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The Effect of Diet on Antioxidant Status in Patients with Galactosemia

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Galactosemia is an autosomal recessively inherited disorder of galactose metabolism. It has good prognosis, if detected in neonatal period or early infancy. Treatment consists of life long dietary restriction of galactose. Present study included eight patients with galactosemia on dietary treatment, five of them had galactose-1-phosphate uridylyltransferase deficiency known as classical galactosemia and three had uridine-diphosphate galactose-4' epimerase deficiency. Clinical evaluation of patients under galactose restricted diet and assessment of the antioxidant status in response to dietary therapy was done. Delayed milestones were present in all patients, jaundice at birth was present in 4 and low birth weight was present in 3 patients. Craniofacial dysmorphism was present in 5 patients. Hepatomegaly was present in 6 patients. MRI of the brain showed brain atrophy in 3 patients and demyelination in 2 patients. There was cataract in 7 patients. The levels of zinc, copper, iron, calcium, phosphate, magnesium, selenium, manganese, β -carotene and vitamin A were evaluated in the blood of galactosemic patients on galactose restricted diet and a comparison between trace elements, β -carotene and vitamin A in studied patients with galactosemia and controls was done. Copper, calcium, phosphate, manganese and β -carotene levels in blood were significantly decreased in our patients ($p < 0.001$) than in controls. These findings suggest that patients on galactose restricted diet are at risk of oxidative stress. The data emphasize the importance of dietary supplementation with an antioxidant containing β -carotene, calcium, copper, selenium and manganese to inhibit oxidative stress in these patients. Consequently this will minimize the neurological deficits improve bone mineralization, reduce the development of retinopathy and damage to liver cells in patients with galactosemia.

Key words: Galactosemia, dietary therapy, antioxidants, trace elements, oxidative stress

INTRODUCTION

Galactosemia is an autosomal recessive inherited disorder of galactose metabolism, which occurs as a consequence of a deficiency of one of three principal enzymes involved in the metabolism of galactose, through its conversion to glucose. These enzymes are galactose-1-phosphate uridylyltransferase (GALT), uridine-diphosphate galactose-4' epimerase and galactokinase (Wasilenko *et al.*, 2005). The most common deficiency in all communities is that of the transferase enzyme and it is this enzyme deficiency that underlies "classical galactosemia" (OMIM, 2004; Bosch *et al.*, 2002).

Because milk is the staple of an infant's diet, early diagnosis and treatment of this disorder is absolutely essential to avoid serious lifelong disability. Despite the treatment, long term complications occur such as disturbed mental and motor development, decreased bone mineral density and learning disorders (Arm, 2003).

Failure to thrive is the most common initial clinical symptom of galactosemia. Vomiting or diarrhoea usually begins within a few days of milk ingestion. Jaundice of intrinsic liver disease may be accentuated by the severe haemolysis occurring in some patients (Huidekoper *et al.*, 2005). The pathophysiology of cataracts is one of the well characterized aspects of galactosemia. Cataracts have been observed within a few days of birth. These may be found only on slit-lamp examination and missed with an ophthalmoscope, since they consist of punctate lesions in the fetal lens nucleus (Holton *et al.*, 2001). The most vexing problem facing patients with galactosemia is the effect of galactose deficiency on brain development and function. The patient may have decreased IQ and/or learning disability. Clinically evident speech delay and cerebellar signs are more frequent than other findings. Abnormal white matter signal is found in most subjects, but this abnormality does not correlate with cognitive outcome. Webb *et al.* (2003) noted that verbal dyspraxia (chaotic speech) is found in many galactosemic patients.

Oxidative stress may be implied in the pathogenic mechanisms of galactosemia. The lower activities observed in patients on natural protein restriction may likely be due to a deficient bioavailability of antioxidant cofactors (Vilaseca-Busca *et al.*, 2002). Dietary supplementation with an antioxidant containing β -carotene and selenium inhibits oxidative stress in galactosemia. Antioxidants inhibit the abnormal metabolic process that contributes to the development of the disease (Kowluru *et al.*, 2000).

