Serum markers of bone turnover in children and adolescents with classic galactosemia

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ABSTRACT

Purpose: Classic galactosemia is an inherited metabolic disease resulting from galactose-1-phosphate uridyltransferase (GALT) deficiency. Dietary lactose exclusion reverses many clinical manifestations of acute phase of the disease. Unfortunately most of the patients, despite dietary treatment, develop long-term complications among them disturbances of bone mineralization resulting in decrease of bone mineral density (BMD). The aim of our study was to assess bone formation and resorption processes with bone turnover markers in children and adolescents with galactosemia.

Materials and methods: We studied 62 galactosemic children (mean age±SD 5.9±2.7 years) and adolescents (mean age±SD 15.6±2,4 years). The clinical diagnosis had been confirmed by the absence of GALT activity in erythrocytes. All patients were diagnosed in the neonatal period and had good dietary control. Healthy children (n=70) were the reference group. Serum osteocalcin (OC), bone alkaline phosphatase (BALP), collagen type I crosslinked C-telopeptide (CTX-I), 25(OH)D metabolite of vitamin D were determined by ELISA assays.

Results: We observed similar mean values of bone formation markers in children with galactosemia as compared to the age-matched controls. The level of bone resorption marker CTX-I in these patients was lower by about 20% (p<0.001) than in healthy children. On the contrary we obtained slightly higher values of CTX-I in adolescents with galactosemia in comparison to the age-matched controls. In these patients the values of OC and BALP were significantly higher than in healthy adolescents (111.8±52.1μg/L versus 82.3±43.0 μg/L, p<0.02; and 95.4±45.7 U/L versus 72.6±40.6 U/L, p<0.05 respectively).

Conclusion: Our results suggest that bone turnover in galactosemic patients elevates from childhood to adolescence, whereas in healthy individuals there is a decline during aging. Further studies on adults with galactosemia are necessary to assess bone status in these patients.

Key words: bone markers, galactosemia, children, adolescents

INTRODUCTION

Biochemical bone markers are a valuable noninvasive tools useful in management of metabolic bone diseases. Among markers that may reflect both modeling and remodeling bone processes bone fraction of alkaline phosphatase (BALP) as well as osteocalcin (OC) and collagen type I cross-linked C-telopeptide (CTX-I) can be measured in blood [1]. BALP and OC are released into the circulation by osteoblasts predominantly in the osteoid formation and during the matrix mineralization phases respectively. During the bone resorption process as a result of the osteoclast mediated degradation of type I collagen CTX fragments are released into circulation. These fragments have high bone specificity, because osteoclasts are not active in the degradation of other type I collagen-containing tissues [2]. Physiologically, these three markers were increased in children particularly during the first year of life and during puberty. In postpubertal period a gradual decrease of bone turnover markers to values observed in young adults was demonstrated [3-5].

Deficiency of galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12) activity is the most common disorder of galactose metabolism and is responsible for classic galactosemia (McKusick 230400). The deficiency of GALT leads to accumulation of galactose-1-phosphate and to oxidation and reduction of galactose to galactonate and galactitol respectively. Infants with galactosemia usually exhibit feeding difficulties, jaundice, vomiting, diarrhea, sepsis, liver diseases, renal tubular dysfunction, some degree encephalopathy and growth retardation. Acute neonatal or infantile manifestation is reversed with galactose- and lactose-restricted diet [6]. Unfortunately, most of the patients despite dietary treatment develop long-term complications including mental retardation, ovarian failure as well as disturbances of bone metabolism resulting in decreased of bone modeling and growth [7,8]. In many patients with galactosemia, growth is delayed in childhood and early adolescence, but the final height is usually normal [9,10]. The pathological mechanism leading to a diminished bone mineral content in this metabolic disease is not well known. Dietary deficiencies and/or intrinsic defect in the galactosylation of the bone collagen matrix may be risk factors for osteopenia and osteoporosis in later life of galactosemic patients [11]. Biochemical evidence for abnormal galactosylation is found in the altered isoform patterns of serum transferrin, β-hexosaminidase and follicle stimulating hormone [12-14]. Moreover, N-acetylgalactosamine and galactosyl residues were reduced in lymphocytes and brain lipids of galactosemic infants in comparison to the controls [15]. It has been hypothesized that in classic galactosemia as a result of deficient galactose residues abnormal collagen is also formed, thereby interfering with bone formation and mineralization [11]. According to Kaufman et al. [11] who used computed tomography, prepubertal as well as postpubertal patients of both sexes had bone mineral density (BMD) below the level observed in the control group. This abnormality was more pronounced in adults than in children. Decreased bone mass in young children with galactosemia was also found by Rubio-Gozalbo et al. [16] and Panis et al. [17]. Changes in profile of bone turnover markers in patients with galactosemia mainly children in prepubertal period were observed [17,18]. The aim of presented study was to assess bone formation and resorption processes with bone turnover markers in children and adolescents with galactosemia.

