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Research Article

Classical Galactosaemia and Bone Mineral Density

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Abstract

Objective: Classic galactosaemia (CG), due to Galactose-1-phosphate uridylyltransferase (GALT), deficiency presents in neonates after exposure to galactose. On galactose restricted and lactose free diet, the mainstay of treatment of CG, initial symptoms recede but patients still experience long term complications including impaired bone health. Decreased bone mineral density (BMD), has been observed in pre-pubertal children increasing the risk of osteoporosis and fractures later in life. We present longitudinal and individual serial data on bone health in CG patients by retrospective analysis of clinical and biochemical data.

Patients and methods: A total of 47 CG patients between the ages of 10-18 years old from a single centre were included. Data on clinical presentation, comorbidities, mobility status, BMD, hormone replacement therapy (HRT), and fracture data was collected. In addition, biochemical results including: Vitamin D, calcium, galactose-1-phosphate, alkaline phosphatase and sex hormone levels were collected and analysed.

Results: There was significant difference (p=0.02), in BMD age matched Z-scores of female and male patients. There was modest improvement in BMD in female patients on hormone replacement therapy. Vitamin D insufficiency was observed and treated in 34% of the subjects. Calcium and ALP remained within normal range.

Conclusions: Patients in this study demonstrated low BMD median age matched Z-score (-1.7 SD). Impaired bone health is a common feature in children with CG. This occurs despite prompt initiation and good compliance to treatment. The aetiology of reduced BMD in galactosaemia has not been fully elucidated. Understanding the pathogenic mechanism will inform development of improved therapeutic strategies.

ABBREVIATIONS

DEXA: Dual Energy X-Ray Absorptiometry Imaging; HRT: Hormone Replacement Therapy; BMD: Bone Mineral Density; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; ALP: Alkaline Phosphatase; GAL-1-P: Galactose-1-Phosphate; GALT: Galactose-1-Phosphate Uridylyltransferase

INTRODUCTION

Classic galactosaemia (CG, *OMIM 230400*), is a rare inborn error of carbohydrate metabolism caused by homozygous or compound heterozygous mutations in the galactose-1-phosphate uridyltransferase (*GALT,*) gene. It has a prevalence in Western countries of 1:16,000 - 1:60,000 live births and is the most frequent galactose metabolism disorder [1].

The enzyme deficiency leads to failure to metabolize Galactose-1-Phosphate (Gal-1-P), which consequently accumulates and generates alternate pathway metabolites (galactitol, galactonate). Even though the full pathogenesis of the disease is not yet known, it is likely that disturbances in the glycosylation of glycoproteins and the accumulation of Gal-1-P and its metabolites are responsible for the clinical manifestations of the disease [2].

CG presents in the neonatal period after exposure to galactose-containing milk. Symptoms appear in the first few weeks of life and include refusal to feed, vomiting, jaundice and lethargy/ encephalopathy. Other findings may include hepatomegaly, oedema and ascites. Escherichia coli sepsis, congenital cataracts and proximal renal tubulopathy are also known associations of galactosaemia [2].

The treatment is based on galactose restricted and lactose free diet that should be commenced immediately should CG be suspected whilst waiting for confirmation of diagnosis. This diet should be continued for life, restricting the main sources of lactose and galactose (predominantly present in dairy products) and ensuring ample calcium and Vitamin D intake [3].

When the appropriate diet is started early enough, initial symptoms disappear within days. However, it is still uncertain what the most effective long term treatment for this disorder is, and long term complications are seen even in patients compliant to treatment. Complications include: cognitive and intellectual difficulties, speech and language delay, neurological and/or movement disorders, psychosocial deficits, primary ovarian insufficiency and osteopenia [1,4].

An association between CG and decreased bone mineral

density (BMD) was first reported by Kaufman et al. in 1993 [5]. Subsequent reports showed that a more evident decrease was seen mostly in adults [6], however, low BMD is already present in pre-pubertal children, increasing the risk of osteoporosis and fractures later in life [7-9].

Bone health could be affected in CG patients due to a number of factors: nutritional deficiencies (e.g. calcium), due to dietetic restrictions, and Vitamin D deficiency; low oestradiol levels in women with primary ovarian insufficiency; limited physical activity due to neurological complications; and potentially intrinsic factors involving aberrant glycosylation of glycoproteins and collagen [10]. These variants may result in an imbalance between bone resorption and bone formation processes. Bone turnover in CG patients has been reported to increase from childhood to adolescence whereas in healthy individuals it declines [11].

