INTRODUCTION

Glutaric aciduria type 1 (GA1) is an autosomal recessive disease caused by defects of the mitochondrial matrix protein glutaryl-CoA dehydrogenase (GCDH), an enzyme involved in the degradation of the amino acids lysine, hydroxylysine and tryptophan ***(Kölker et al., 2006).*** It has an estimated prevalence of 1 in 100,000 newborns ***(Lindner et al., 2004).***

Mutations in the GCDH gene lead to the accumulation of glutaric acid (GA), 3-hydroxyglutaric acid (3OHGA), and in some patients also glutaconic acid in body fluids and tissues. Several underlying mechanisms of GA1 have been discussed. It has been assumed that 3OHGA is the GA1-specific metabolite crucial for the disease-specific symptoms because GA also accumulates in other organic acidurias ***(Ullrich et al., 1999).***

The elevated glutaric acid, 3-hydroxyglutaric acid, glutaconic acid, and glutarylcarnitine can be detected by gas chromatography/mass spectrometry (organic acids) or tandem mass spectrometry (acylcarnitines) ***(Chace et al., 2003).***

Based on the urinary excretion of metabolites, GA1 patients are classified into high and low excretor groups. The low excretor patients are more difficult to diagnose despite having the same clinical picture and prognosis of high excretor patients ***(Kölker et al., 2011).***

MRI plays an important role in the diagnostic work-up. Many characteristic findings have been reported focusing on striatal changes, subdural collection and widening of both Sylvian fissures ***(Hedlund et al., 2006).***

GA1 patients are prone to the development of encephalopathic crises triggered by catabolic stress induced by fever, infections, vomiting and/or diarrhea. During these catabolic crises, concentrations of the metabolites show a further increase. The development of an encephalopathic crisis is accompanied by destruction of striatal neurons with a subsequent irreversible disabling movement disorder, especially dystonia. Children affected by GA1 are at risk for such a crisis during a time window from 3 to 36 months of age. After completion of the third year of life an encephalopathic crisis occurs only sporadically ***(Kölker et al., 2006).***

Treatment with a lysine-restricted diet, carnitine supplementation and aggressive emergency treatment has been shown to significantly improve outcome in GA ***(Kölker et al., 2011).***

The GCDH gene is localized on chromosome 19p13.2 and encodes a Flavin adenine dinucleotide-dependent mitochondrial matrix protein that is involved in the degradative metabolism of L-lysine, L-hydroxylysine and L-tryptophan ***(Fu et al., 2004)***.

The GCDH gene stretches over 7 kb on chromosome 19p13.2 and comprises 11exons ***(Schwartz et al., 1998)***. The gene product is a polypeptide of 438 amino acids, of which 44 N-terminal residues are removed after mitochondrial import; 63 different disease causing mutations in the gene have been described so far ***(Goodman et al., 1998)***. Single common mutations are found in genetically homogeneous communities such as the Amish of Pennsylvania, but GA1 in general is quite heterogeneous; the most frequent mutation in whites, R402W, has been identified on 10-20% of alleles ***(Biery et al., 1996).***

AIM OF THE WORK

In this study, we aim to describe and correlate the clinical, biochemical and molecular characteristics of Egyptian children with glutaric aciduria and as well their demographic criteria.

REVIEW OF LITERATURE

Glutaric aciduria type 1

Glutaric aciduria type I (GA I) is an autosomal recessive inborn error of metabolism caused by a deficiency of the enzyme glutaryl-CoA dehydrogenase ***(Sarngi et al., 2015).***

The active GCDH enzyme represents a mitochondrial homotetramer that dehydrogenates glutaryl-CoA to crotonyl-CoA in the chemical reaction pathway of lysine, hydroxylysine, and tryptophan, within the mitochondria ***(Anikster et al., 1996).***

The metabolic block results in accumulation of glutaric and 3-hydroxyglutaric acids in addition as glutarylcarnitine in body fluids ***(Busquets et al., 2000).***

Molecular Genetics

GCDH gene is localized on chromosome 19p13.2 and encodes a flavin adenine dinucleotide-dependent mitochondrial matrix protein that is concerned within the degradative metabolism of L-lysine, L- hydroxylysine and L-tryptophan ***(Fu et al., 2004; Greenberg et al., 1995***).

