1 gene **(4)**.

GSD type I, Glucose 6-Phosphatase Deficiency (von Gierke Disease) is the most common and one of the most severe GSDs, with over 80% of the cases attributed to a deficiency in the enzyme glucose-6-phosphatase α (G6Pase α; Type I a) **(5)**.

GSD type II, Acid α-Glucosidase (GAA) deficiency (Pompe Disease) results in an intra-lysosomal accumulation of glycogen, resulting in lysosomal dysfunction and destruction **(6)**.

GSD type III, Glycogen debranching enzyme deficiency (GDE) (Cori/Forbes Disease) has accumulation of abnormal glycogen with very short outer chains **(7)**.

GSD type V, Muscle glycogen phosphorylase deficiency (McArdle Disease) has two isoforms of glycogen phosphorylase encoded by two separate genes. Deficiency in the muscle isoform results in impaired glycogenolysis and leads to GSD type V **(8)**.

GSD type VI, Liver glycogen phosphorylase deficiency (Hers' Disease). The disease can start in early childhood but is significantly more benign. Individuals typically present with growth retardation and hepatomegaly. Ketotic hypoglycemia is common, as well as hyperlipidemia **(9).**

GSD type IX is a hereditary deficiency of glycogen phosphorylase kinase B that affects the liver and skeletal muscle tissue. It is inherited as an X-linked or autosomal recessive manner its diagnosis consists of Complete blood count, urine analysis, histological study of liver via biobsy ,genetic testing ,physical examination **(10)**.

GSD type XI (Fanconi Bickel syndrome) is caused by a mutation in the gene which encodes glucose-transporter protein 2 (GLUT2). Therefore, glucose builds up in the liver and kidneys and stored as glycogen and cause the symptoms **(11)**.

The aim of this prospective study was to diagnose molecularly some types of GSDs screened clinically by the basic symptoms and signs and confirmed by metabolic screen.

SUBJECTS AND METHODS

Twentyfour neoborns, infants and children suspected of having GSD referred to Genetic Unit, Gastroenterology Unit, NICU of Mansura university children's hospital (MUCH) in period of (September,2021 -2023).

Inclusion criteria: symptoms of GSD, hypoglycemia, hepatomegaly, doll like facies with or without cardiac affection.

Exclusion criteria: Other causes of hypoglycemia as endocrine causes, an infant of diabetic mother, hereditary fructose intolerance, Causes of hepatomegaly known to be due to Gaucher and Niemann-pick disease or others causes as viral hepatitis, other infection hepatitis and Myocardial dysfunction as a part of congenital heart disease, HOCM, IDM, myocarditis.

Method

All cases were subjected to:

Clinical evaluation (complete history taking and general examination), Routine laboratory tests and Two mL blood on EDTA were taken for DNA extraction and analysis and another 2 mL on heparine non-routine enzyme tesing.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocyte. Blood samples (1ml for each sample) were withdrawn from all patients on EDTA-prepared tubes and preserved in -20°C. The analysis was performed using the DNA Purification Capture Column Kit (Bioflux), Cat No. BSC06S1 **(12)**.

Agarose gel electrophoresis (12): In order to verify the presence of DNA in a good quantity, agarose gel electrophoresis technique using 0.8-1% agarose gel was used.

Solutions for gel electrophoresis: Tris Phosphate buffer 10 x (TPE 10 x), Loading solution and Ethidium bromide (0.5mg/ml).

Figure (1): Electrophoretic Tray for agarose gel electrophoresis.

Figure (2): Equipment for DNA analysis.

Polymerase chain reaction of the extracted DNA:

Analysis statistics: Cento Metabolic (sequencing including NGS-based CNV analysis) and Targeted nucleotides covered ≥ 20x 99.85%.

RESULTS

Table (1): Types of glycogen storage diseases among studied cases.