To our knowledge previously published data on bone health in CG patients is only based on analysis of cross sectional studies. We present longitudinal and individual serial data of our CG population over a period of ten years (2010-2020).

MATERIALS AND METHODS

Patients

A retrospective analysis of clinical and biochemical data was performed for all CG patients followed up in a single centre (Great Ormond Street Hospital, UK). These patients were between 10-18 years old during the observation period of 2010 to 2020. Clinical data was reviewed using electronic medical records. This included demographics, clinical presentation, comorbidities, mobility status, bone mineral density, use of hormone replacement therapy (HRT), and occurrences of fractures. Patients' mobility status was assessed by their clinician and grouped in one of three categories: (1) Assistance required to mobilise, (2) normal mobility (i.e. able to do an average amount of physical activity appropriate for age), and (3) patients who participate in high intensity physical activities.

Biochemical monitoring data reviewed were: Vitamin D, calcium, Gal-1-P, alkaline phosphatase (ALP), Follicle stimulating hormone (FSH), Luteinizing hormone (LH), oestradiol and testosterone levels.

All galactosaemia patients were seen in joint endocrine and metabolic clinics once to twice yearly. In female patients the decision to start HRT was guided by clinical examination of pubertal progress, measurements of FSH, LH and oestradiol; and results of pelvic ultrasound scans to monitor follicle activity and uterine dimensions.

DEXA (Dual energy X-ray absorptiometry), scans and biochemical monitoring (as above) were performed annually on all patients (Table 1). Annual DEXA scans were commenced at the age of 10 years for female patients and age 14 years for male patients.

Ethical approval was not required as this was an anonymised retrospective study.

DEXA scans

Dual Energy X-ray Absorptiometry (DEXA), scans of hip and lumbar spine were performed on the Lunar Prodigy Advance (*GE Healthcare*), by trained radiographers according to the manufacturer's protocol and International Society for Clinical Densitometry guidelines [12,13]. Lumbar spine areal BMD (BMD), measurements were obtained in g/cm2.

Biochemical analysis

GALT was measured by spectrophotometry using a Tecan infinite M200 pro plate reader.

Range: Non-galactosaemics 15-35 micromol/h/gHb; Heterozygote range 5.64-16.92 micromol /h/gHb; Classical galactosaemics < 5 micromol /h/gHb.

Gal-1-P was measured by spectrophotometry using a Tecan infinite M200 pro plate reader. Normal controls <0.1 micromol/gHb; Galactosaemics on treatment 0.1-0.57 micromol/gHb; Untreated galactosaemics up to 10.8 micromol/gHb.

Total 25-hydroxyvitamin D (D2 + D3), was measured using a chromatography-mass spectrometry method on a Waters Xevo TQ-S tandem mass spectrometer. Normal range in children: 50 - 120 nmol/L; Insufficient: 25-50 nmol/L; Deficient: <25 nmol/L.

Statistics

Baseline data are presented as percentages, mean and range, or median and interquartile ranges (IQR) where appropriate. Significance of difference was evaluated by the Mann-Whitney test. A p-value of <0.05 was considered statistically significant.

Statistical analyses were conducted with the statistical program Prism 8 (GraphPad Software, San Diego, CA, USA).

RESULTS

Demographics

A total of 64 patients with CG were identified (32 males and 32 females), from 57 families. Six females and 11 males were excluded from analysis due insufficient clinical information leaving 47 patients for analysis. All patients were diagnosed within the neonatal period with the exception of 6 patients (2 females), who were diagnosed on early sibling screen and 1 female patient who presented with isolated cataracts at 7 months.

At presentation, 28 patients had liver dysfunction (12 associated with failure to thrive, 3 associated with vomiting, 2 with sepsis, 3 with cataracts, 1 with renal tubulopathy). 2 patients presented with sepsis only. 1 patient presented with isolated failure to thrive. 6 patients were asymptomatic at presentation and were diagnosed on early sibling screen. For 10 patients, their initial presentation was unknown (Table 2).

GALT enzyme activity measured in 32 patients showed median GALT of 0.8 micromol/hr/g (IQR 0, 1.7).

All patients were commenced on a galactose restricted and lactose free diet at time of diagnosis and all patients showed resolution of hepatopathy and renal tubulopathy within days of starting treatment.

The median duration of follow up for all patients was 14.1 years (IQR 12.1, 17.1).

