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Screening for Frequency of Occurrence of Mutations of Exon 28 of ABCC8 Gene in Egyptian Patients with Congenital Hyperinsulinism

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ABSTRACT

Background: Congenital hyperinsulinism (CHI) is a severe inherited form of hyperinsulinemic hypoglycemia (HH). It can occur as a result of several gene mutations. The most prevalent are mutations of the ABCC8 gene, which codes for the sulphonylurea receptor 1 subunit (SUR-1) of the potassium-sensitive ATP channels located on the pancreatic B-cells.

Aim of the study: to identify mutations of exon 28 of the ABCC8 gene in patients with CHI.

Patients and Methods: Thirteen patients diagnosed with CHI, aged from 1 day to 18 years, following up in the Diabetes, Endocrine and Metabolism Pediatric Unit, Abo ElReesh Children's Hospital, Cairo University were recruited. Clinical and biochemical data were collected through history taking, physical examination, and revising patients' medical records. Genetic analysis of exon 28 of the ABCC8 gene was done using DNA sequencing.

Results: The results of the DNA sequencing of exon 28 of the ABCC8 gene and its intronic boundaries detected no abnormalities in the study group except for one case which revealed an intronic homozygous variant on intron 28 (rs1954399854) of uncertain significance. **Conclusion:** Mutations of the ABCC8 gene account for around 40-50% of CHI cases. To our knowledge, there are no sufficient studies in the Egyptian population to detect mutations of the ABCC8 gene, which necessitated conducting this study. Exon 28 of the ABCC8 gene was the only exon tested due to limited resources and self-funding. This did not reveal significant mutations. Further research is warranted to detect other ABCC8 gene mutations in the Egyptian population.

Keywords: CHI, Octreotide, Exon 28 ABCC8 gene.

INTRODUCTION

Persistent hypoglycemia is a serious illness leading to lifelong neurological sequelae. The most frequent cause of persistent hypoglycemia is hyperinsulinemic hypoglycemia (HH). Congenital hyperinsulinism (CHI) is the most severe form of HH[(1)]. CHI is a group of heterogeneous diseases characterized by frequent attacks of hypoglycemia due to inappropriate insulin production from the B-cells of the pancreas. This group of disorders can result in serious neurological sequelae in pediatric patients which necessitates prompt diagnosis and management[(2)].

CHI has an estimated incidence of 1/50,000 newly-born and reaches 1/2500 in areas with elevated rates of consanguineous marriage[(3,4)]. Dysregulation of insulin production is due to mutations in 12 distinctive genes (ABCC8, KCNJ11, PMM2, GLUD1, PGM1, GCK, HK1, HADH, SLC16A1, HNF1A, UCP2, and HNF4A) [(5)]. Recently, mutations in two novel genes forkhead box A2 transcription factor (FOXA2) and calcium voltage-gated channel subunit alpha1 D (CACNA1D) and genes have been associated with CHI[(6)].

Around 40-50% of CHI cases were accounted for by more than 200 different mutations in ABCC8 (encoding for the SUR1 subunit of the K-ATP channel) versus 30 distinct KCNJ11 mutations (encoding for the Kir6.2 subunit of the K-ATP channel)[(7)], 5-10% of the cases were accounted for by other genes. While the remaining 40% remain unaccounted for[(8)].

The main clinical presentation of CHI is hypoglycemia. This hypoglycemia may not be symptomatic (detected as a finding during glucose checking) but it may also lead to life-endangering apnea, status epilepticus, or coma[(3)]. In older patients, the clinical signs may be less prominent, but they are typically severe during the first month of life. The disease severity may even differ within the same family[(9)]. Therefore, early detection and prompt management are necessary to avoid brain insult[(10)].

In the case of CHI with mutations inherited paternally, especially in the ABCC8 gene, they have a higher probability of focal lesions occurring in the pancreas. Recently, the diagnosis of these patients has been easier due to recent improvements in molecular genetic analysis and 18-fluro L-dihydroxy phenylalanine (18F-DOPA) positron emission tomography (PET CT) imaging[(11,12)].