Iron homeostasis and galactose metabolism are linked with one another because galactose metabolism is

defective when iron availability is restricted. Iron deficient galactosemic patients might be more severely compromised than iron repleted individuals (Shi *et al.*, 2003).

Treated galactosemic children are at risk of abnormal bone mineralization which may predispose them to osteoporosis and fracture in adult life (Rubio-Gozalbo *et al.*, 2002). Also, calcium is important in relation to maintenance of lens transparency (Kamei *et al.*, 1987).

Nutrition plays a vital role in maintaining physical growth and development throughout life. The dietary modifications form the mainstay to disease stabilization and control. A modified diet is essential for the patient's survival and adequate mental development (Badawy *et al.*, 2003). In the last decade there has been tremendous progress in cloning of various genes, identification of their protein products and detection of different mutations in many metabolic disorders. With improved understanding of the underlying metabolic pathways, dietary interventions may be more appropriately targeted (Bavdekar *et al.*, 2002).

MATERIALS AND METHODS

The study included eight galactosemic patients on galactose restricted diet (seven males and one female). They were recruited between 2003 and 2005 from the Clinical Genetics Department, National Research Centre, Cairo, Egypt. All the patients have been diagnosed in the neonatal period and had good dietary control. Their age of onset ranged from 10 days to 30 days.

The control group consisted of ten healthy controls who were matching for age and sex.

Each patient was subjected to meticulous clinical examination to detect any malformation or anomaly. Three generations pedigrees were constructed and analysed with special emphasis on positive parental consanguinity and similarly affected sibs in the family.

In addition the following investigations were done to all the patients:

- Abdominal sonography.
- MRI brain.
- Complete eye evaluation.
- Cytogenetic analysis of peripheral blood lymphocytes from the patients was done using G-banding technique.
- Biochemical assessment of: Trace elements (Zn, Cu, Fe, Ca, P, Mg, Se and Mn) and antioxidants (β -carotene and vitamin A).

The levels of plasma zinc and copper were measured according to the methods of Homsher and Zak (1985) and Abe *et al.* (1989), respectively. The determination of iron was according to the method of Tabacco *et al.* (1981). Calcium and phosphorus were measured according to the method of Gitelman (1967) and Gamst and Try (1980), respectively. The level of magnesium was measured according to the methods of Gindler and Heath (20). The level of selenium and manganese were measured with a Varian Spectr AA 220 atomic absorption spectrometer equipped with a graphite-surnace tube atomizer (GTA). Samples and standard were injected into GTA using an auto sampler (GTA 100). The levels of β -carotene and vitamin A were determined according to the method of John *et al.* (1963).

- Determination of total galactose (galactose and galactose 1-phosphate) in dried blood samples according to the method of Diepenbrock *et al.* (1992). The enzyme levels of galactose-1-phosphate uridylyltransferase and uridine diphosphate galactose-4-epimerase were estimated according to the method of Shin (1991).

RESULTS

This study was carried out on eight galactosemic patients on restricted galactose dietary treatment (seven were males and one female). Positive consanguinity was present in 5 patients (62.5%), there was no similarly affected members in the families.

Table 1 shows the clinical findings in the studied patients. There were five patients with Galactose 1-uridylyl Transferase Deficiency (GALT) and the other three with epimerase deficiency. The mode of inheritance in both groups is autosomal recessive. Among the groups, seven were males and one female with parental consanguinity in five patients (62.5%). Delayed milestones were present in all patients, jaundice at birth was present in 4 (50%) and low birth weight was present in 3 patients (37.5%). Craniofacial dysmorphism was present in 5 patients (62.5%) in the form of frontal bossing, depressed nasal bridge and low set ears. In patient (1), there was incomplete simian crease in left hand and bilateral talipes equinovarus. In patient (4) there was arachnodactyly of the fingers and dentinogenesis imperfecta. In patient (6) there was bilateral simian crease, rocker bottom feet and left inguinal hernia. In patient (7) there was bilateral clinodactyly of fourth finger. However, chromosomal analysis of all patients was normal.