Table 1: Monitoring parameters for galactosaemia patients.

Monitoring Parameter for Galactosaemia patients		Female	Male
Biochemistry (6-12 monthly)	Vitamin D	X	X
	Calcium	X	X
	Gal-1-P	X	X
	ALP	X	X
	Testosterone		X
	FSH	X	
	LH	X	
	Oestradiol	X	
Imaging (12 monthly)	Pelvic ultrasound	X	
		X	X
Neurocognitive assessment	School performance, psychosocial development	X	X
Dietetic Review (6-12 monthly)	Diet compliance, nutritional review	X	X
Endocrinology Review (6-12 monthly)	Pubertal staging	X	X

Abbreviations: Gal-1-P: Galactose-1-phosphate, ALP: alkaline phosphatase, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, DEXA: Dual energy X-ray absorptiometry imaging.

Table 2: Patients' Characteristics.

Patient characteristics (Total n=47)	Female (n=26)	Male (n=21)
Clinical		
Presentation -Early sibling screen (asymptomatic) -Liver dysfunction -isolated liver dysfunction -with FTT -with vomiting -with sepsis -with cataracts -with renal tubulopathy -Isolated sepsis -Isolated failure to thrive -Unknown	 2 17 4 8 2 0 2 1 1 0 6	 4 11 3 4 1 2 1 0 1 1 4
Patients on HRT (mean age of onset; years)	16 (12.8)	1 (14)
Vitamin D supplementation	25	20
Fractures (no. of patients; age) -Finger -Forearm	 1 (14y) 1 (4y)	 1 (11y)
Monitoring period (years) (median, IQR)	13.6 (12.3, 15.6)	16.0 (12.2, 17.7)
Biochemical (median, IQR)		
Gal-1-P (umol/gHb)	0.29 (0.18, 0.40)	0.29 (0.15, 0.39)
Vitamin D (nmol/L)	78 (56, 106)	74 (54, 90)
ALP (U/L)	170 (120, 229)	180 (146, 243)
Calcium (mmol/L)	2.4 (2.34, 2.47)	2.4 (2.32, 2.48)
Imaging		
BMD age matched Z-score (median, IQR)***	-1.9 (-2.5, -1.1)	-1.3 (-2.0, -0.4)

Abbreviations: Gal-1-P: Galactose-1-phosphate, ALP: alkaline phosphatase, BMD: bone mineral density, HRT: hormone replacement therapy, ***P=0.02

Bone mineral density

The median BMD age matched Z-score of the L2-L4 region for all patients was -1.7 SD (IQR -2.2, -0.9) (Figure 1).

Female (n=26)

Twenty-six female galactosaemia patients were analysed. The median BMD age matched Z-score of the L2-L4 region was -1.9 SD (IQR -2.5, -1.1) (Figure 2). The worst BMD age matched Z-score for all female patients showed a median score of -2.0 SD (IQR-2.5, -1.27). 50% of female patients had at least one BMD age matched Z-score of less than -2.0 SD in the follow up period.

Sixteen patients were commenced on hormone replacement therapy (HRT), at a mean age of 12.8 years (range 10-14 years). For patients who commenced on HRT, the pre-HRT median BMD age matched Z-score was -1.9 SD (IQR -2.25, -1.67), and the post-HRT median BMD age matched Z-score was -1.9 SD (IQR -2.45, -1.25). There was no significant difference between the pre-HRT and post-HRT BMD Z-scores. 5/16 female patients who were commenced on HRT to induce puberty had serial BMD age matched Z-score measurements showing a modest increase in BMD Z-score (Figure 3).

Two patients sustained fractures, one at age 14 years (right finger fracture) and the other at 4 years (left forearm).

Male (n=21)

Twenty-one male CG patients were analysed. One patient was started on testosterone at the age of 14 years due to delayed pubertal status unrelated to his diagnosis of CG. The median BMD age matched Z-score of the L2-L4 region was -1.3 SD (IQR -2.0, -0.42) (Figure 1 and 2). The worst BMD age matched Z-score of all male patients showed a median score of -1.3 SD (IQR -2.2, -0.35).

Four patients (28%), had at least one BMD age matched Z-score of less than -2.0 in the follow up period.

One patient sustained fracture to his finger at 11 years old.

