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Original Article

Basic laboratory bone profile in Greek patients with galactose metabolic disorders

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Abstract

Objective: the investigation of the relationship between different basic biochemical parameters of bone metabolism in galactosaemia patients. **Methods:** Study participants included twenty two patients with an average age 7.97±5.1 years, early diagnosed by newborn screening and dietary treated. Serum biochemical factors including calcium, phosphate, magnesium, alkaline phosphatase (ALP), creatinine, albumin, lipidaemic factors, vitamin-D, hemoglobin and ferritin were determined during a routine biochemical physical examination. Z-BMI, Z-Wt and Z-Ht were estimated for the assessment of normal or delayed growth. **Results:** Estimated biochemical variables did not demonstrate significant differences between patient groups (e.g. males-females, galactosaemia subtypes). Creatinine manifested lower values whereas ALP manifested highest values as compared to reference ranges. Significant differences were observed with respect to BMI estimates as well as Vitamin D vs. age, body weight vs. height in females. Vitamin D was negatively correlated to creatinine and HDL. Calcium was negatively correlated to creatinine, in all patients. **Conclusion:** These findings suggest that there is a place for periodic monitoring of basic biochemical bone profile in galactosaemic patients, in order to detect early subtle changes in bone metabolism.

Keywords: Galactosaemia, Vitamin D, Creatinine, BMI, HDL

Introduction

Galactosaemia is an inborn error of metabolism that results from impaired activity of any of the three enzymes of the Leloir pathway: a) galactokinase (GALK), b) galactose-1-phosphate uridylytransferase (GALT) and c) UDP galactose-4'-epimerase(GALE)[1].

Classic Galactosaemia (CG) (OMIM 230400) is the most clinically severe type of the disorder resulting in a deficiency of the GALT enzyme in erythrocytes, caused by homozygous or compound heterozygous mutations in the GALT gene. Classic Galactosaemia occurs in approximately 1/23.000- 40.000 newborns and leads to an accumulation of galactitol, galactose, galactonate and to a decreased production of UDP-GAL, which plays a very important role in glycoprotein and glycolipids formation in various tissues[2]. The clinical manifestations in newborn period-if untreated-may include: cataracts, jaundice, vomiting, hepatomegaly, liver failure and cirrhosis, *pseudotumor cerebri*, *Fanconi syndrome*, mental retardation, sepsis due to *Escherichia Coli* and death. In childhood and adolescence may present with adverse effects on the ovaries (primary ovarian failure) and bone, among other tissues[2-4].

The Galactokinase (GALK) deficiency (OMIM 230200) incidence is 1/50000-1000000 and causes excess accumulation of galactose and galactitol. The only consistent abnormality due to GALK deficiency is cataract formation[5,6]. The UDP galactose-4'-epimerase (GALE) deficiency (OMIM 230350), may present similar

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	n	Age (years)	Body Weight (kg)	Height (m)	BMI
Males	9	8.78±5.31	28.18±15.55	1.25±0.21	16.58±3.1
Females	13	7.42±4.9	23.31±12.1	1.11±0.3	18.38±3.8
GALT	15	9.7±5.04	29.04±14.6	1.28±0.2	16.67±2.96
GALK	5	4.2±2.4	18.3±7.2	0.92±0.24	21.73±2.68
GALE	2	4.5±3.5	14.8±6.25	0.99±0.27	14.7±1.7
TOTALS	22	7.97±5.2	25.3±13.82	1.17±0.27	17.65±3.62

Table 1. Biometric data of patients participated in the present study.

symptoms to those of classical galactosaemia: cataracts, hepatomegaly, jaundice, hypotonia, developmental delay or no symptoms. Its incidence is 1/6.200-64.800 and, due to decreased or absent GALE activity, there are increased levels of galactose-1 phosphate, galactitol, UDP-galactose and diminished levels of UDP-Glucose[6,7]. Two other galactose metabolic disorders (OGMD) which are rare and with good clinical outcome are called *Duarte* variant and exist in two different forms, Duarte1 and Duarte2[8].

The treatment includes a strict, lifelong galactose/lactose free diet, especially for the newborns suspected for the type of Classical Galactosaemia (GALT), even if the diagnosis is not already confirmed by enzymatic or mutation analysis[1].

Among other long-term complications of the disease, decreased Bone Mineral Density (BMD) is frequently encountered in Classic Galactosaemia, especially in adult men and women but is already described in prepubertal children[9,10].

The pathophysiological mechanism is not fully understood but a number of studies have revealed that several factors could negatively affect bone metabolism in this disease. These factors include, among others, the dietary restrictions, which may result in nutritional deficiencies[4,11], hypergonadotrophic hypogonadism[9,12], abnormal glycosylation of collagen or other glycoproteins[4,9] and the decreased concentrations of insulin-like growth factor (IGF-1) and insulin-like growth factor-binding protein-3(IGFBP-3)[4,11].