MATERIALS AND METHODS

Our study group consisted of 62 galactosemic children (age range 2-20 years; 30 girls, 32 boys) treated at the Department of Pediatrics of the Institute of Mother and Child in Warsaw. The patients were divided into two subgroups: galactosemic children (age range 2-9 years; 15 girls, 17 boys) and galactosemic adolescents (age range 10-20 years; 15 girls, 15 boys) (Table 1). The clinical diagnosis was confirmed by the absence of galactose-1-phosphate uridyltransferase activity in erythrocytes [19]. All patients were diagnosed in the neonatal period and had good dietary control. Children and adolescents with galactosemia were receiving daily 400-800 mg and 800-1200 mg calcium respectively. They had an adequate physical activity and none of them had a concomitant disease that could affect bone metabolism. Healthy children sent to our laboratory for routine analytical control (n= 70; 35 girls, 35 boys) were the reference group which showed normal physical development and had no diseases that could affect bone metabolism. None of them was receiving any medication. The control group was divided into subgroups: healthy children (mean age±SD 6.4±2.6; 20 girls, 20 boys) and healthy adolescents (mean age±SD 15.6±2.4; 15 girls, 15 boys) (Table 1). Pubertal status was classified according to the method of Tanner and Whitehouse [20]. One girl with galactosemia was receiving hormone supplementation. There were no patients with untreated delayed puberty. Weight and height were assessed as the number of standard deviation from mean of a Warsaw reference population (Z score) [21]. This study had been approved by the Ethics Committee of the Institute of Mother and Child.

Venous blood samples were obtained from all children and adolescents after an overnight fasting between 8^{oo} and 10^{oo} h and then centrifuged (1000 g for 15 min at 4^{0}C). Serum concentrations of calcium, phosphate, and 25-hydroxycholecalciferol - 25 (OH) D vitamin were determined. Remaining samples of serum were frozen at -20^{0}C and stored for up to three months before measurements of OC, BALP and CTX-I.

BALP activity was evaluated by enzyme immunoassay (Alkphase-B kit, Metra Biosystems, USA). The sensitivity of this assay is 0.7 U/L and the intra- and interassay CVs are below 2.7%. Total OC as sum of uncarboxylated and carboxylated forms was analysed immunoenzymatically using the N-Mid Osteocalcin ELISA kits (Nordic Bioscence Diagnostics, Denmark) which is based on the application of two highly specific monoclonal antibodies against human OC which recognising the midregion (20-29 AA) and the N-terminal region (10-16 AA) of osteocalcin. The sensitivity of this assay is 0.5 μg/L, the intra- and interassay imprecision CVs are less than 4% and 7% respectively. Serum CTX-I concentration was measured using Serum CrossLaps One Step ELISA assay (Osteometer, Biotech, Denmark). This method is based on highly specific monoclonal antibody against a β-aspartate isomerized form of the sequence EKAHD-β-GGR derived from the C-terminal telopeptide region of the type I collagen α1-chain. According to the manufacturer, the intra- and interassay imprecision (CVs) are less than 3% and 10.9% respectively. The lower detection limit is 293 pmol/L. 25(OH)D vitamin was determined by the kit from Biomedica (Austria). The detection limit is 0.6 ng/ml and intra- and interassay imprecision (CVs) are less than 10.8% and 10.5% respectively. Calcium and phosphate were assayed by standard kits with Cobas Integra analyser (Roche, Switzerland).