The first line of treatment is diazoxide, which reverses the effects of glucose-induced channel closure by activating intact K-ATP channels. Nevertheless, it is ineffective in treating patients with focal CHI and diffuse CHI brought on by recessive inactivation mutations in ABCC8 and KCNJ11[(10)]. A second line therapy is octreotide which is a long-acting somatostatin analogue that hinders insulin production[(13)]. Surgery is used in the management of patients presenting with focal lesions in the pancreas[(14)] and when medical treatment fails to control hypoglycemia[(15,16)].

To our knowledge, there is no sufficient research conducted to detect mutations of the ABCC8 gene, which accounts for around half of the cases of congenital hyperinsulinism (CHI). This study aimed to identify mutations in the exon 28 ABCC8 and its intronic boundaries.

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PATIENTS AND METHODS

The current cross-sectional study took place in the Diabetes, Endocrine, and Metabolism Pediatric Unit (DEMPU) at Abo ElReesh Children's Hospital, Cairo University during the period from January 2021 and January 2022. Thirteen patients diagnosed with congenital hyperinsulinism (CHI) aged between 1 day and 18 years were included.

The following criteria were fulfilled to diagnose CHI: Persistent, recurrent hypoglycemia occurring during an intravenous glucose infusion rate (GIR) of more than 8 mg/kg/min; (2) presence of measurable insulin levels (any detectable value); (3) raised C-peptide ≥ 0.5 ng/ml and (4) lowered ß-hydroxybutyrate levels in serum <1.8 mM. Blood glucose levels: ≤2.8 mmol/L (≤50 mg/dL) in the first 48 hours of life, and ≤3.3 mmol/L (≤60 mg/dL) thereafter were used according to the Pediatric Endocrine Society[17].

Patients with the following conditions were excluded: perinatal stress (intrauterine growth restriction, birth asphyxia/ischemia, hypothermia, maternal preeclampsia/eclampsia of hypertension, polycythemia, meconium aspiration syndrome, and erythroblastosis fetalis), other endocrinal causes of hypoglycemia like hypopituitarism, hypothyroidism or adrenal insufficiency, patients with syndromes affecting development such as Beckwith-Wiedemann, Kabuki or Sotos syndrome, patients with metabolic disorder, infants of a diabetic mother, and critically ill patients.

Ethical approval

The study received approval from the Institutional Review Board of Cairo University and was carried out in accordance with the Helsinki Declaration. After the explanation of the study methodology to the patients and their parents or legal guardians, informed consent and/or assent was attained.

The following data were gathered from the participants' medical records, and by conducting interviews, followed by clinical examination of patients, and collecting blood samples:

- Demographic data were collected by reviewing medical records and conducting interviews with the families: age, age at presentation of hypoglycemia, administration of medications during pregnancy, perinatal risk factors, gestational age, birth weight, mental and motor development, current manifestations of hypoglycemia, details of therapy including type and dose, family history of low plasma glucose or diabetes, unexplained infant deaths, seizures or sudden infant death syndrome (SIDS).
- A thorough clinical examination of patients was performed to exclude other causes of hypoglycemia. Anthropometric data were collected including (weight and weight SDS, length and length SDS [below 3 years of age] height and height SDS [above 3 years of age], body mass index, and body mass index SDS) and the values were plotted on Egyptian growth curves.
- Radiological data were obtained from the patients' medical records: findings detected using either abdominal ultrasound or CT scan were used.
- Laboratory investigations: results of the following blood work performed during episodes of hypoglycemia were retrieved from the patient's medical records including levels of plasma glucose, B-hydroxybutyrate, ammonia, lactate, pyruvate, blood gases, liver function tests plasma insulin, C-peptide, cortisol, ACTH, thyroid profile, growth hormone, serum electrolytes (for calculation of the anion gap), and toxicology studies (salicylate, ethanol, sulfonylurea) if suspected.
- Urine samples were collected in clean bottles and were analysed immediately during the attack of hypoglycemia to detect ketones using urine analysis reagent strips.
- Blood samples were collected to be screened for mutations of exon 28 of the ABCC8 gene using the DNA sequencing method.