The aim of the present study was to investigate the basic metabolic bone profile in galactosaemic patients.

Patients and methods

Patients and anthropometry

Twenty two, early-diagnosed patients with a disorder of galactose metabolism, with an average age of 7.97±5.1 years (9 males-13 females) were included in the present study. They were all detected by the Greek National Newborn Screening Program, in the Institute of Child Health ("Aghia Sofia" Children's Hospital, Athens, Greece) and follow a strict galactose/lactose-free diet with Galactomin-17 (SHS-Scientific Hospital Supplies, Liverpool, UK). This product is a non-soya, casein based, lactose-free formula, enriched with vitamins and minerals (Ca: 426 mg/100 gr powder and Vitamin D: 9.3 μg/100 gr powder).

In the context of their regular follow up, special attention is paid to growth, therefore all patients undergo anthropometry measurements (weight, length/height, body mass index), initially on a monthly basis for the first three years of life and then yearly, until they reach adulthood, according to the local protocol. Patients' biometric data are summarized in Table 1. None of them receives any supplementation for calcium or/and vitamin D, at the time of the study.

Total galactose screening assay

An enzymatic colorimetric assay (Gal MMR 2000, R&D Diagnostics) is used for the quantitative determination at 550nm of Total Galactose (Galactose and Galactose-1-phosphate) concentration in neonates. The assay is performed in dried blood spot samples, collected in filter paper cards (Guthrie Card-whatman 902).The normal ranges for our laboratory are 1 to 7 mg/dl.

Biochemical-hematological investigations

Fasting serum samples for routine biochemistry and complete blood count are obtained from all patients, every 3 months till 2 years, every 6 months from 2 to 6 years and every year for children older than 6 years, in order to detect, if present: a) delayed growth and b) poor dietary compliance to the diet. According to the local protocol the biochemical profile include not only the bone but also the lipid profile. The determined parameters which were included in the study were Calcium (mg/dl), Phosphorus (mg/dl), Magnesium (mg/dl), Albumin (gr/dl), Alkaline Phosphatase (IU/l), Triglycerides (mg/dl), Total Cholesterol (mg/dl), HDL (mg/dl), LDL (mg/dl), Creatinine (mg/dl), 25(OH)D (ng/ml), Hemoglobin (gr/dl) and ferritin (μg/lt). The routine Biochemistry was performed in an automatic biochemical analyzer Advia 1800 (Siemens). Hematological parameters were determined using an automatic hematological analyzer (Advia 2120i, Siemens).

Biomarker, units, reference ranges	Vitamin D (ng/ml)	Ca^{+2} (mg/dl) 8.7-10.8	P^{+4} (mg/dl) 4.0-6.0	Mg^{+2} (mg/dl) 1.5-2.3	ALP (U/lt) 60-240	ALB (gr/dl) 3.4-4.8	CHOL (mg/dl) 120-200	Creatinine (mg/dl) 0.20-1.0	Triglycerides (mg/dl) 30-130	HDL (mg/dl) 35-84	LDL (mg/dl) <150	Hb (gr/dl) 11.5-14.5	Feritin (ug/lt) 10-150	Z-BMI	Z-Wt	Z-Ht
Males	30.98± 7.3	9.8± 0.2	4.6±0.4	1.97±0.02	243.78± 171.02	4.5±0.32	144± 27.06	0.42±0.2	64.44±39.03	57.89± 16.82	73.11± 15.3	12.33±1.82	96.79± 54.4	-0.52±0.43	-0.42±0.37	-0.004± 0.01
Females	27.6± 8.2	9.93± 0.33	4.92±0.65	1.9±0.055	313.46± 197.6	4.54±0.3	146.15± 27.63	0.45±0.12	63.84±37.51	56± 14.79	75.46± 24.54	14.06±6.3	55± 5.28	-0.46±0.75	-0.51±0.68	-0.05± 0.07
GALT	27.53± 8.9	9.82± 0.22	4.82±0.44	1.94±0.05	306.2± 217.45	4.5±0.32	148.6± 31.42	0.44±0.17	59±31.32	60.53± 14.58	73.8± 23.69	13.89±6.04	82.32± 45.74	-0.59±0.39	-0.67±0.49	-0.009± 0.01
GALK	33.66± 3.37	9.88± 0.25	4.44±0.48	NaN	245± 110.99	4.7±0.21	142.2± 10.28	0.44±0.13	61±16.68	51.4± 12.48	80.4± 13.36	12.08±0.51	46.89± 22.63	0.62±0.78	0.14±0.39	-0.11± 0.1
GALE	28.1± 3.1	10.25± 0.55	5.45±0.48	NaN	225.5± 44.5	4.45±0.25	128± 14	0.37±0.1	110±75	42±18	65±12	12.55±0.55	50.3± 16.7	-1.3±0	-0.52±0.47	-0.01± 0.008
TOTAL	28.98± 8.01	9.9± 0.3	4.8±0.6	1.94±0.05	284.95± 190.29	4.52±0.3	145.28± 27.42	0.43±0.16	641±38.14	56.77± 15.68	74.5± 21.28	13.35±5.05	73.8± 43.5	-0.48±0.63	-0.47±0.57	-0.03± 0.06