Mutations in the GCDH' gene prevent production of the enzyme or result in the production of a defective enzyme with very low residual activity, or an enzyme with relatively high residual activity (***Christensen et al., 2004).***

The gene spans about 7 kb on chromosome 19p13.2 and is composed of 11 exons and 10 introns ***(Goodman et al., 1998).*** The encoded protein comprises 438 amino acids. After import into mitochondria 44 N-terminal amino acid residues are cleaved off ***(Biery et al., 1996).*** More than 200 disease-causing mutations are known ***(Zschocke et al., 2000).***

The most common mutation is R402W in exon ten that accounts for less than twenty percent of mutations. The R402W missense mutation retains only about three percent of enzyme activity while other missense mutations (such as A421V in exon eleven) retain significant residual enzyme activity (up to about forty percent of normal) when expressed in E- coli ***(Goodman et al., 1998).*** It is unclear whether or not individuals with ‘‘leaky’’ mutations (such as A421V, prevalent with in the old-order Amish of Pennsylvania) have an increased probability of remaining asymptomatic, that is, not having striato-nigral degeneration.

There is correlation between residual enzyme activity and biochemical phenotype, that is, urinary excretion of glutaric acid. Patients with a mild mutation (such as R227P and V400M) on at least one chromosome might have low or normal urinary excretion of glutaric acid ***(Pineda et al., 1998).***

By contrast, patients with severe mutations such as R402WorA293T on both alleles have no residual activity and show the typical urinary metabolite pattern. However, there is

no association between phenotype and severity of the genetic lesion ***(Christensen et al., 2004),*** since siblings within the same family and with the same mutation and genetic background can have inconsistent phenotypes.

In addition, patients with different mutations and very different biochemical phenotype (urinary excretion of glutaric and 3-OH-glutaric acid) can presents similarly. Therefore, non-genetic factors, among which fever, infections, and fasting, play an important role in precipitating neuronal damage in glutaric acidemia type 1.

The combined worldwide frequency of GA-1based on newborn screening by MS/MS of 2.5 million children is 1:100,000 infants ***(Lindner et al., 2004).***

The disorder is very frequent (up to 1:300) among certain ethnic groups such as the Old-Order Amish community of Pennsylvania ***(Morton et al., 1991)*** and the North American Ojibway Cree in Canada ***(Haworth et al., 1991; Greenberg et al., 1995).*** In Sweden, it affects 1:30,000 newborn ***(Kyllerman and Steen, 1980)*** and about 1:50,000 in the USA ***(Goodman and Frerman, 2001).***

The GCDH founder mutation among the Lancaster Amish is a C-to-T change at nucleotide 1296 within exon 11 that causes an A-to-V change at amino acid 21.The mutation is postulated to impair tetramer assembly ***(Goodman et al., 1998).***

The GCDH activity in fibroblasts of affected Amish patients is 0–12% of control values ***(Morton et al., 1991).*** The majority of affected Amish infants are homozygous for c.1262C->T, and a significant number of affected non-Amish patients are heterozygous for this base change. In non-Amish infants an array of mutations affect 9 of the 11 exons in the GCDH gene. Compound heterozygosity is common. From 15 non-Amish haplotypes, c.1262C->T is found in 9 of 30 mutant GCDH genes, consistent with the early European origin of this mutation ***(Goodman et al., 1998; Busquets et al., 2000; Zschocke et al., 2000).***

Biochemistry

Biochemically, GA-I is characterized by an accumulation of glutaric acid (GA), 3- hydroxyglutaric acid (3-OH-GA), glutaconic acid (less frequently), and glutarylcarnitine (C5DC).These can be detected in body fluids (urine، plasma, CSF) and tissues by gas chromatography/mass spectrometry (GC/MS) or electrospray-ionization tandem mass spectrometry (MS/MS) ***(Baric et al., 1999; Chace et al., 2003).***