	Enzyme deficient	N=24	%
GSD Ia (Von Gierke disease)	Glucose 6 Phosphatase	6	25.0
GSD Ib	Glucose 6 phosphate Translocase	3	12.5
GSD II (Pompe)	Acid α Glucosidase	6	25
GSD III	Glycogen debranching enzyme	3	12.5
GSD IX	Glycogen phosphorylase kinaseB	2	8.3
GSD XI (Fanconi Biekel syndrome)	Glucose -transporter protein2	4	16.7

Distribution of types of GSDs among patients. The most common in our patients were GSD Ia, GSD II (pompe)**.**

Table (2): Molecular diagnosis (mutations) of included patients.

GSDs Type	Total number of cases	Total number of mutation type	Nomenclature of mutation	Number of patients having mutation (% of mutations in relation to same GSD type)
GSD Ia	6	3	Japanese mutation, 727G-T, G6PC, IVS4, G-T, +86 arg83-to-cys (R83C)	3 (50%) 1(16.7%) 2(33.3%)
GSD Ib	3	2	W118R mutation Homozygous 758G-A transition in exon 6 of the G6PC3 gene, resulting in an arg253	1 (33.3%) 2 (66.7%)
GSD II	6	2	Homozygous state c.1464dupC (p.(Asp489Argfs*17)) a single basepair substitution of G to A at position 271	1 (16.6%) 5(83.4%)
GSD III	3	1	Homozygous c.17_18delAG	3(100%)
GSD IX	2	1	C-to-T transition in exon 8, resulting in a gln1009-to-ter (Q1009X) substitution	2 (100%)

GSD XI (Fanconi Biekel)	4	2	SLC2A2 gene for GLUT2 protein synthesis 1 two bases (GA) deleted (Frameshift mutation) 2 Homozygous: c.253_254delGA / c.253_254delGA 2 Heterozygous c.253_254delGA/ Wild	4 patients with two bases (GA) deleted (Frameshift mutation) 2 Homozygous: (50%) c.253_254delGA / c.253_254delGA 2 Heterozygous (50%) c.253_254delGA/ Wild

Most of GSD Ia have Japanese mutation, 727G-T (three patients OF total six patients 50%), Most of GSD IB have homozygous 758G-A transition in exon 6 of the G6PC3 gene, resulting in an arg253(66.6%). Most of GSD II have a single basepair substitution of G to A at position 271(66.6). All patients of GSD III have homozygous c.17_18delAG mutation. All type GSD IX have C-to-T transition in exon 8, resulting in a gln1009-to-ter (Q1009X) substitution. All patients of GSD IX SlC2A2 gene for GLUT2 protein synthesis 1(50 % Homozygous: c.253_254delGA / c.253_254delGA and 50% heterozygous c.253_254delGA/ Wild).

Table (3): Relation between type of glycogen storage diseases and demographic characteristics of the studied cases.

	GSDIa	**GSD Ib**	**GSD II**	**GSD III**	**GSD IX**	**GSD XI Fanconi Biekel**	**test of significance**
Age of diagnosis (days)	90 (5-150)	90 (60-120)	10 (4-30)	120 (90-150)	60 (30-90)	135 (90-150)	KW=13.83 P=0.017*
Current age/Years	2.5 (1.5-6)	2.5 (1.5-3.0)	1.5 (0.5-2.0)	4 (0.5-7.0)	3.5 (1.0-6.0)	2 (1.5-3.5)	KW=4.18 P=0.524
Sex							
Male	3(50)	1(33.3)	3(50)	2(66.7)	0	3(75.0)	MC=3.67
Female	3(50)	2(66.7)	3(50)	1(33.3)	2(100)	1(25.0)	P=0.598
Residence							
Urban	1(16.7)	1(33.3)	0	0	0	2(50)	MC=6.0
Rural	5(83.3)	2(66.7)	6(100)	3(100)	2(100)	2(50)	P=0.306

This table showed that age, sex, residence of patients with statistically significant difference of age of diagnosis with early age of diagnosis of GSD type II and older age of diagnosis of GSD III and XI.

Table (4): Relations among GSDs types and anthropometric measurements of the studied cases.