Table 2. Summary of biochemical parameters expressed as mean ± standard deviation (25(OH)-vitamin D (ng/ml)<12=deficiency; 12-20=insufficiency; >20=sufficiency)[28].

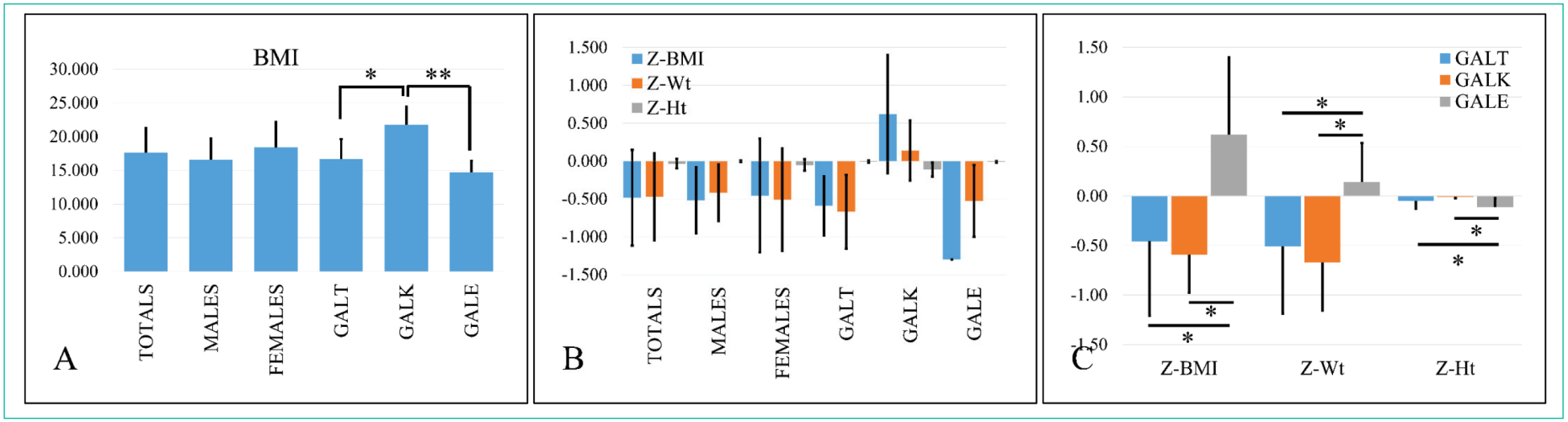

Figure 1. Descriptive statistics of BMI measurements (**A**) as well as Z-BMI, Z-Wt and Z-Ht (**B**). GALT and GALK manifested negative values for Z-BMI, Z-Wt and Z-Ht while GALK manifested positive values for Z-BMI, Z-Wt and Z-ht. Significant differences were observed between GALK and GALT as well as GALK and GALE subtypes (**A**). Further on, similar significant differences were observed with respect to Z-BMI, Z-Wt and Z-Ht (**C**). (* depicts a significance at the p<0.05 level and ** depicts a significance at the p<0.01 level. Totals indicate the mean of all samples irrespectively of gender or galactosaemia subtype. Legend: **BMI**: Body-Mass Index, **GALT**: galactose-1-phosphate uridylytransferase, GALK: galactokinase, **GALE**: UDP galactose-4'-epimerase).