Analysis of genetic material:

Three milliliters of venous blood were withdrawn in a sterile vacutainer comprising ethylene diamine tetra acetate "EDTA". They were either processed fresh or were stored at -20ºC and the following was done: extraction of DNA, measurement of DNA concentration, amplification of DNA by polymerase chain reaction (PCR), detection of amplified product, purification of DNA and detection of purified product, DNA cycle sequencing, second cleaning, long-read capillary electrophoresis, and data analysis and interpretation.

- **DNA extraction** was done using the DNA extraction kit QIAamp DNA Blood Mini Kit (Catalog no. 51104QIAGEN®) (Hilden, Germany)[18].

- **Measurement of DNA concentration**:
  The quantity of DNA was performed by Qubit™ dsDNA HS assay Kit (Catalog no. Q32852) using Qubit™ 2.0 Fluorometer supplied by Invitrogen™ life Technologies[19].

- **Enzymatic amplification** was performed by PCR using HotStarTaq® MasterMix Kit (250 units) Catalog no. 203443 supplied by QIAGEN® and BioradT100™ thermal cycler. PCR could intensify a specific segment of DNA in vitro utilizing two site-specific primers that hybridize into opposite strands of DNA (Table 1). PCR was performed in three distinct steps; denaturation step, annealing step, and extension step, which are repeated multiple times[20].

Table (1): Forward and reverse primers sequence for ABCC8 (exon 28)[21]

	Forward primer (5' to 3')	Reverse primer (5' to 3')
ABCC8 exon 28	AGTCTGGGCAACAGTGAGAC	TAGGGCGGTGGAATAAGATG

- **Detection of amplified product:** in agarose gel using ultra-violet transillumination.
- Then **purification of amplified product** was done using Pure DireX PCR Clean-Up and Gel Extraction Kit
- **DNA cycle sequencing** followed using Frederick Sanger's enzymatic dideoxy technique of DNA sequencing based on the chain-terminating dideoxynucleotide analogues[22].
- **Second cleaning** (Dye terminator removal):
- Using BigDye® XTerminator™ purification kit supplied by Applied Biosystems.

- **Long-read capillary electrophoresis:**
  - The extension products were divided by size based on their total charge.
  - The size-divided fluorescently tagged DNA fragments traveled across a laser beam's path just before they reached the positive electrode. The dyes on the pieces glowed when exposed to the laser beam. The fluorescence was picked up by an optical detecting device on Applied Biosystems genetic analyzers.
  - The data were saved in a *.ab1 file once the data collection software digitized the fluorescence signal.
  - Analysis of coding regions as well as flanking intronic regions of exon 28 of the ABCC8 gene (NG_008867.1) was done with variants map position on the latest assembly: GRCh38.7 sequence or ABCC8 Refseq Gene (LRG-790).
  - Analysis was done by BLAST [Basic Local Alignment Search Tool] (www.ncbi.nlm.nih.gov). The nomenclature was according to the Human Genome Variation Society (HGVS)[23].

Statistical Analysis

Microsoft Access was used to enter the data. To facilitate data manipulation, data were gathered and coded. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 22 on Windows 7 (SPSS Inc., Chicago, IL, USA). Simple descriptive analysis in the form of percentages and numbers was applied to qualitative data. Mean and standard deviation (SD) or standard deviation score (SDS) or median and interquartile range (IQR) were used to represent the quantitative data.

RESULTS

Characteristics of the study group:

The current cross-sectional study included 13 patients, 8 (61.5%) males, and 5 (38.5%) females, diagnosed with CHI–who were followed up at Diabetes, Endocrine and Metabolism Pediatric Unit (DEMPU), Abo ElReesh Children's Hospital. The participants were recruited over one year.

The characteristics of the study group are demonstrated in table (2) and figure (1). The age of the subjects ranged between (17 days and 38 months) with a mean ± SDS of (13.9 ± 13.3) months old. The anthropometric measurements of the study patients at the time of recruitment were as follows: (1) 11 of them (84.6%) having normal weight SDS. (2) 9 patients (69.2%) having normal height SDS, 3 patients were <-2 SDS for height and one was > +2 SDS for height.

