CHARACTERIZATION OF HNF1-A GENE MUTATIONS IN EXON 5 IN A SAMPLE OF EGYPTIAN CHILDREN WITH MATURITY ONSET DIABETES OF THE YOUNG

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Abstract

Background: Maturity-onset diabetes of the young (MODY) is a group of inherited disorders of non-autoimmune diabetes mellitus which usually present in adolescence or young adulthood.

Patients and Methods: This cross-sectional study included 20 patients diagnosed with diabetes mellitus following up at Diabetes, Endocrine and Metabolism pediatric unit (DEMPU) Children hospital, Cairo University. The range of patients' age was 3 -18 years with mean diabetes duration of 1 year and 5 months. Genetic analysis of exon 5 of HNF1A gene was done using DNA sequencing method after a written consent was taken from the patient's guardian.

Results: The mean age of patients is 12.1 years with range of 3 -18 years, mean age at diagnosis 10.77 years with mean diabetes duration 1 year and 5 months and mean HBA1c is 9%. All participants have family history of diabetes in at least 3 generations most of them diagnosed before age of 35 years as well as negative auto antibody (Anti GAD and Anti islet), measurable c peptide with mean of 0.45 nmol/litre. All patients showed no mutation in Exon 5 of HNF1A.

Conclusion: Clinical suspicion of MODY 3 requires studying the whole gene for 10 exons and if negative, genetic testing for GCK, HNF1B, and HNF4A is highly recommended due to similarities in phenotype.

Keywords: Diabetes Mellites, MODY, MODY3, HNF1A, EXON 5

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1. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long- term damage, dysfunction, and failure of different organs through several pathogenic processes. Most affected organs are the eyes, kidneys, nerves, heart, and blood vessels (1).

Maturity-onset diabetes of the young (MODY) is a group of inherited disorders of non- autoimmune diabetes mellitus which usually present in adolescence or young adulthood, characterized by impaired insulin secretion, with minimal or no defects in insulin action (1).

MODY people account for 1–2% of all diabetic patients, they are often misdiagnosed as having type 1 or type 2 diabetes due to the presence of overlapping clinical symptoms (2).

MODY3 resulting from the HNF1A gene mutations are considered the most common form of monogenic diabetes with percentage of 30%- 60% of MODY cases (3).

HNF1A consists of ten exons, coding 631 amino acids and has three different isomers namely: isoform A (exons 8–10), isoform AB (exon 7) and isoform ABC (exons 1–6) formed by alternative splicing and polyadenylation (4).

HNF1A-MODY Diabetes mellitus appears generally at the age of 6–25 years with modest osmotic symptoms (polyuria, polydipsia) or as asymptomatic postprandial hyperglycemia without ketosis or ketoacidosis. C-peptide readings are lower than in healthy persons, but greater than for T1D, lack of pancreatic islet antibodies (5).

The HNF1A-MODY is treated differently according on the patient's age and HbA1c levels. Diets without excess saccharides may be temporary effective if HbA1c is less than 6.5% (DCCT). Sulfonylurea derivatives may be an effective therapy for high HbA1c levels (6).

Prior to changing the medication, DNA analysis is essential to validate the HNF1A-MODY. This group of individuals responds well to low doses of sulfonylureas, which are considered first-line therapy; in some instances, insulin will be required over time (1).

Aim of the work was to study the characterization of HFN1A mutations in Exon 5 in MODY3 in a sample of Egyptian children of pediatric and adolescent age group. Study the association between mutations in EXON 5 in patients with MODY 3 and clinical as well as laboratory parameters.

2. PATIENTS AND METHODS

This was a cross-sectional study that was conducted at the period between June 2019 and December 2021. Twenty patients (9 males and 11 females) diagnosed with diabetes mellitus were studied for the presence of genetic variations in Exon 5 of HNF1A gene. A retrospective analysis was done for the clinical, biochemical, phenotypical characteristics and outcomes in children with criteria eligible for MODY screening whom presented to the clinic at Diabetes, Endocrine and Metabolism Pediatric Unit (DEMPU), Cairo university children Hospital (CUCH), (Abo Elreesh Al-mounira). The inclusion and exclusion criteria are listed below, all the recruited patients fulfilled the screening criteria and an informed written consent was taken from the patient's guardians.

Inclusion criteria: Age 10-18 years, both sexes were included and patients suspected to have MODY3 as per the following criteria (12), Positive family history of diabetes in young age in 3 successive generations, negative Glutamic acid decarboxylase antibodies (GADA), Pancreatic islet-cell antibodies (ICA), low dose insulin (less than 0.5 U/kg/day) and measurable C-peptide in the presence of hyperglycemia (> 0.2nmol/L).