	AGE	Vitamin D (ng/ml)	Ca (mg/dl)	P (mg/dl)	ALP (U/lt)	ALB (gr/dl)	CHOL (mg/dl)	Creatinine (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Hb (gr/dl)	Body Weight (kg)	Height (m)	BMI	Z-Wt	Z-Ht
AGE	1.0000																
Vitamin D (ng/ml)	-0.6729	1.0000															
Ca (mg/dl)	-0.4145	-0.0085	1.0000														
P (mg/dl)	0.1001	-0.1229	0.1796	1.0000													
ALP (U/lt)	0.3272	-0.3852	0.1891	0.0677	1.0000												
ALB (gr/dl)	-0.3008	0.0774	0.0643	-0.2353	-0.1010	1.0000											
CHOL (mg/dl)	0.3695	-0.3124	0.0803	-0.2561	0.6084	-0.0786	1.0000										
Creatinine (mg/dl)	0.7884	-0.6126	-0.4800	-0.0390	0.2849	-0.0986	0.2292	1.0000									
Triglycerides (mg/dl)	-0.4317	0.2509	-0.0234	0.2323	-0.3276	0.3457	-0.2496	-0.3883	1.0000								
HDL (mg/dl)	0.2667	-0.4158	0.3364	-0.3001	0.3645	-0.0425	0.6502	0.0686	-0.4524	1.0000							
LDL (mg/dl)	0.3367	-0.2080	-0.0958	-0.1264	0.3556	-0.0023	0.8199	0.2680	-0.0939	0.2806	1.0000						
Hb (gr/dl)	0.4111	-0.4282	0.2048	0.2553	0.3035	-0.1644	0.0212	0.5025	-0.5140	0.1791	-0.0113	1.0000					
Body Weight (kg)	0.9408	-0.6414	-0.4158	0.0313	0.2691	-0.2423	0.3237	0.7373	-0.3507	0.2160	0.3563	0.3029	1.0000				
Height (m)	0.9737	-0.6915	-0.3974	0.0729	0.2758	-0.3080	0.3301	0.7572	-0.4416	0.2454	0.3280	0.3817	0.9508	1.0000			
BMI	-0.0411	-0.0497	0.0330	-0.2313	-0.0305	0.2615	-0.0407	0.2274	0.3163	-0.0444	-0.0435	0.0792	0.1049	-0.0619	1.0000		
Z-Wt	-0.5633	0.3917	0.3206	-0.3348	-0.2896	0.2008	-0.1654	-0.4196	0.3833	-0.1197	-0.1331	-0.2986	-0.3130	-0.4792	0.5113	1.0000	
Z-Ht	0.1871	-0.1494	-0.2108	-0.0422	-0.1110	-0.2141	0.1250	-0.0288	-0.0973	0.1054	0.1230	-0.3331	0.3165	0.3113	-0.4532	0.1255	1.0000

Table 3. Pearson's Correlation analysis. Green boxes indicate a significant positive correlation, while red boxes indicate a significant negative correlation (marked *rho* values are at the $p<0.05$ significance level).