Table (2): Demographic data of the study group

Variables	Mean ± SDS	Range
Birth weight (kg)	3.2 ± 0.81	1-4
Weight (kg)	8.4 ± 7.1	2.4-15
Weight SDS	0.35 ± 0.29	0 - 0.7
Height/length (cm)	69.3 ± 14.6	43-87
Height/length SDS	1.1 ± 1.4	0.10 - 2.7
BMI (kg/m^2)	16.3 ± 2.8	9.7-20.9
BMI SDS	1.4 ± 1.2	0.1 - 2.9

SDS: Standard deviation score

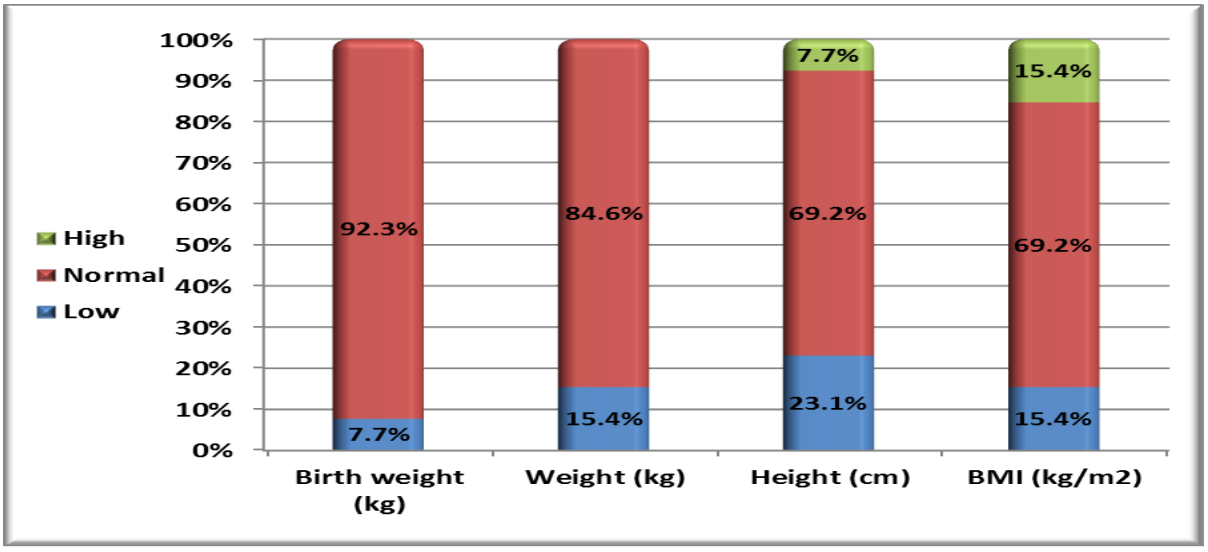

Figure (1): Frequency of anthropometric measures standardized score in our study group.

Around 61.5% of the study population were the product of consanguineous marriage. Most of the study group (84.6%) were delivered at full term and 69.2% were delivered by cesarean section. Ninety- two percent of the recruited subjects had normal birth weight. Only one patient had a history of unexplained infant death in the family. None of the patients were infants of diabetic mothers. Regarding the age of presentation, 8 out of 13 patients presented during the first week of life. The presenting symptoms were mainly neurological in the form of seizures. Neuro-development assessment of the study group revealed that 46.2% had delayed motor development. None of the cases had defective hearing (Table 3).

In our study, nine patients (69.2%) were treated using octreotide {3 of them (33.3%) responded well to treatment while 6 patients (66.7%) failed to achieve normoglycemia in spite of reaching maximum therapeutic dose}, one patient (7.7%) used diazoxide, achieved normal blood glucose level complicated with mild hypertrichosis but no further side effects were observed, one patient (7.7%) received both octreotide and diazoxide for treatment, and two patients (15.4%) did not receive any treatment {parents refused medical or surgical management against medical advice}. None of the patients had pancreatectomy done (Table 3).