Other contributing factors to bone health

Calcium intake: All patients received regular dietetic reviews to ensure optimal nutritional and calcium intake and

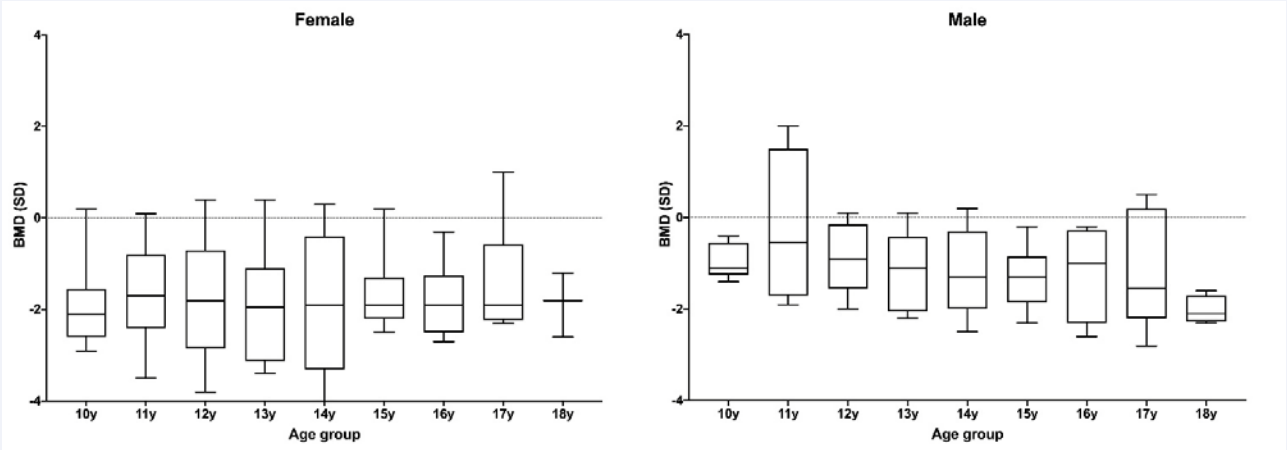

Figure 1 Bone mineral density of female and male classic galactosaemia patients according to age group.

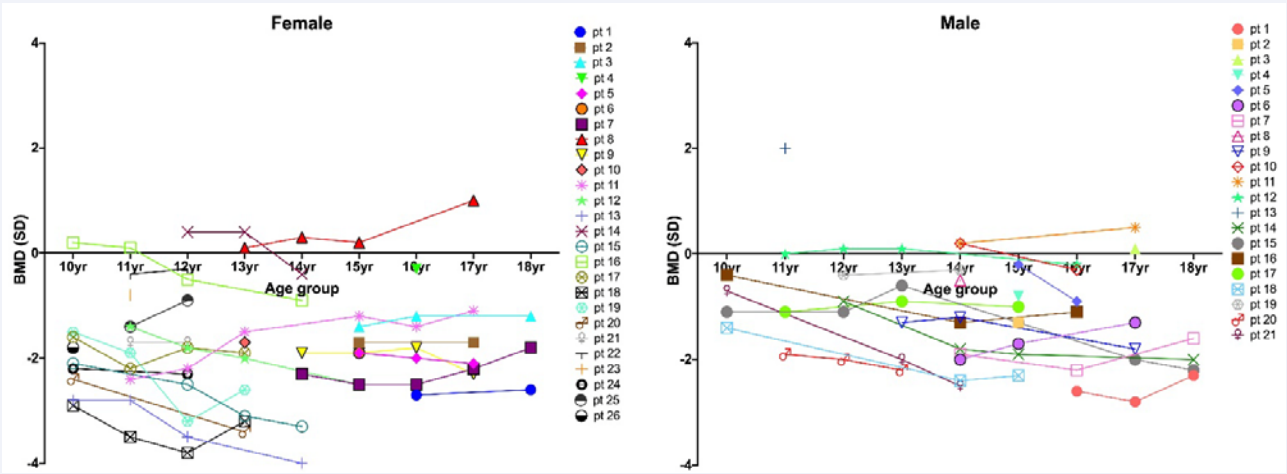

Figure 2 Longitudinal BMD age matched Z-scores in female and male patients.