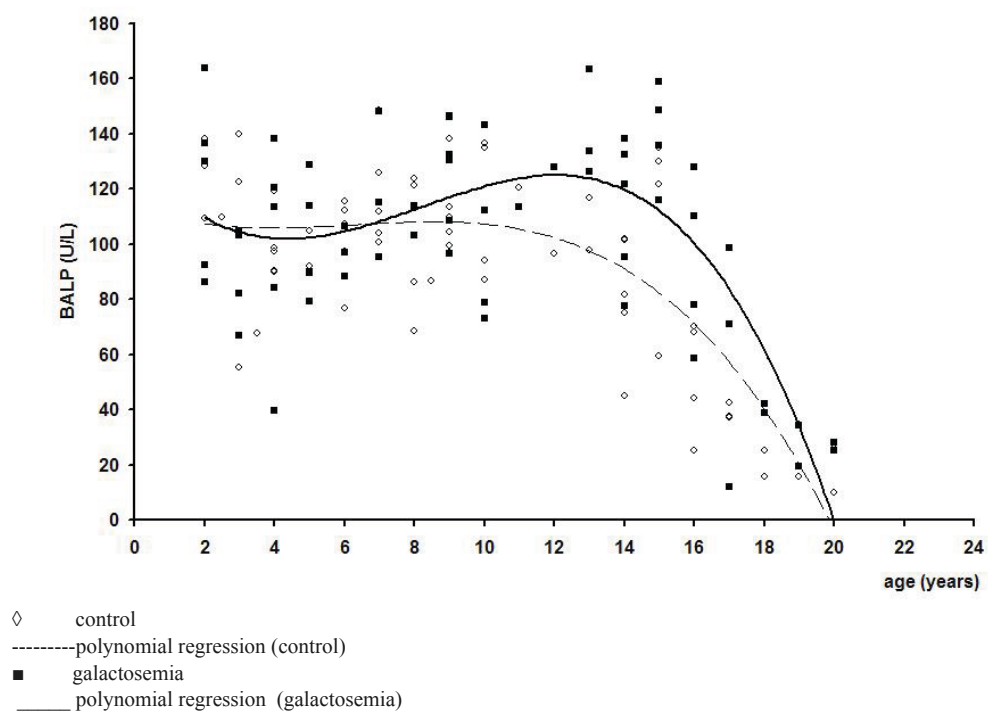*Figure 1.* **Individual serum BALP activities in galactosemic and healthy children.**

◊   control
---------polynomial regression (control)
■   galactosemia
_____ polynomial regression (galactosemia)

Figure 2. **Individual serum OC concentrations in galactosemic and healthy children.**