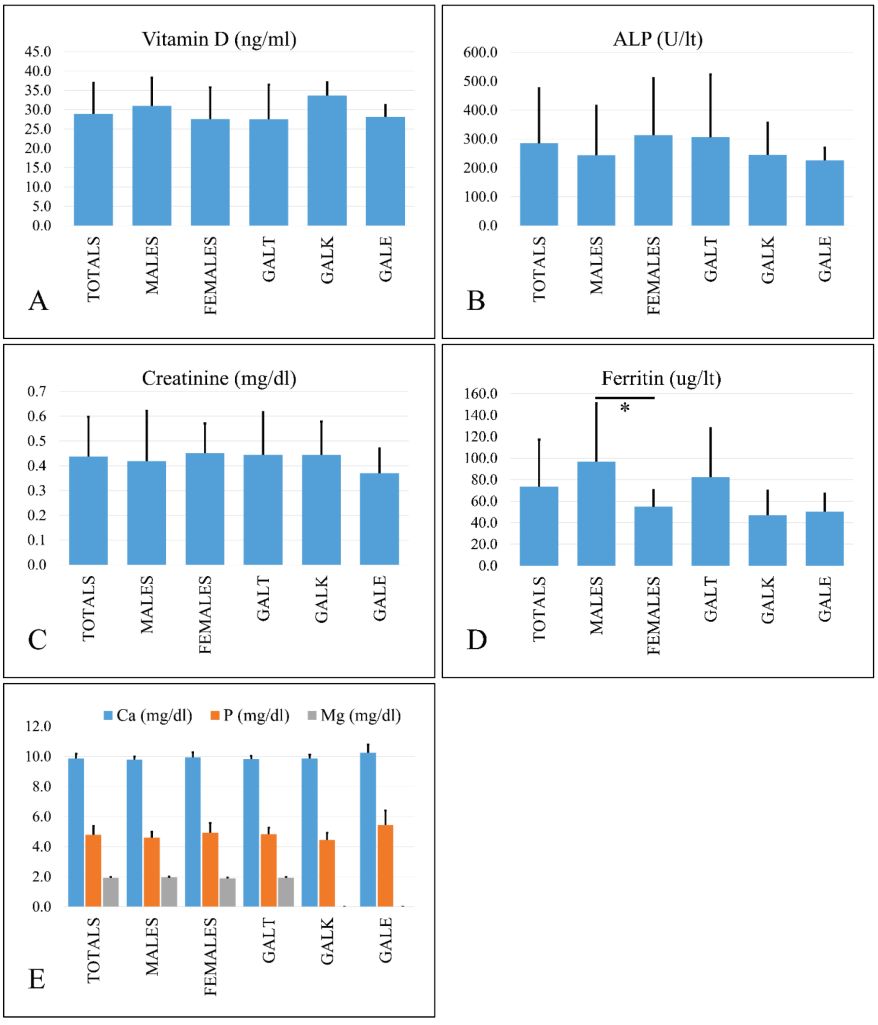

Figure 2. Descriptive statistics of Vitamin D (**A**), ALP (**B**), Creatinine (**C**), Ferritin (**D**) and Ca^{+2}, P^{+4}, Mg^{+2} (**E**). No significant differences were detected among the groups under investigation such as males and females as well as galactosaemia subtypes. There was one significant difference, where males manifested higher levels of ferritin as compared to females (D) (* depicts a significance at the p<0.05 level. Totals indicate the mean of all samples irrespectively of gender or galactosaemia subtype. Legend: **ALP**: Alkaline Phosphatase, **Ca**: Calcium, **P**: Phosphorus, **Mg**: Magnesium).

Data analysis

The multi-parameter analyses were performed with the MATLAB® simulation environment (The Mathworks, Inc., Natick, MA). The one- and two-way ANOVA tests were used to test the mean differences between groups. To facilitate comparisons between different age groups, all growth data were converted to Z-scores, using the Greek growth charts. Continuous variables are expressed as median ± standard deviation unless indicated differently. Correlations between variables were calculated using *Pearson's* Correlation coefficient. Linear regressions were performed using the $y=ax+b$ and/or the $y=ax^2+bx+c$ form and curves were estimated using chi-square approach. K-means, clustered scatter plots as well as Hierarchical Clustering algorithms were implemented with the MATLAB® simulation environment (The Mathworks, Inc., Natick, MA).

Results

Study population overview and subgroup analysis

All galactosaemic patients were under strict diet (lactose/galactose free diet) and in particular, those patients diagnosed with the classical type of the disease. That way t-Galactose concentrations is retained within reference values (T-Gal<7 mg/dl). Comparison of all anthropometric and biochemical parameters, related to bone and lipid metabolism of the patients versus reference population,

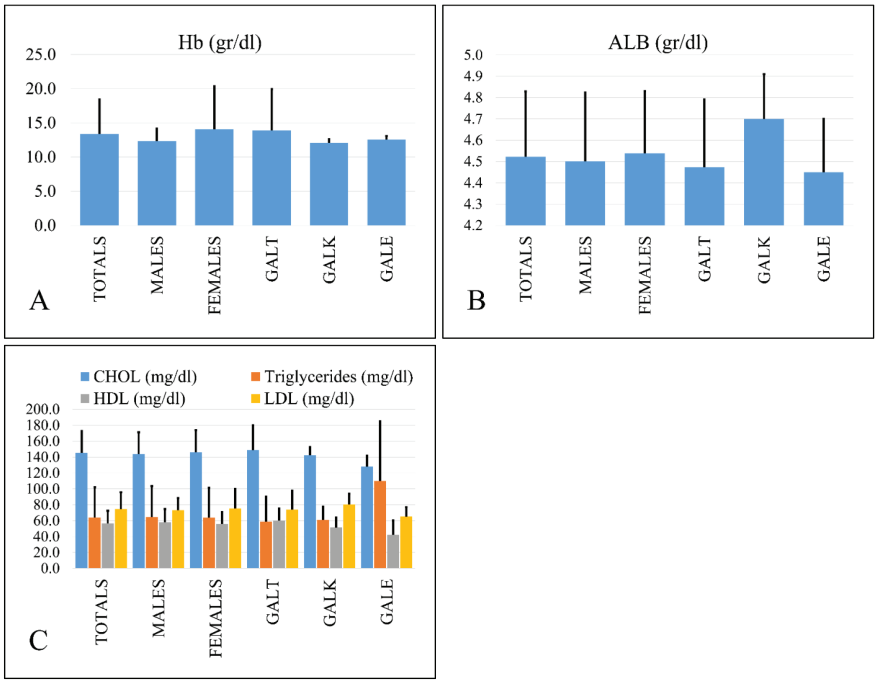

Figure 3. Descriptive statistics of Hemoglobin (**A**), Albumin (ALB) (**B**) and Cholesterol (CHOL), Triglycerides, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) (**C**). No significant differences were detected among the groups under investigation such as males and females as well as galactosaemia subtypes (Totals indicate the mean of all samples irrespectively of gender or galactosaemia subtype. Legend: **Hb**: Hemoglobin, **ALB**: Albumin, **CHOL**: Cholesterol, **HDL**: High Density Lipoprotein, **LDL**: Low Density Lipoprotein).