Table (3): Demographic data, disease characteristics, and neuro-developmental evaluation in the study group

	Variables (n=13)	**Frequency**	
		Number	**%**
Consanguinity	Negative	5	38.50%
	Positive	8	61.50%
Family history	Negative	12	92.30%
	Positive	1	7.70%
Gestation	Preterm	2	15.40%
	Full term	11	84.60%
Mode of delivery	Vaginal	4	30.80%
	Cesarean section	9	69.20%
An infant for a diabetic mother	No	13	100%
	Yes	0	0%
Pancreatectomy	No	13	100%
	Yes	0	0%
Age of presentation	Less than 1 week	8	61.50%
	Less than 6 months	2	15.40%
	More than 6 months	3	23.10%
	Median /IQR (days)	3/ 7.5	
Presenting symptoms	Seizures	11	84.60%
	Apnea	2	15.40%
	Lethargy	8	61.50%
	Poor feeding	5	38.50%
Type of treatment	No treatment	2	15.40%
	Sandostatin	9	69.20%
	Diazoxide	1	7.70%
	Sandostatin and diazoxide	1	7.70%
Motor development	Abnormal	6	46.20%
	Normal	7	53.80%
Mental development	Abnormal	1	7.70%
	Normal	12	92.30%
Speech	Delayed	5	38.50%
	Normal	8	61.50%
Hearing	Abnormal	0	0%
	Normal	13	100%
Vision	Abnormal	1	7.70%
	Normal	12	92.30%

Data are represented as frequency and percentage, and median /IQR. IQR: Interquartile range.

Investigations done during the attack of hypoglycemia to diagnose CHI:

During the episodes of hypoglycemia, all the participants exhibited detectable to high levels of serum insulin and C-peptide. Growth hormone and cortisol responses to spontaneous hypoglycemia were variable. All of the patients had normal TSH and normal free T4 levels except for one patient who exhibited low levels of serum TSH (0.5 uIU/mL) and free T4 (0.7 ng/dL). Similarly, all patients had blood ammonia and lactate levels within the normal range except for one case that showed high levels of serum ammonia and lactate. Ketone bodies could not be detected in urine during hypoglycemia in the recruited patients.

Participants had normal kidney function tests (KFTs), liver function tests (LFTs), blood gases, and electrolytes (sodium and potassium) (Table 4). None of them had abnormal radiological findings on abdominal ultrasonography, computed tomography of the abdomen, and MRI of the abdomen.

Table (4): Laboratory investigations of the study group during the attacks of hypoglycemia

Variables	Mean ±SD, Median/IQR	Reference Range
Glucose profile		
Blood glucose (mg/dL)	28.9 ± 4.5*	20 - 47
Serum Insulin (uIU/mL)	16.4 /18.4**	3.6 - 42.3
C-peptide (ng/mL)	4 / 3.8**	1 – 8.2
Growth hormone (ng/mL)	6.9 / 20.4**	0.28 - 41
Hormonal profile		
Serum TSH (uIU/mL)	2.56 / 2.9**	0.40 - 11.2
Serum T4 (ng/dL)	0.92 ± 0.28*	0.7 – 2
Serum cortisol (ug/dL)	22 / 26.5**	0.80 - 40
Plasma ACTH (pg/mL)	26.3 ± 3.5*	8.1 - 41.3
Serum electrolytes		
Sodium (mmol/L)	137.8 ± 2.03*	135 - 142
Potassium (mmol/L)	4.08 ± 0.39*	3.6 – 5
Liver and kidney function tests		
Serum ammonia (pg/dL)	123 / 97.6**	41.7 - 364
Serum lactate (mg/dL)	10.4 / 8.2**	3.3 – 45.8
Creatinine (mg/dl)	0.52 ± 0.13*	0.40 - 0.70
BUN (mg/dl)	8.5 / 3.7**	3.7 - 17.7
ALT(U/l)	23.1 ± 4.5*	11 - 36
AST(U/l)	31.7 ± 5.3*	18 - 49
Acid-base balance test		
pH	7.4 ± 0.05*	7.31 - 7.5
PCO_2 (mmHg)	36.7 ± 4.8*	27.1- 44.3
HCO_3 (mmol/L)	22.9 ± 1.8*	18.4 - 25.2

Data are represented as * mean ±SD, ** median /IQR, and range. IQR: Interquartile range.