Exclusion criteria: Patients with secondary cause of diabetes, patients with history of diabetic ketoacidosis and patients with features suggestive of type 2 diabetes (obesity, acanthosis nigricans) were excluded.

All patients were subjected to. meticulous history taking & thorough clinical examination and anthropometric measurements.

Laboratory workup: Results of laboratory investigations that were collected from patients' records included the followings: C-peptide: fasting level in which all results were converted to nmol/L standard unit and measurable c-peptide was considered above 0.2nmol/l. Lipid profile including: serum Triglycerides normal value ranging from(37-148mg/dl), total cholesterol with desirable level below 200mg/dl, HDL-cholesterol (40-60mg/dl) and LDL-cholesterol optimal below 100 mg/dl. Glycated hemoglobin (HBA1c): the most recent one within 3 to 6 months. The target is < 7% (7). Glutamic acid decarboxylase antibodies (GADA), pancreatic islet-cell antibodies (ICA). Liver function: aspartate aminotransferase (AST) with normal value (0-31U/L), alanine transaminase (ALT) with normal value (0-31U/L). Kidney function: serum creatinine with normal value range (0.5- 1mg/dl)

Genetic analysis: Genetic analysis of exon 5 of HNF1A gene was done using DNA sequencing method according to the following steps:

Sample Collection and processing: Three milliliters venous blood was withdrawn in a sterile vacutainer

containing Ethylene Diamine Tetra Acetate "EDTA". They were either processed fresh or were stored at -20 ºC and were subjected to the following: DNA extraction and measurement of DNA concentration, DNA amplification by Polymerase Chain Reaction (PCR) and amplified product detection, DNA purification and purified product detection, DNA cycle sequencing, second Cleaning, long-Read Capillary Electrophoresis **Figure (1).** and data analysis and interpretation.

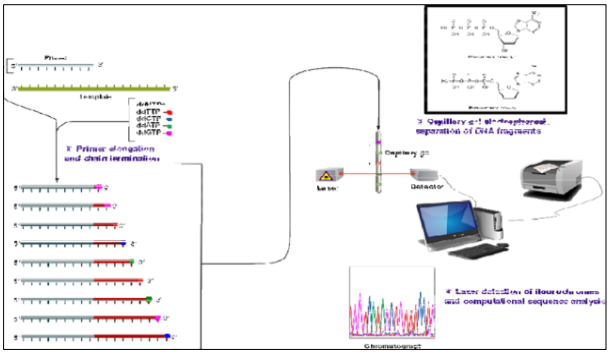

Figure (1): Fluorescent sequencing process

STATISTICAL ANALYSIS:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non- parametric Mann-Whitney test (8).

3. RESULTS

This cross-sectional study included 20 patients diagnosed with diabetes mellitus following up at Diabetes, Endocrine and Metabolism pediatric unit (DEMPU) in Cairo University Children Hospital (CUCH). The presence of genetic variations in Exon 5 of HNF1A gene using DNA sequencing methods.

Table (1): Demographic and diabetes mellitus clinical data of the studied group (n=20)

	Mean ±Standard Deviation	**Median**	**Range**
Age (years)	12.16 ±3.58		(3.-18)
Age at diagnosis (years)	10.76 ±3.10		(3-15.6)
Diabetes mellitus duration (months)*		10.5	
Frequency and percentage**			
	Count (n=20)		**Percentage**
Sex **- Male**	9		45%
-Female	11		55%
Consanguinity **- Yes**	3		15%
-No	17		85%
Family history (in 3 generation) **-Yes**	20		100%
Presenting symptoms **-Polyuria**	15		75%
-Polydipsia	13		65%
-Weight loss	5		25%
-DKA	2		10%
-Accidental	2		10%
Hospital admission **-Yes**	3		15%
Current treatment **-No**	4		20%
-Insulin	13		65%
	Mean ±SD	**Median**	**Range**

		0.56*		
-Oral hypoglycemic drugs	1			5%
-Both	2			10%

Data were represented by Mean ±Standard Deviation and range
* Data were represented by median **Data were presented by frequency

All included patient(n=20) had positive family history of DM in 3 successive generations with different presentations but polyuria constituted most common presenting symptom. Most of patients were on insulin (65%) and (5%) where on OHG as shown in **table (1)**

Table (2): Glycemic indices among recruited patient

	Mean± Standard Deviation	**Median**	**Range**
c peptide (nmol/L) *		0.43**	0.02-1.1
HBA1c	9±2.21		5.5-12
Frequency and percentage**			
	Count (n=20)	**Percentage**	
HbA1c below 7.5%	6	30%	
HbA1c above 7.5%	14	70%	
Anti-GAD(GAD)			
-Negative	20	100%	
Anti-Islet cell (ICA)			
- Negative	20	100%	