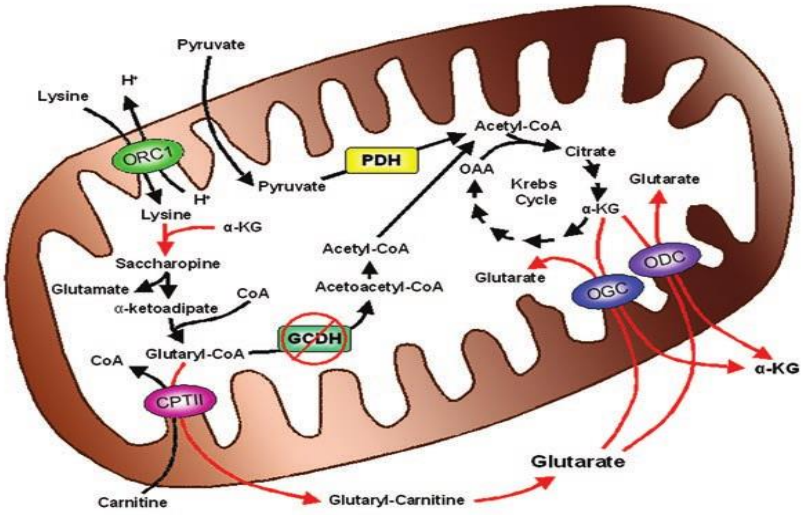

Fig. (1): Proposed mechanism of mitochondrial dysfunction. Normal mitochondrial metabolism involves lysine uptake through the ornithine carrier (ORC1) with proton exchange. Lysine degradation is initiated by combination with α-ketoglutarate (α-KG) to form saccharopine. Dehydrogenation of saccharopine forms glutamate and aminoadipic semialdehyde, which is further oxidized to α-ketoadipate. Glutaryl-CoA is then formed from oxidation of α-ketoadipate with addition of free CoA. GCDH is required for complete oxidation with the formation of acetyl-CoA that can enter the Krebs cycle. GCDH deficiency results in disruption of normal lysine breakdown, which may cause saccharopine to accumulate and sequester α-KG. Alternatively, accumulating free glutaric acid (glutarate) may deplete α-KG levels by a strict counter-exchange mechanism via the oxodicarboxylate carrier (ODC) and to a lesser extent through the oxoglutarate carrier (OGC) that normally functions in the malate/aspartate shuttle. Depletion of α-KG prevents regeneration of oxaloacetate (OAA) needed to combine with acetyl-CoA to form citrate for continued Krebs cycle function. Acetyl-CoA is unable to enter the Krebs cycle and accumulates. CPTII, (carnitine palmitoyltransferase II); PDH, (pyruvate dehydrogenase) ***(William et al., 2007).***

Two biochemically defined subgroups of patients have been described based on urinary metabolite excretion of GA, i.e., low and high excretors ***(Baric et al., 1999).*** The low excreting patients have the same risk of developing striatal

injury as the high excretors ***(Christensen et al., 2004; Kölker et al., 2006)*** and must not be considered to have a“ mild” clinical phenotype.

Since the description of two index patients in 1975 ***(Goodman et al., 1975)*** more than 500 patients have been reported worldwide. Five genetic isolates with a high carrier frequency (up to 1:10) and incidence (up to 1:250): the Amish Community in Lancaster County, PA, USA ***(Morton et al., 1991)***, the Oji-Cree First Nations in Manitoba and Western Ontario, Canada ***(Haworth et al., 1991),*** the Irish Travellers in the Republic of Ireland and UK ***(Naughten et al., 2004),*** and the Lumbee Indian Tribe Carolina in North ***(Basinger et al., 2006)***, and the Xhosa and other subgroups of the South African black population ***(van derWatt et al., 2010).***

Signs and symptoms

There is marked variation in the clinical expression and severity of the disease, even within families ***(Haworth et al., 1991).***

Most, however, present in infancy with an acute encephalopathy, often triggered by infection or other minor illness ***(Monavari et al., 2000)***. The outcome is often poor, with a previously well child suffering spastic cerebral palsy, choreoathetosis, dystonia and, occasionally, mental retardation, although typically intellectual function is preserved ***(Brismar and Ozand, 1995).***

Macrocephaly is a very frequent finding, present at birth or developing in the first weeks of life ***(Forstner et al., 1999).***

Babies with glutaric acidemia type 1 often are born with unusually large heads (macrocephaly). Macrocephaly is amongst the earliest signs of GA1. It is thus important to investigate all cases of macrocephaly of unknown origins for GCDH deficiency ***(Martınez-Granero et al., 2005).***

In neonates and infants, unspecific neurologic symptoms such as muscular hypotonia and delayed motor development occur in about half of all individuals with GA-I, whereas others are asymptomatic. Macrocephaly is a frequent (75 %) but nonspecific finding and is present at or shortly after birth ***(Bjugstad et al., 2000; Renaud, 2012).***

Considering a 3 % frequency of macrocephalic individuals in the general population, the positive predictive value (PPV) of macrocephaly for GA-I is low.

Indeed, the infant may be asymptomatic but referred for evaluation of isolated macrocephaly. Less commonly, the disease may manifest a more slowly progressive course with the gradual development of disability. Diagnosis may also be made soon after birth by screening the siblings of affected children.

Untreated, approximately 90% of patients will develop neurological disease during a finite period of brain development (age 3-36 months) following an acute

encephalopathic crisis often precipitated by gastroenteritis, intercurrent febrile illness, immunization, or surgical intervention ***(Hoffmann et al., 1991; Kölker et al., 2006).***

The characteristic neurological sequela of these crises is acute bilateral striatal injury and, subsequently, a complex movement disorder. Dystonia is the dominant extrapyramidal symptom, usually superimposed on axial hypotonia ***(Hoffmann et al., 1991; Kyllerman et al., 1994; Heringer et al., 2010).*** With aging, there is a tendency for a fixed dystonia and akinetic-rigid Parkinsonism to develop ***(Gitiaux et al., 2008).***