	GSDIa	**GSD Ib**	**GSD II**	**GSD III**	**GSD IX**	**GSD XI (Fanconi Biekel)**	**test of sig.**
Weight centile							
≤5	1(16.7)	2(66.7)	3(50)	0	1(50)	2(50)	MC=33.8
5th-10th	3(50)	0	2(33.3)	1(33.3)	1(50)	1(25)	P=0.289
>10TH	2(33.4)	1(33.3)	1(16.7)	2(66.6)	0	1(25)	
Height centile							
≤5th	1(16.7)	0	0	0	0	3(75)	MC=43.28
5th-10th	0	3 (100)	1(16.7)	0	0	1(25)	P=0.06
>10th	0	0	5(83.4)	3(100)	2(100)	0	
Head circumference							
≤5TH	0	0	0	0	0	0	MC=20.30
<5TH-10TH	2(33.3)	0	0	0	0	2(50)	P=0.439
>10TH	4(66.7)	3(100)	6(100)	3(100)	2(100)	2(50)	
Anthropmetric measurements after six months							
Weight centile at six months							
≤5th	1(16.7)	2(66.7)	3(50)	0	1(50)	2(50)	MC=31.07
5th-10th	1(16.7)	1(33.3)	2(33. 3)	1(33.3)	1(50)	2(50)	P=0.412
>10th	4(66.6)	0	1(16.7)	2(66.6)	0(50)	0	

Height centile at six months **$\leq 5^{th}$** **5th-10th** **$>10^{th}$**	 1(16.7) 0 5(83.4)	 1(33.3) 2(66.6) 0	 0 4(66.7) 2(33.4)	 0 1(33.3) 2(66.6)	 0 3(100) 0	 4(100) 0 0	 MC=37.9 P=0.152
Head circumference centile at six months **$\leq 5^{th}$** **5th-10th** **$>10^{TH}$ centile**	 0 0 6(100)	 0 0 3(100)	 0 0 6(1100)	 0 0 3(100)	 0 0 2(100)	 0 2(50) 2(50)	MC=15.95 P=0.101

There was no statistical significance between patients according to weight, height /recumbent length, head circumference centiles at time of diagnosis and on follow up of patients after 6 months.

Table (5): Relations among GSDs types according to history, clinical manifestations of the studied cases.

	GSD Ia **(6)**	**GSD Ib** **(3)**	**GSD II** **(6)**	**GSD III** **(3)**	**GSD IX** **(2)**	**Fanconi Biekel syndrome GSDXI** **(4)**	**test of sig.**
Consanguinity	3(50)	2(66.7)	6(100)	3(100)	0	4(100)	MC=12.44 P=0.029*
Jaundice	1(16.7)	1(33.3)	0	0	0	0	MC=4.36 P=0.498
Hepatomegally	6 (100)	3(100)	5(83.3)	3(100)	2(100)	4(100)	MC=3.13 P=0.680
Neurologic symptoms and signs	5(83.3)	1(33.3)	5(83.3)	0	2(100)	4(100)	MC=12.71 P=0.026*
Hypotonia	4(66.7)	1(33.3)	6(100)	0	2(100)	4(100)	MC=14.32 P=0.014*
Respiratory manifestations	3(50)	0	6(100)	0	2(100)	4(100)	MC=17.60 P=0.003*
Cardiomyopathy	1(16.7)	0	6(100)	0	0	0	MC=19.97 P=0.001*
Rachitic manifestation	0	0	0	0	0	4(100)	MC=24 P<0.001*
Renal manifestations	0	0	0	0	0	4(100)	MC=24 P<0.001*

Consanguinity is significant, neurologic symptoms and signs (hypotonia) has significant results and respiratory manifestations are significant. Cardiomyopathy has significant results. Renal and rachitic manifestation is significant results in Fanconi Biekel.

Table (6) mortality among patients with GSDs.

Living patients	**Died patients**
20	4 (16.7%) Three Patients are Pompe (50% of Pompe disease patients) with median age of 7.5 months with cardiorespiratory failure and one patient is FanconiBiekel syndrome (25% of patients diagnosed FBS) with median age of 4 years with respiratory failure.