◊   control
---------polynomial regression (control)
■   galactosemia
_____ polynomial regression (galactosemia)
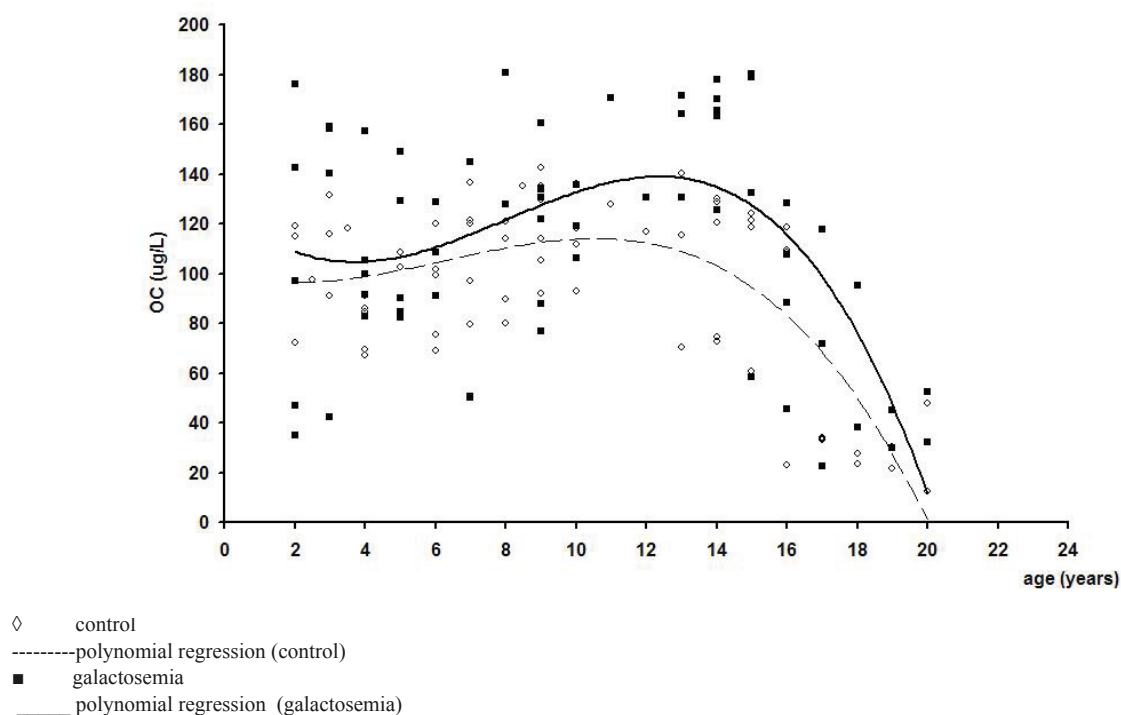
Figure 3. **Individual serum CTX concentrations in galactosemic and healthy children.**

◇   control

--------polynomial regression (control)

■   galactosemia

_____ polynomial regression (galactosemia)
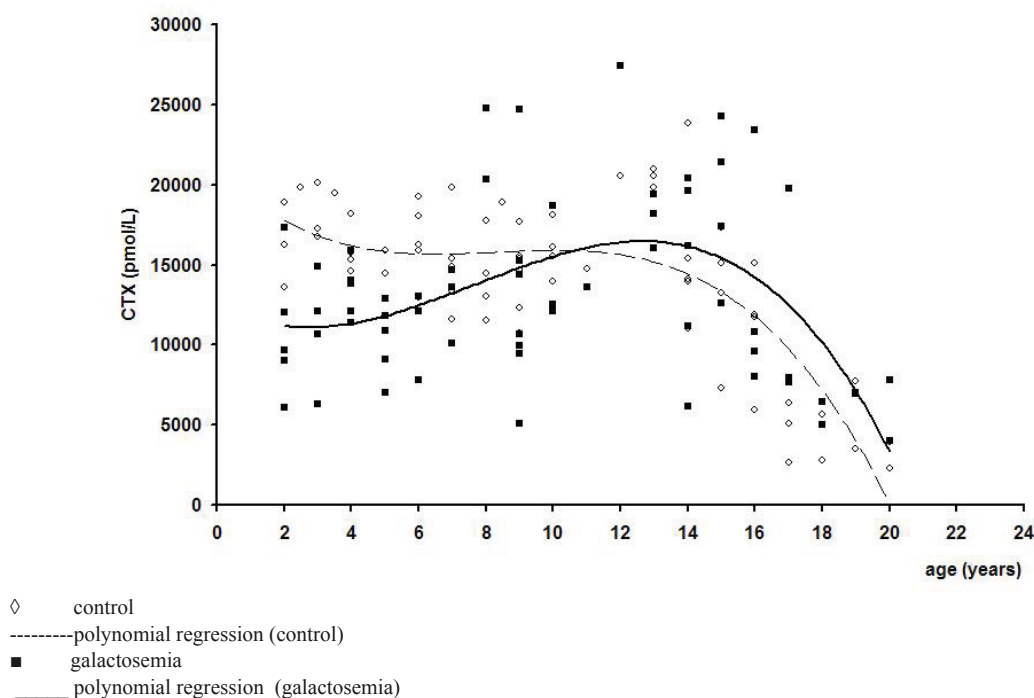
Table 1. **Characteristics of the study groups.**