Hepatomegaly was present in 6 patients (75%), patient (8) showed paucity of the interlobular ducts in



Fig. 1: MRI T₂ axial section showing white matter demyelination around the occipital horn of lateral ventricle in patient number 1

his liver biopsy. He had no cardiovascular, vertebral or renal abnormalities which excludes Alagille syndrome. MRI of the brain showed brain atrophy in 3 patients (3, 5 and 6), demyelination in 2 patients (1 and 8) and degeneration of basal ganglia in one patient (7). There was cataract in 7 patients (87.5%) three of them had squint on top (1, 3 and 5). The ERG in patient (8) showed diffuse retinal pathology affecting rods and cones.

The Table 2 shows significant decrease in the level of Cu, Ca, P, selenium, Mn and β -carotene in patients compared to the control group.

MRI T₂ axial section showing white matter demyelination around the occipital horn of lateral ventricle Fig. 1.

DISCUSSION

Galactosemia is an autosomal recessive disorder of galactose metabolism caused by a deficiency of one of three principal enzymes involved in the metabolism of galactose through its conversion to glucose. These enzymes are galactose-1-phosphate uridylyltransferase (GALT), uridine-diphosphate galactose-4' epimerase and galactokinase. In our study, galactose-1-phosphate uridylyltransferase enzyme was deficient in five patients while epimerase deficiency was present in three. Because galactosemia is a relatively rare disease with an average of 3 new cases per year in our clinic, the patients group participating in this study was small.

Table 1: Clinical picture and enzyme deficiency

Serial No.	Sex	Parental consang	Clinical picture	Abdominal sonar	MRI brain	Eye findings	*Total galactose	Enzyme deficiency
1	M	-ve	Delayed milestones, frontal bossing, incomplete simian crease and Bilateral talipes equinovarus	hepatomegaly	Demyelinating lesion	Cataract and squint	28	Epimerase deficiency
2	M	+ve	Delayed milestones	hepatomegaly	Normal	Cataract	22	**Uridyl trans. deficiency
3	M	-ve	Jaundice at birth, low birth weight	hepatomegaly	Mild brain atrophic changes	Cataract and squint	12	Epimerase deficiency
4	M	+ve	Jaundice at birth, low birth weight Arachnodactyly Delayed milestones Dentinogenesis imperfecta	hepatomegaly	Normal	Normal	14	Uridyl trans. deficiency
5	F	-ve	Jaundice at birth Delayed milestones frontal bossing depressed nasal bridge	hepatomegaly	Frontal and central brain atrophy	Cataract and squint	12	Uridyl trans. deficiency
6	M	+ve	Delayed milestones seizures low birth weight semian crease, rocker bottom feet, inguinal hernia	Normal	Frontal and central brain atrophy	Bilateral cataract	13	Uridyl trans. deficiency
7	M	+ve	Delayed milestones frontal bossing low set ears bilateral clinodactyly of 4th finger	Normal	Degeneration of basal ganglia	Bilateral cataract	15	Uridyl trans. deficiency
8	M	+ve	Jaundice at birth, delayed milestones microcephaly	Hepatomegaly Liver pathology showed paucity of the interlobular bile ducts.	Demyelinating lesion	Bilateral cataract ERG:diffuse retinal pathology affecting rods and cones	32	Epimerase deficiency

* Total galactose in blood (normal value: 0-5 mg dL⁻¹) **Uridyl transferase deficiency

Table 2: Comparison between trace elements, β-carotene and vitamin A in studied patients with galactosemia and controls

Variables	Patients	Controls	p-value
	Mean±SE N = 8	Mean±SE N = 10	
Zn (µg dL ⁻¹)	70.8±11.0	88.7±6.4	0.2
Cu (µg dL ⁻¹)	75.5±7.4	113.4±5.8	0.003*
Fe (mg dL ⁻¹)	64.8±8.5	73.9±6.5	0.7
Ca (mg dL ⁻¹)	9.1±0.3	10.4±0.5	0.03*
P (mg dL ⁻¹)	6.9±0.6	8.3±0.1	0.01*
Mg (mg dL ⁻¹)	2.6±0.1	2.3±0.1	0.08
Selenium (µg dL ⁻¹)	69.9±5.4	84.5±3.2	0.03*
Mn (µg dL ⁻¹)	16.7±2.4	23.6±0.3	0.0001*
β-carotene (µg dL ⁻¹)	59.3±6.1	92.7±5.8	0.002*
Vitamin A (µg dL ⁻¹)	31.8±1.7	31.3±2.1	0.7