Outcome of patients with GSD with mortality 16.7% of total GSD patients mainly patients with Pompe disease as halve of patients with Pompe disease died.

DISCUSSION

The median age of initial diagnosis as awhole is around 90 days with statistically significant difference (p=0.017*) among subtype of GSDs being early in GSDII (10 days), late in GSDs III, XI (120,135 days respectively) which was reported by **(13)**. Median age of patients at time of this study is around two years.

Eighteen patients (75% of patien Weight is ≤5th centile in nine patients (37.5%) and eight patients have weight at 5 th -10th (33.4%) and seven patient have weight at>10th (29.2%) at time of examination ts) are outcome of conanginous marriage.

Height /recumbent length is ≤5th centile in four patients (16.7%), at 5 th -10th in five (20.8 %), at >10 in fifteen patients (62.6%).

Head circumference measurements are at 5th -10th centile in two patients (8.4%) and > 10 th in twentytwo patients (91.6%).

With follow up of anthropometric measurement after six months of initial assessment, the results showed that head circumference is between 5th -10th centile in two patients (8.3%) and more than 10th centile in 22 patients (91.66). Weight centile is less than 5th in eight patients (33.3%) and between 5th -10th centile in nine patients (27.5%) and more than 10th centile in seven patients (29.2%). Height is less than 5th centile in six patients (25%) and between 5th-10th centile in seven patients (29.1%) and more than 10th centile in eleven patients (45.83%). Patients with GSDs had stunted growth as agreed with GSDs which is agreed with **(9)**.

Molecular diagnosis was done in our study and showed six patients are type Ia, three patients (50%) had Japanese mutation [727G-T], two of them (33.3%) have [arg83-to-cys (R83C)], another patient (16.7%) has G6PC, [IVS4, G-T, +86].

Akanuma et al. **(14)** identified G6PC mutations in all alleles from 51 unrelated Japanese patients with GSD Ia. A total of seven mutations were identified, including three novel mutations. The most prevalent mutation, 727G-T, accounting for 88 of 102 mutant alleles, creates an aberrant 3-prime splice site within exon 5 and it isaccounting for most of our study patients which were diagnosed as GSD Ia. The authors demonstrated that ectopically transcribed G6Pase mRNA can be detected in lymphoblastoid cells and may be used for the characterization of mutations that affect mRNA splicing. They concluded that noninvasive molecular diagnosis may ultimately replace the conventional method of enzymatic diagnosis that requires liver biopsy in Japanese patients.

In all of 13 unrelated Korean patients with GSD Ia, Ki et al. **(15)** identified mutant alleles of the G6PC gene. Three known mutations and 2 novel mutations were identified. The most frequent mutant allele was 727G-T, present in 21 of 26 alleles (81%), which was slightly lower than that in Japanese, where it was present in 86 alleles (92%), but much higher than that in Taiwan Chinese (present in 44.4% of alleles).

As regard to results of current study which detected arg83-to-cys (R83C) in third of patients having GSD Ia (33.3%).Lei et al. **(16)** used SSCP analysis and DNA sequencing to characterize the G6PC gene of 70 unrelated patients with enzymatically confirmed diagnosis of type Ia GSD and detected mutations in all except 17 alleles (88%). They uncovered 16 mutations that were shown by expression to abolish or greatly reduce G6Pase activity and that, therefore, were responsible for the clinical disorder. R83C (613742.0002) and Q347X (613742.0004) were the most prevalent mutations found in Caucasians; 130X (613742.0001) and R83C were most prevalent in Hispanics; R83H was most prevalent in Chinese. The Q347X mutation was identified only in Caucasians, and the 130X mutation was identified only in Hispanic patients.

We concluded that, the most frequent mutant allele was 727G-T (50%) in Egyptian patients which were included in our study.