Characteristics	Children		Adolescents	
	Galactosemic	Healthy	Galactosemic	Healthy
Number (n)	32	40	30	30
Age (years)	5.9 ± 2.7	6.4 ± 2.6	15.6 ± 2.4	15.6 ± 2.4
Gender (F/M)	15/17	20/20	15/15	15/15
Tanner stage: 1,2,3,4,5	32, 0, 0, 0, 0	35, 5, 0, 0, 0	3, 0, 5, 10, 12	0, 0, 3, 10, 17
Weight Z score	-0.9 ± 0.5*	0.1 ± 0.7	-1.0 ± 0.64*	-0.2 ± 0.4
	(-1.7 to 0.3)	(-1.2 to 1.0)	(-2.4 to 0.5)	(-0.8 to 0.6)
Height Z score	-0.6 ± 1.0*	-0.1 ± 0.6	-0.6 ±0.8*	-0.1 ± 0.5
	(-2.7 to 0.9)	(-1.0 to 1.2)	(-1.9 to 0.8)	(-0.8 to 1.0)
BMI	14.7 ± 1.2*	16.3 ± 1.8	17.4 ± 2.7*	20.1 ± 1.9

Data are given as mean ± SD. Minimum and maximum values are shown in the parenthesis.

*$p < 0.001$ in children and adolescents groups in comparison to the healthy groups.

Normal distribution of the analyzed data was verified with the Kolmogorov-Smirnov test. All data were compared by Student's t-test. Pearson correlation was computed between concentration of bone turnover markers in studied groups children and adolescents. Differences were regarded as statistically significant at $p < 0,05$.

RESULTS

Serum levels of bone turnover markers for galactosemic individuals and healthy children and adolescents are shown in Fig.1 – Fig.3. We observed that BALP values tend to be similar in both children groups (age range 2-9 years). Only 2 patients with galactosemia had BALP values out of control range (55.3 – 148.8 U/L, Table 2). In galactosemic children more different results for OC levels were found. Among 32 patients, 5 had lower and 8 had higher levels of OC than healthy children (range 67.2 – 142.7 µg/L, Table 2). However, mean values of both bone formation markers were similar in patients and the age-matched controls (Table 2). The level of bone resorption marker CTX-I was lower by about 20% ($p < 0.001$) in the galactosemic group than in controls. No correlations between tested markers were observed.

The values of BALP and OC in adolescents with galactosemia (age range 10 -20 years) were higher by about 30% ($p < 0.05$) and 35% ($p < 0.02$) respectively than in age matched healthy subjects (Table 2). CTX-I values tend to be slightly higher (about 15%) in patients than in the age-matched controls. Significant positive correlations between all tested markers in adolescents with galactosemia (n=30; r=0.64-0.78, p<0.0001) as well as in the reference group (n=30; r=0.82-

Table 2. **Serum bone turnover markers in galactosemic patients.**

Group	BALP(U/L)	OC(µg/L)	CTX(pmol)
Galactosemic children	108.5 ± 26.7	111.9 ± 39.4	12435 ± 4456***
	(66.8 – 164.1)	(50.3 – 180.6)	(6100 – 24787)
Healthy children	105.6 ± 21.2	104.9 ± 21.0	15778 ± 2464
	(55.3 – 148.8)	(67.2 – 142.7)	(10706 – 20087)
Galactosemic adolescents	95.4 ± 45.7*	111.8 ± 52.1**	13722 ± 6474
	(11.9 – 163.3)	(30.1 – 180.4)	(3994 –27419)
Healthy adolescents	72.6 ± 40.6♦♦♦	82.3 ± 43.0♦	12016 ± 6326♦♦
	(10 – 134.8)	(12.2 – 140.1)	(2325 – 23836)

Results were expressed as mean value ±SD
Minimum and maximum values are shown in the parenthesis

* $p < 0.05$ between galactosemic adolescents and healthy adolescents
** $p < 0.02$ between galactosemic adolescents and healthy adolescents
*** $p < 0.001$ between galactosemic children and healthy children
♦ $p < 0.05$ between healthy adolescents and healthy children
♦♦ $p < 0.01$ between healthy adolescents and healthy children
♦♦♦ $p < 0.001$ between healthy adolescents and healthy children

0.85, $p < 0.0001$) were found.