*p-value is significant if ≤0.05

Parental consanguinity was present in five patients (62.5%). Several families with epimerase deficiency have been reported, but most are from highly consanguineous families and there does not appear to be a consistent phenotype which can clearly point to epimerase deficiency (Schulz *et al.*, 2005).

Long-term results of treatment have been disappointing; IQ is low in many despite early and seemingly adequate therapy (Bosch *et al.*, 2004). For example, the retrospective study by Schweitzer *et al.* (1993) reports the cause of the unsatisfactory outcome of seemingly good control of galactose intake and the disturbances in long-term development despite treatment was unclear. Possibilities include chronic intoxication by galactose metabolites or deficiency of

galactose-containing glycoproteins and/or glycolipids as a result of an over restrictive galactose-free diet. However, Badawy *et al.* (2003) found that 80% of patients had improved both in mental and motor functions with galactose free diet.

Increased oxidative stress may be involved in the complications of galactosemia (Schulpis *et al.*, 2005). Antioxidant supplementation can help alleviate the subnormal activities of antioxidant defense enzymes (Renu *et al.*, 1999).

The results of assessment of the antioxidant minerals (Zn, Cu, Fe, Ca, P, Mg, Se and Mn) and antioxidants (β-carotene and vitamin A) showed significant decrease in Cu, Ca, P, Se, Mn and β-carotene.

Kowluru *et al.* (2001) mentioned that β-carotene and selenium prevent galactosemia-induced elevation of retinal oxidative stress. Therefore, supplementation with antioxidants such as alpha-tocopherol, N-acetyl cysteine, ascorbic acid, beta-carotene and selenium can offer an achievable and inexpensive adjunct therapy to help inhibit the development of retinopathy as they are neuroprotective.

In this study, 6 patients presented with hepatomegaly (75%). Surgical biopsy of the liver showed marked fatty infiltration, periportal fibrosis and cirrhosis. Pozzato *et al.* (2005) studied patients with galactosemia who had enlarged liver but the patients did not show increased liver echogenicity. Marcoux *et al.* (2005)

mentioned that galactosaemia reveals usually in the second and third weeks of life with a severe liver dysfunction. They reported a case of congenital galactosaemia with an early onset liver failure. Therefore, evaluation of patients with galactosaemia should not be limited to enzymatic analysis, but should also include hepatic imaging; especially ultrasonography (Nishimura *et al.*, 2004). Patient number 8 showed paucity of the interlobular bile ducts in his liver biopsy. This coincides with the findings of Balistrari (1987) who mentioned that some syndromes characterised morphologically by intrahepatic cholestasis may be associated with paucity of the interlobular bile ducts. Schildkraut (2003) mentioned that the association of certain metabolic diseases with paucity of interlobular bile ducts carries a grave prognosis. Combined therapy with Coenzyme Q 10, Zn, Mg and selenium were effective in reducing damage to liver cells (Maritim *et al.*, 2003).

MRI of the brain showed brain atrophy, demyelination and degeneration of basal ganglia. Similar findings were mentioned by Mizoguchi *et al.* (2001) who described manganese elevation in blood and magnetic response imaging changes in the basal ganglia of his patients.

These findings suggest that treated galactosemic patients are at risk of oxidative stress and abnormal bone mineralization. Therefore, therapeutic intervention in these cases should be more appropriately targeted. Present data emphasise the importance of antioxidants and trace elements to minimize the neurological deficits and improve bone mineralization, inhibit the development of retinopathy and reduce the damage to liver cells in patients with galactosaemia.

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