During the course of the disease, 9 out of the 13 cases experienced recurrent hypoglycemia, 6 cases had recurrent convulsions and 4 cases required recurrent hospital admission. Only one patient did not survive one of the attacks of hypoglycemia (Table 5).

Table (5): Frequency of complications among the study group.

Variables (n=13)	Frequency	
	Number	%
Recurrent admissions		
No	9	69.2%
Yes	4	30.8%
Recurrent hypoglycemia		
No	4	30.8%
Yes	9	69.2%
Recurrent convulsions		
No	7	53.8%
Yes	6	46.2%
Surviving		
Not survived	1	7.7%
Survived	12	92.3%

Data are represented as frequency and percentage.

Analysis of genetic material:

The results of DNA sequencing of exon 28 and its intronic boundaries revealed an intronic homozygous variant on intron 28 (rs1954399854) in one patient, while all other patients showed normal exon 28. On sequencing his DNA of exon 28 of the ABCC8 gene, we found an intronic homozygous Variant on intron 28 (rs1954399854), where there was G to A nucleotide substitution at nucleotide position 77550.

- rs1954399854:
- NC_000011.10:g.17404353G>A
- NG_008867.1:g.77550C>T
- NM_000352.6:c.3557+159C>T

On analysis on Varsome.com, it was reported as a variant of uncertain significance.

DISCUSSION

Hyperinsulinemia hypoglycemia (HH) is the most common cause of persistent hypoglycemia in pediatric patients, the most serious forms are known as congenital hyperinsulinism (CHI)[1]. Mutations of the ABCC8 gene are responsible for around half of the patients. Symptoms of hypoglycemia, the main feature of CHI, range from asymptomatic, accidental discovery on routine blood glucose checking to life-endangering conditions such as apnea, coma, or status epilepticus[3]. Management includes medical (diazoxide and octreotide) and surgical treatment[10,13-16]. This study aimed to detect mutations in the exon 28 ABCC8 and its intronic boundaries in the studied cohort.

The mean age ± SDS among the recruited patients was 13.9 ± 13.3 months ranging between 17 days and 38 months. Around 38.5% were females and 61.5% were males. A study conducted in the UK to detect variation in glycemic outcomes of patients with focal CHI revealed similar findings of male predominance, around 76% were males[24]. A high prevalence of consanguineous marriage among families of participants (61.5%) was observed in the current study. Similarly, a study conducted including a large number of Iranian patients with CHI showed 48% consanguinity[25]. Two participants of the current study group (15.4%) were preterm versus 11 (84.6%) were full-term. Research that studied the genetic properties of patients with CHI in a tertiary center in India showed similar results with 2 patients (4.76%) who were premature and those families had two affected siblings, which is similar to the current study where only one case (7.7%) had a positive family history of CHI[26].

Around 61% of cases presented during the first week of life, 15.4% presented between the first week and 6 months of age, and 23.1% (3 patients at the age of 9 and 18 months) presented after the age of 6 months, which is similar to the results of the study conducted including 44 patients with CHI in Iran, which revealed that 81.8% were diagnosed before the first year[25].

The neuro-developmental assessment of the current study showed that 46.2% of cases had delayed motor milestones, 7.7% had delayed mental milestones, 38.5% had a speech delay, and 7.7% had abnormal vision. Similarly, the study conducted in the UK on patients with focal CHI reported abnormal neurodevelopment in 22% of patients[24]. This indicates that CHI can sustain high-morbidity with lifelong consequences that necessitates early diagnosis and management.