We observed statistically significant differences in serum concentrations of tested bone markers between healthy children and adolescents. The values of BALP, OC and CTX-I were lower in adolescents than in children by about 30% ($p < 0.001$), 20% ($p < 0.05$) and 20% ($p < 0.01$) respectively. On the contrary, similar values of tested bone markers in galactosemic children and adolescents were obtained. Indices of bone turnover were intercorrelated in healthy group (n=70; r=0.69-0.71, $p < 0.0001$) and in the group of galactosemic patients (n=62; r= 0.42-0.50, $p < 0.001$).

In galactosemic children and adolescents the median values of calcium 2.46 mM (2.20-2.77), phosphorus 1.56 mM (1.01-1.92), 25OH-D vitamin 27.3 µg/L (17.2-59.3) were within the normal ranges.

DISCUSSION

During childhood and adolescence bone turnover markers are released into the circulation as a result of bone modeling and remodeling processes. The use of these biochemical parameters allows the assessment of antiresorptive treatment or disease progression in children with metabolic bone disease [22]. Several studies have shown an association between biochemical markers of bone turnover and fracture risk mainly in adults [23,24]. According to Szulc et al. [25] insufficient accumulation of skeletal mass in patients through childhood may predispose them to fractures in later life.

Our results suggest that bone metabolism abnormalities in children with galactosemia may be a result of imbalance between bone resorption and bone formation processes. These abnormalities occur already in the prepubertal period and relate mainly to bone resorption. The lower values of CTX-I and additionally NTX (N-terminal telopeptide) in children with galactosemia at age 3-17 years were previously observed by us and also by other authors [10-12]. Since these changes are observed in the prepubertal period their connection with abnormal galactosylation of the bone collagen matrix may be a result of deficiency of GALT activity. However in our study CTX-I concentration in adolescents with galactosemia tend to be higher than in age-matched controls. Since in our patients no abnormalities of calcium, phosphorus and vitamin D were found, we suggest that disturbance in bone resorption process may be connected with other deficiencies caused by restricted diet. The role of some intrinsic factors can not be excluded as well.

In our study in children with galactosemia mean values of OC and BALP were similar to the age-matched healthy subjects. This is in agreement with the results of Rubio-Gozalbo et al. [16] who studied the group of 11 patients in the age range 2-18 years. Normal values of hydroxyproline, pyridinoline, osteocalcin and procollagen I carboxyterminal propeptide (PICP) in 5 patients in the age range 5-13 years observed also Fernandez Espuelas et al. [26]. However, Panis et al. [17] found decrease of OC as well as NTX and CTX-I in group of 40 children among them 31 were in prepubertal period. In our study we also observed changes in the pattern of bone formation markers in some children with galactosemia. The lower values of OC were often accompanied by the lower concentration of CTX-I. These may lead to the reduction of bone turnover in these patients.

The bone turnover markers in adolescents with galactosemia have not yet been studied extensively. In our study we observed that, these patients had higher concentration of the three tested markers than age-matched controls, which suggested a higher rate of bone turnover with predominance of the bone formation process. It is not clear if higher bone turnover in galactosemic adolescents may be associated with reduced bone mass and higher risk of osteoporosis in later life. Further studies of adults with galactosemia are necessary to assess bone status in these patients. We do not exclude that

higher bone turnover in galactosemic adolescents may reflect the adaptive mechanism ensuring proper bone mass after disturbances of bone metabolism in early childhood.

In our study the comparison between children and adolescents within both groups demonstrates age dependent changes in bone metabolism. Mean values of bone formation and resorption markers in adolescents with galactosemia were similar to those obtained in galactosemic children, although high variation in levels of these markers among age range were observed. On the contrary healthy adolescents in comparison to healthy children showed significantly lower physiological values of the three tested markers. This is in agreement with observations of other authors [3,4].

CONCLUSIONS

In conclusion, our results suggest that bone turnover in galactosemia patients elevates from childhood to adolescence, whereas in healthy individuals there is a decline during aging.

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