is presented in Table 2. Interestingly, serum Creatinine values were in the lower normal limits. In addition, 26% of GALT patients demonstrated Vitamin D levels below 20 ng/ml. Further on, ALP levels were found in the upper normal limits. No significant differences were detected among the subgroups under investigation that is males vs. females or between galactosaemia subtypes. Mean BMI was estimated for the whole population. A separate analysis was performed, based on gender (males vs. females) and galactosaemia subtype (Figure 1A). Further on, the mean values for all aforementioned comparisons have been estimated for Z-score values of BMI, Wt and Ht, i.e. Z-BMI, Z-Wt and Z-Ht, respectively (Figure 1B). Statistically significant differences were observed with respect to BMI between GALT and GALK patients as well as GALK and GALE patients (Figure 1A). In addition, significant differences were observed between GALT and GALK patients as well as GALK and GALE patients with respect to Z-BMI, Z-Wt and Z-Ht (Figure 1C).

The mean values of biochemical factors, which include Vitamin D (Figure 2A), Alkaline Phosphatase (ALP) (Figure 2B), Creatinine (Figure 2C), Ferritin (Figure 2D) and Ca^{+2}, P^{+4}, Mg^{+2} (Figure 2E) have been also estimated. No significant differences were detected among the aforementioned subgroups under investigation that is males vs. females as well as galactosaemia subtypes. There was only one significant difference, where males manifested higher levels of Ferritin, as compared to females (Figure 2D). Moreover, no significant differences were observed with respect to haemoglobin (Hb) (Figure 3A), albumin (ALB) (Figure 3B), cholesterol (CHOL), Triglycerides, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) (Figure 3C).

Pearson's correlation analysis

Since no significant differences were detected, we have investigated for possible correlations between different parameters. In particular, a negative correlation appeared between age and vitamin D (*rho*= -0.67), while positive correlations appeared between age and creatinine (*rho*=0.79), body weight (*rho*=0.94), height (*rho*=0.97) and Z-Wt (*rho*=0.56). Further on, negative correlations appeared between vitamin D and creatinine (*rho*=-0.61), HDL (*rho*= -0.41), Hb (*rho*= -0.42), body weight (*rho*= -0.64) and height (*rho*= -0.69). Also, negative correlations appeared between Calcium and creatinine (*rho*= -0.61) and body weight (*rho*= -0.41). Positive correlations were manifested between ALP and Cholesterol (*rho*=0.6), cholesterol and HDL (*rho*= -0.65) as well as LDL (*rho*=0.81), creatinine and body weight (*rho*=0.73) as well as height (*rho*=0.75). No

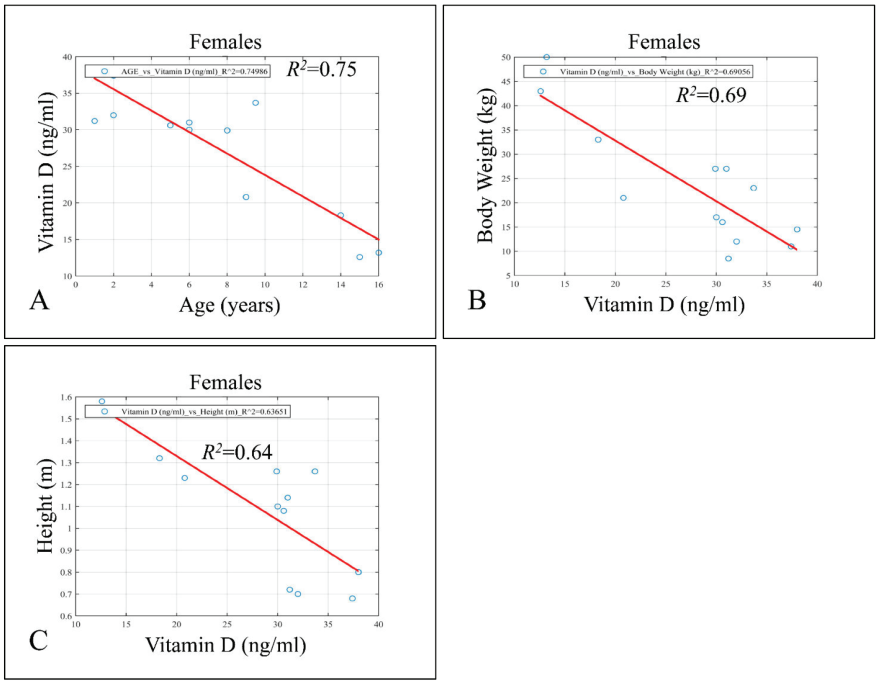

Figure 4. Regressions of age vs. vitamin D (R^2=0.75) (**A**), vitamin D vs. body weight (R^2=0.69) (**B**) and vitamin D vs. height (R^2=0.64) (C) in female patients. Significant correlation was observed among all studied variables (regressions are at the p<0.05 significance).

significant correlation was found between Vitamin D and BMI both in males and females. *Pearson's* correlation analysis results are summarized in Table 3.