As for the different laboratory investigations done during the attack of hypoglycemia, all participants had low levels of blood glucose with a mean ± SDS of 28.9 ± 8.5 mg/dl, associated with detectable to high levels of serum insulin and C peptide with a median of 16.4 uIU/ml (range: 3.6-42.3 uIU/ml), and 4 ng/ml (range:1-8.2 ng/ml), respectively without ketone bodies in urine. This confirms the diagnosis of hyperinsulinemic hypoglycemia. Similarly, a Chinese study included 25 patients with CHI detected a median level of insulin and C peptide of 15.7 µIU/mL (range, 2.5–110.6 µIU/mL) and 3.5 ng/mL (range,1.2–8.1 ng/mL), respectively[27]. The Growth hormone response during the attacks of hypoglycemia in the current study's population was variable ranging from 0.28 to 41 ng/ml with a median of 6.9 ng/ml despite of normal GH secretion in those patients detected using confirmatory tests. This result could be explained by the pulsatile nature of GH secretion with the

short half-life (10 minutes). Additionally, the GH response to spontaneous hypoglycemia was found to be significantly lower than induced hypoglycemia due to the slower rate of decline in blood glucose during spontaneous hypoglycemia[28]. The faster the descent of blood glucose, the better is the response.

Despite of the variable serum cortisol response during the attack of hypoglycemia in this study group (median: 22 ug/dl, range: 0.8-40 ug/dl), the serial hormonal profile found no abnormalities in cortisol levels. This could be explained by the blunted cortisol secretion in response to the slow decline of blood glucose in case of spontaneous hypoglycemia compared to induced hypoglycemia [28,29].

This study reported elevated levels of serum ammonia in one case. Similarly, a systematic review conducted to include cases diagnosed with CHI in China from 2002 to 2016 reported high levels of serum ammonia in a subgroup of their patients' pool[30]. Mutations in the GLUD1 gene were associated with hyperinsulinemic hypoglycemia and hyperammonemia. This could be explained by the fact that GLUD1 encodes GDH activity with controls the rate of ammonia production and hence mutations of GLUD1 lead to hyperammonemia[31,32]. Other studies involved patients with congenital hyperinsulinism and normal blood ammonia levels detected no mutations in the GLUD1 gene[33,34,35]. This proves significant associations between hyperammonemia and mutations in the GLUD1 gene in patients with CHI.

Normal radiological findings by (US, MRI, or CT) were observed in the current study. This questions their role in the diagnosis of CHI. 18-F-fluoro-L-DOPA positron emission tomography scan (PET-scan) is the main radiological investigation for cases with CHI however it is not available in our institute.

Genetic analysis of exon 28 and its intronic boundaries revealed an intronic homozygous variant on intron 28 (rs1954399854) in one patient while all other patients could not show any mutations in exon 28. This variant was found to be of uncertain significance. A study conducted in western Saudi Arabia revealed mutations in exon 28 in 2 of the recruited cases[35].

Diazoxide is the main medical treatment for cases with CHI, however, in Egypt, due to its unavailability, octreotide is used. Treatment has several modalities but in Egypt we have diazoxide so it is a statement. In our study, nine patients (69.2%) were treated using octreotide {3 of them (33.3%) responded well to treatment while 6 patients (66.7%) failed to achieve normoglycemia in spite of reaching maximum therapeutic dose}, one patient (7.7%) used diazoxide, achieved normal blood glucose level complicated with mild hypertrichosis but no further side effects were observed, one patient (7.7%) received both octreotide and diazoxide for treatment, and two patients (15.4%) did not receive any treatment {parents refused medical or surgical management against medical advice}. None of our patients underwent total or subtotal pancreatectomy.

CONCLUSION

Mutations of the ABCC8 gene account for around 40-50% of cases of congenital hyperinsulinism. To our knowledge, there are no sufficient studies conducted in the Egyptian population to detect mutations of the ABCC8 gene in cases with CHI, which necessitated conducting this study. Genetic analysis of exon 28 of the ABCC8 gene did not reveal significant mutations in the studied cohort. Further research is warranted to detect other ABCC8 gene mutations in the Egyptian population.

Limitations

A small number of patients were included in the study due to the rarity of the disease. One exon was studied (exon 28 of the ABCC8 gene) due to limited resources and the study being self-funded. Genetic analysis should be performed on a wider scale (whole exon sequencing or preferably whole genome sequencing due to the involvement of multiple genes in the pathology).

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- **Conflict of interest:** There are no conflicts of interest to declare.

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