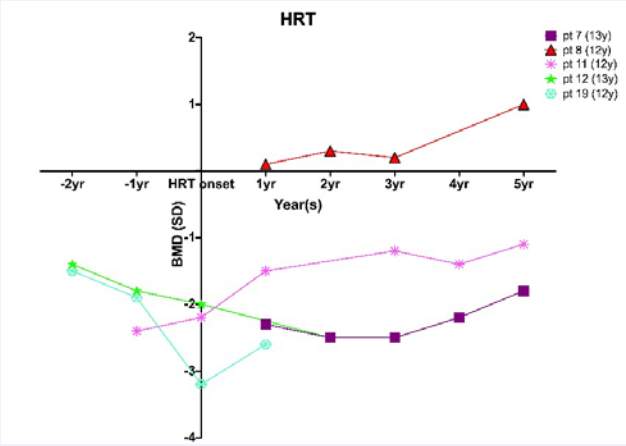

Figure 3 Longitudinal BMD age matched Z-scores in 5 female patients pre and on HRT (age of HRT commencement in brackets).

were commenced on calcium supplementation as appropriate. The median calcium level was 2.4mmol/L (IQR 2.34, 2.48).

Vitamin D: All patients were on regular Vitamin D supplementation (except for 2 patients due to poor compliance). Patients were commenced on Vitamin D supplementation from as early as the age of 4 (range 4- 14 years old). The median level of Vitamin D was 77nmol/L (IQR 54, 99). Suboptimal Vitamin D levels (<50nmol/L), were observed in 34% of patients (9 female; 7 males), at any point in the 10 year follow up period.

Physical activity: The majority of CG patients had normal mobility and were able to do an average amount of physical activity appropriate for age. 3 patients (1 male), required assistance with mobility, the 2 female patients required assistance as one had suffered from ischaemic stroke and the other had brain structural abnormalities (unrelated to CG). 4 patients (1 male), participated in higher intensity physical activity.

DISCUSSION

CG is a rare inborn error of carbohydrate metabolism due to a deficiency in the galactose-1-phosphate uridylyltransferase (*GALT*), enzyme. This leads to failure to metabolize Galactose-1-Phosphate (*Gal-1-P*), which consequently accumulates and generates alternate pathway metabolites (galactitol, galactonate). Lifelong dietary restriction of galactose has been the therapeutic basis for CG. This is extremely effective in eliminating acute clinical presentations, however despite early and lifelong dietary treatment, many patients go on to experience long term complications (e.g. impaired bone health, cognitive disability, speech problems, neurological and/or movement disorders) [14].

A reduction in BMD was first described in 1993 [5], and subsequent reports have shown evidence of this feature in patients with CG [6-9]. It is uncertain whether this is secondary to the restricted diet, a primary intrinsic effect or a combination of both.

In this paper, we have presented 10 year longitudinal and individual serial patient data on BMD in CG patients from a single centre. In our cohort of 47 patients, when analysed in gender groups, both groups display comparable biochemical markers for Gal-1-P, Vitamin D, ALP and calcium levels. Sixteen female patients commenced on HRT.

Comparing BMD age matched Z-scores of female and male patients in our cohort, we documented a significant difference (p=0.02). To our knowledge gender based comparison of BMD age matched Z-scores have not been reported previously. This study also demonstrates serial DEXA scan study results in 5 patients who commence on HRT. Results showed that in 4 of 5 patients, BMD age matched Z-scores improved over time with the most significant improvement from -2.4 SD to -1.1 SD.

Patients in this study showed diminished BMD median age matched Z-score of -1.7 SD (IQR -2.2, -0.9). A meta-analysis by van Erven et al., showed that in 112 CG patients the mean BMD age match Z-score was -0.7 (95% CI -0.88, -0.52) [15]. In contrast to these included studies, our patients' demonstrated lower BMD age matched Z-scores. We do not believe that this is attributed to different therapeutic approaches but could be secondary to a smaller cohort size and/or genetic heterogeneity.

Impaired bone health in CG is a well-recognised long term complication and its aetiology remains to be elucidated. To support the best possible outcome of bone health in CG, regular monitoring of bone health parameters, nutritional support including calcium and Vitamin D supplementation, regular physical activities and the commencement of HRT in females as appropriate in a timely fashion, are all crucial factors.

CONCLUSION

Our study showed that decreased BMD is a common feature in CG paediatric patients. This finding has been supported by previous cohort studies. Diminished BMD occurs despite patients being on optimal diet and nutritional supplements, being physically active and receiving prompt treatment for primary ovarian failure.

The aetiology of reduced BMD in galactosaemia remains to be fully explained. A better understanding of the underlying pathogenic mechanism will inform future development of improved therapeutic strategies.

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