Regression analysis

Based on the aforementioned estimated correlations, regression analysis was performed between biomarkers in order to further detect relations among studied variables. In the female population significant regressions appeared between age and vitamin D (R^2=0.75) (Figure 4A), vitamin D and body weight (R^2=0.69) (Figure 4B) and vitamin D and height (R^2=0.64) (Figure 4C). Finally, regressions in the total population with respect to Vitamin D vs. Creatinine (R^2=0.233) (Figure 5A), Vitamin D vs. HDL (R^2=0.19) (Figure 5B) and Calcium vs. Creatinine (R^2=0.20) (Figure 5C) manifested significant negative correlations.

Discussion

In this small cohort of patients, no significant differences were observed between biochemical indices. Interestingly, galactosaemia subtypes seemed to influence growth, as there was a significant difference in anthropometric Z-scores, especially of BMI. This observation could be important considering that BMI and BMD are positively correlated, according to the literature[13,14], but the sample size is too small for safe conclusions (n_{GALE}=2, n_{GALK}=5).

Vitamin D was negatively correlated to age, body weight and height in female patients.Previous studies have reported a growth delay in childhood and early adolescence and it is not clear whether this finding is related to nutritional deficiencies or to intrinsic factors of the disease[4,13,15]. Especially,for the females, a slow growth velocity is expected due to the endocrine complications. A balanced nutrition, with adequate calcium and close monitoring of their values, along with good dietary compliance and regular exercise are all measures to ensure satisfactory growth in this particular population.

Creatinine serum values were observed in the lower limit normal, in the study population (Table 3).It has been described that one of the clinical manifestations of galactosaemia is Fanconi syndrome, especially in GALT subtype[6]. In this disease, the accumulation of Gal-1-Phospate in cells, when a patient doesn't follow a strict lactose/galactose free diet may cause severe damage in brain, liver and kidneys[5,6]. This finding is not directly related to Fanconi disease, but each regular measurement in combination with other factors, such as mono-disaccharides in urine, could be very useful in early diagnosis of this syndrome, which has an implication in skeletal growth and damage[16].

In the subgroup of GALT patients, 26% of them had vitamin D concentration lower than 20 ng/ml. Previous studies have assessed the vitamin D levels in GALT patients, too. Some of them have reported concentrations in the

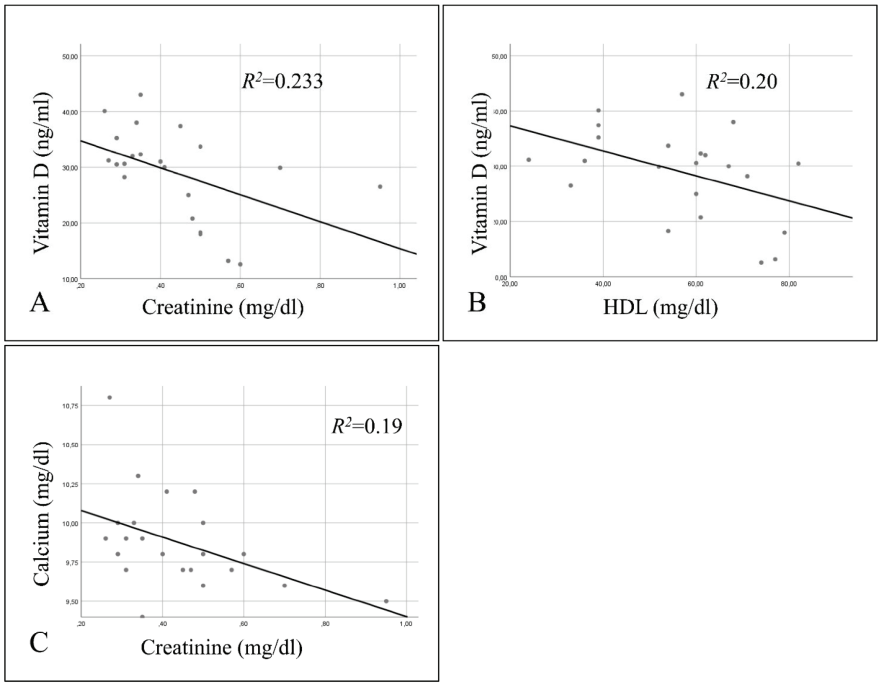

Figure 5. Regressions of Creatinine vs. vitamin D (R^2=0.233) (**A**), HDL vs. vitamin D (R^2=0.20) (**B**) and Creatinine vs. Calcium (R^2=0.19) (C) in the total population (regressions are at the p<0.05 significance).

low reference range and others, measured values within normal ranges[17,18]. The different approach of vitamin D reference values and units by the researchers still remains an important methodological issue. In GALT patients also, nutrition and physical status are not very well balanced as well as nutritional counseling. Low levels of vitamin D are reported in this population due to a) decreased physical activity[19], which results in less exposure to sunlight or b) diet deficits (low intake of Ca and/or vitamin D)[20].