Morbidity and mortality is high in patients who have had a crisis ***(Kyllerman et al., 2004; Kölker et al., 2006).*** In 10-20% of patients, neurologic disease has been demonstrated in the absence of any documented encephalopathic crisis and has been termed insidious-onset ***(Busquets et al., 2000; Hoffmann et al., 1996)*** and late onset ***(Bähr et al., 2002; Külkens et al., 2005).***

Furthermore, insidious onset in newborn screening cohorts has been observed in individuals not adhering to current dietary recommendations ***(Heringer et al., 2010).*** In any case, an acute encephalopathic crisis with striatal necrosis is the major prognostic factor of morbidity and mortality in GA-I ***(Kölker et al., 2011).*** However, in a minority of cases, the onset is insidious with gradual disease progression and the absence of an acute encephalopathic episode or major pathological

changes of basal ganglia in imaging, but, as a rule, presence of other hallmark GA-I clinical and radiological signs, such as macrocephaly and widening of Sylvian fissures ***(Strauss et al., 2003; Kölker et al., 2011).***

Late-juvenile or adult-onset GA-I is extremely rare. There have been scant reports in the medical literature, and in fact, some cases reported as "adult-onset GA-I" were simply childhood-onset GA-I diagnosed in adulthood ***(Prevett et al., 1996; Twomey et al., 2003; Fernandez-Alvarez et al., 2003; Harting et al., 2009; Chen et al., 2011).***

According to the few reports of truly adult-onset GA-I (onset at the age of 18 years or older), (1) the patients were paucisymptomatic or asymptomatic at the time of diagnosis, (2) the disease followed either an acute encephalopathic or a non-encephalopathic clinical course, and (3) in all cases supratentorial, diffuse, symmetric U-fiber -sparing leukoencephalopathy involving periventricular and deep white matter was seen in cranial MRI. ***Bahr et al.*** reported the case of a 19-year-old woman without macrocephaly, who presented with recurrent headaches, mild ophthalmoparesis, and hyperreflexia ***(Bahr et al., 2002).***

Fernandez-Alvarez et al. described the case of an adolescent girl without macrocephaly and a history of postural hand tremor that by the age of 19 had developed focal dystonia and orofacial dyskinesia ***(Fernandez-Alvarez et al., 2003).***

Kulken et al. reported the interesting case of 15 years old boy with macrocephaly, psychomotor retardation, progressive vertigo, and intermittent severe diffuse headaches often induced by physical exercise and relieved by rest ***(Kulkens et al., 2005).***

Sonmez et al. described the case of a 20-year-old man with a 6-month history of recurrent headaches and hyperactive muscle stretch reflexes ***(Sonmez et al., 2007).***

Finally, a Chinese patient has been described with a purported ischemic cerebral stroke in young adulthood, whereby the correct diagnosis of GA-I was later made ***(Chen et al., 2011).***

More remarkable was a case reported by ***Kulkens et al.*** of a 66-year-old man macrocephalic since infancy with a history of severe intermittent headaches since the age of 35 and a complex progressive neuropsychiatric syndrome since the age of 50 encompassing hand tremor, seizures, dementia, and aggressive behavior with acoustic and visual hallucinations ***(Kulkens et al., 2005).***

In most of these atypical late-onset cases, diagnosis was incidental and suggested by MRI findings and metabolic screening. By contrast, ***Prevett et al.*** reported the case of a young lady with mild psychomotor retardation, gait disturbance, and orofacial dyskinesia presenting at seven years of age ***(Prevett et al., 1996).***

Frequency of epilepsy is increased in patients with GA-I, and seizures might even be the initial clinical presentation ***(Kölker et al., 2015a; McClelland et al., 2009; Young-Lin et al., 2013; Zaki et al., 2014).***

Subdural haemorrhage may occur at any age in GA-I but peaks during the age of maximal extent of macrocephaly, i.e., late infancy ***(Brismar and Ozand, 1995; Köhler and Hoffmann, 1998; Woelfle et al., 1996).*** Minor head traumas with disruption of elongated bridging veins have been suggested as underlying mechanisms. The exact frequency of subdural bleeding is unknown, since affected patients may remain asymptomatic. Subdural haemorrhage in GA-I may be mistaken with shaken baby syndrome and vice versa ***(Hartley et al., 2000; Morris et al., 1999).***

Bilateral temporal fluid collections including the anterior temporal CSF spaces and the Sylvian fissure have been described and are highly suggestive for GA-I ***(Lütcherath et al., 2000).*** While typically bilateral, they may be asymmetric and even space occupying, with hydrocephalus ***(Jamjoom et al., 1995).***

Bitemporal arachnoid cysts have been described in some affected patients and should result in a high suspicion for GA-I ***(Hald et al., 1991; Jamjoom et al., 1995; Lücherath et al., 2000; Martinez-Lage et al., 1994),*** whereas unilateral arachnoid cysts are a rare occurrence in this disease.