Data were represented by Mean± Standard Deviation and range
*Data were presented by median
**Data is presented by frequency and percentage

HbA1c among recruited patient range from (5.5-12%) with mean of 9% ±2.21. value of 0.45nmol/L as well as all have negative auto-antibodies (Anti GAD and Anti Islet antibodies) as shown at **Table (2).**

Table (3): Relation between c-peptide and age of presentation and age of diagnosis

		Age at diagnosis years
c peptide (nmol/l)	**Correlation Coefficient**	0.486
	P value	0.030
	N	20

*p value <0.05 is considered highly significant

There was positive correlation with age of diagnosis and c-peptide with p value (0.03) and correlation coefficient 0.486 as shown at **Table (3).**

Table (4): Mutational analysis for Exon 5 at HNF1A

variant	**Percentage**
Exon 5 at HNFA1 gene mutational analysis	
- Non- Mutant	100%

Patients fulfilled eligible criteria to be screened for HNF1A- MODY (MODY 3) specifically EXON 5 mutational analysis showed no mutation (100%) as shown at **Table (4).**

4. DISCUSSION

This cross-sectional study included 20 patients diagnosed with diabetes mullites following up at Diabetes, Endocrine and Metabolism pediatric unit (DEMPU) Cairo university; Eleven females (55%) 3 of them were non-pubertal, 3 started puberty (stage 3) and 5 are pubertal, Nine males (45%) 3 of them were non-pubertal,3 started puberty (stage 4) and 3 were pubertal..

Valkovicova et al., (5) concluded that Diabetes mellitus appears generally at the age of 6–25 years with modest osmotic symptoms (polyuria, polydipsia) or as asymptomatic postprandial

hyperglycemia without ketosis or ketoacidosis suspected to have MODY3.

Similarly, this study showed that Polyuria constituting the main presenting symptom (75%) together with polydipsia (65%) then weight loss (25%).

Lebenthal et al., (9) noticed that DKA can be a presenting symptom for MODY diabetes although it was previously one of the exclusion criteria and accordingly two patients (10%) were diagnosed by DKA included in this study.

Trhanint et al., (10) conducted a study on 20 Moroccan patients of average age of 19 years whereas the mean age at diagnosis was 17.2 years (range: 11 months–31 years old) with a slight predominance of males (60%).

In contrast to our study where mean age of patients is 12.1 years, mean age of presentation is 10.4 years, mean age of diagnosis 10.7 years with mean diabetes duration 17.7 months (1 year and 5 months) with female predominance (55%).

The later included subjects with hyperglycemia detected before 25 years of age, positive family history of diabetes in at least two generations, and absence of pancreatic auto-antibodies. The mean HbA1c was 8.9% (range, 5.5–14%). The average BMI was 21.24kg/M^2 (range,13–30kg/M^2). The treatment included OHA (oral hypo glycemic agents) alone in one patient (5%), and OHA associated with insulin therapy in 19 subjects (95%).

In this study, genetic analysis of exon 5 of HNF1A gene was normal for all 20 cases included on the study with no genetic variants detected. This result was in agreement with the results done by **Karaca et al., (11)** on 136 unrelated Turkish patients with MODY3; where exon 5 was normal when analyzed with all 10 exons of HNF1 gene. Also, in a study done in Iran by **Moghbeli et al., (13)** where they screened the coding and promoter regions of HNF1A gene for mutations in 34 unrelated Iranian MODY patients. They detected a new missense variant in exon 1 but normal exon 5. Also, in **Iran Mohammadi et al., (13)** revealed normal exon 5 on screening all exons of HNF1A for variant detection. Also, in Hungarian population, a study done by **Gaál et al., (15)** revealed normal exon 5 among the whole HNF1A gene analysis on a study done on 450 patients.

On contrast to the results of this study**, Fathy et al., (16)** revealed 9 variants in exon 5 on 4 patients with MODY3 out of 8 patients included for HNF1A genetic analysis in Egypt; three females and five males aged 13.6 years ± 6.18, and their age at diagnosis was 5.5 years ±3.4, HbA1c ≥ was 10.4% ±2.9, BMI was 26.26± 5.79.

The largest study on the mutational and clinical analysis of suspected MODY patients among Asian population done by **Yorifuji et al., (17)** which included 263 patients where mutations were detected in 101(39%) of the patients only, came with a conclusion that GCK mutations were the most common (55.3%) followed by HNF1A (28.2%), HNF1B (9.7%), and HNF4A (6.8%).

5. CONCLUSION

We concluded that for a better outcome and better understanding about clinical phenotype and genotype among Egyptian pediatric diabetic population with clinical suspicion of MODY3; the whole HNF1A gene with its ten (10) forming exons should be screened simultaneously specially in this new field of research in pediatric Diabetes Mellitus.

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