For completeness, it is worth mentioning that, along with calcium and vitamin D supplementation, vitamin K is being investigated as a possible adjunct towards increasing bone mineral density[4,9,10,13,15]. In particular, *Panis B* and collegues (2006) reported a significant increase in cOC (carboxylated osteocalsin) concentration, in a prepubertal and pubertal children population, when receiving vitamin K combined with calcium and vitamin D[21]. cOC is a marker of bone formation and is required for the binding between calcium and hydroxyapatite in the bone[22].

The mean values of ALP are all upper than reference ranges (Table 2). This result, probably, shows that bone formation in childhood and adolescent is increasing in order to a maximal peak bone mass will be acquired[23], but the values of ALP are in the upper normal reference values for an age-matched normal population. Excluding the case of liver dysfunction for those patients and considering that ALP is one of the bone formation serum markers, these elevated values may be in line with the results of a previous study[17], which suggests that bone turnover is increasing in galactosaemia patients.

It is well known that 25(OH)D and cholesterol have the same precursor which is the 7-dehydrocholesterol (7DHC). Recent studies have investigated the association between the cholesterol and 25(OH)D levels, concluding that there is an inverse relationship between them: increased total cholesterol levels are associated with decreased 25(OH)D concentrations but not vice versa[24,25]. However, most studies which were included in a relevant meta-analysis, reported that there is a positive correlation between vitamin D and HDL[26]. In other words, this association has not been fully elucidated. In our study population, the levels between vitamin D and HDL demonstrated a negative correlation, indicating that in galactosaemia patients when HDL levels are increased, the vitamin D levels are decreased. *Vitezova* and his colleagues (2015), refer in their study that there is an inverse association between HDL and Vitamin D concentrations which may be bidirectional[24]. Furthermore, *Patwarham* and his colleagues (2015) tried to investigate the varying relationship between 25(OH)D, HDL and serum 7-dehydrocholesterol reductase (DHCR7)-which converts 7DHC to cholesterol-with sunlight exposure[27].

A significant negative correlation observed (p<0.05) between serum vitamin D and HDL, under intense sunlight exposure, whereas a positive correlation (p<0.05) was found between them in lower sunlight exposure. This finding has not been reported previously. Apparently, HDL levels seem to have a variation in association with 25(OH) D, when the sunlight exposure conditions are different.

Our results are in accordance with the latter studies above. A question could be posed: is the higher sunlight duration of our country responsible for this adverse trend or the disease itself? Further investigations in this specific population are needed to confirm or not these findings.

The observed correlations between creatinine and vitamin D and calcium levels may underline the importance of renal function as a basic regulator of calcium, phosphorus and magnesium homeostasis with the involvement of other factors like: parathormone (PTH) and vitamin D. Hence, any renal tubular dysfunction may negatively contribute to bone health of galactosaemia patients.

There are several limitations of this study. Galactosaemia is a rare inborn error of carbohydrate metabolism, hence, the small size of study population. Also, serial laboratory monitoring could be more informative in order to draw safer conclusions. Finally, detailed dietary dairies, degree of physical activity and sex hormone determination were not included in the present study.

Notably, monitoring of 25(OH)D is added as a routine biomarker to the follow -up protocol of Greek Galactosaemia Program lately, based on the results of this cohort of patients, ensuring better counseling protocol for bone health assessment. Therefore the derived findings from this study may result in adding new tools in the follow-up protocol, in order to detect, evaluate and possibly prevent bone complications in Galactosaemia patients.

Ethics statement: All measurements were conducted in compliance with the international biomedical studies stipulations, with reference to the Declaration of Helsinki of the World Medical Association (2013). No personal data of patients were kept, while it was impossible to trace back any personal data from the data collected for the present study.

Ethics approval and consent to participate: This work is based on the measurements included in the routine follow-up protocol which is applied to the Greek galactosaemia programme.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Authors' contributions: **MK**: Collected data, performed clinical evaluation, drafted the manuscript. **KHS**: Provided data, critical approval of the manuscript. **AD**: Critical approval of the manuscript, contribution to statistical analysis. **GIL**: performed data analysis, drafted the manuscript, proof-edited the manuscript and gave final permission for publication.*

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