Kishnani et al. **(17)** performed genome wide SNP analysis and mutation detection of target genes in 10 GSD Ia-associated HCA and 7 general population HCA cases. Chromosomal aberrations were detected in 60% of the GSD Ia HCA and 57% of general population HCA. Coincident gain of chromosome 6p and loss of 6q were seen only in GSD Ia HCA (3 cases), with 1 additional GSD I patient showing submicroscopic 6q14.1 deletion. The sizes of GSD Ia adenomas with chromosome 6 aberrations were larger than the sizes of adenomas without the changes (p = 0.012). Expression of IGF2R (FCGR2A; 146790) and LATS1 (603473) candidate tumor suppressor genes at 6q was reduced in more than 50% of seven GSDIa HCA examined. None of the GSD Ia HCA had biallelic mutations in the HNF1A (142410) gene. The authors suggested that chromosome 6 alterations could be an early event in the liver tumorigenesis in GSD I, and possibly in the general population (**18**) .

In current study, three patients were diagnosed GSD type Ib, two of them had homozygous 758G-A transition in exon 6 of the G6PC3 gene, resulting in an arg253-to-his (R253H) (66.7%) and one of them had W118R mutation (33.3%).

Ihara, et al. **(19)** described W118R in one Japanese patient; the entire sequence of the human G6P translocase gene was determined by PCR-directed sequencing. The gene spans approximately 5 kb of genomic DNA and contains eight exons. Analysis of DNA from a Japanese patient with GSD type Ib revealed new compound heterozygous mutations; a T to C transition at cDNA position 521 resulting in W118R, and an A to C transversion at the –2 splicing acceptor site of intron 1. Reverse transcription (RT)-PCR from leukocyte RNA of the patient revealed the abnormally spliced transcript. These results further support the suggestion that the gene is causative for

GSD Ib and should be useful in the molecular diagnosis of such patients **(19)**.

In our study, Six patients with Pompe disease, five of them had a single basepair substitution of G to A at position 271 and one patient with pompe disease has homozygous state c.1464dupC (p.(Asp489Argfs*17))

As was demonstrated in our study, Patients with classic IPD present with symptoms shortly after birth and halve of patients with IPD died with cardiorespiratory failure which is the primary cause of mortality, There is a high mortality rate by one year of age if untreated typically which is agreed with, With the introduction of ERT, survival in patients with classic infantile Pompe disease has increased significantly **(17)**.

As regarding results of current study, there is other study which had similar results as only a single base-pair substitution of an A for a G at base-pair 271 was found, resulting in substitution of asparagine for aspartic acid at codon 91. This amino acid substitution is consistent with the more basic pI of the GAA 2 enzyme. The base-pair substitution also abolishes a Taq-I site, predicting the generation of a larger DNA fragment. This larger Taq-I fragment was also seen in two other individuals expressing the GAA 2 allozyme. A 5' fragment containing the base-pair substitution was ligated to the remaining 3' cDNA from a GAA1 allele and cloned into an expression vector, and the hybrid cDNA was transiently expressed in SV40-transformed GAA-deficient fibroblasts. The enzyme activity exhibited the altered mobility of the GAA 2 allozyme, as demonstrated by electrophoresis in starch gel **(20)**.

In our study, two patients GSD IX had C-to-T transition in exon 8, resulting in a gln1009-to-ter (Q1009X) substitution (100% of GSD IX).

In one study they identified four point mutations in four unrelated XLG I patients: three mutations introduce a premature stop codon, whereas the fourth mutation abolishes a splice site consensus sequence leading to exon skipping. These findings indicate that PHKA2 is the XLG I gene **(21)**.

CONCLUSION

Molecular diagnosis in our study showed that, six patients are type Ia, three patients of them had Japanese mutation, [727G-T] two patients had [arg83-to-cys (R83C)] another one patient had [G6PC, IVS4, G-T, +86] mutation. We also found three patients were GSD type Ib, two of them had homozygous 758G-A transition in exon 6 of the G6PC3 gene, resulting in an arg253-to-his (R253H) and one of them had W118R mutation.

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