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Identification of bone metabolism disorders in patients with Alström and Bardet-Biedl syndromes based on markers of bone turnover and mandibular atrophy

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ABSTRACT

Objectives: Causative variants in genes responsible for Alström syndrome (ALMS) and Bardet-Biedl syndrome (BBS) cause damage to primary cilia associated with correct functioning of cell signaling pathways in many tissues. Despite differences in genetic background, both syndromes affect multiple organs and numerous clinical manifestations are common including obesity, retinal degeneration, insulin resistance, type 2 diabetes and many others. The aim of the study was to evaluate bone metabolism abnormalities and their relation to metabolic disorders based on bone turnover markers and presence of mandibular atrophy in patients with ALMS and BBS syndromes.

Material and methods: In 18 patients (11 with ALMS and 7 with BBS aged 5–29) and in 42 age-matched ($p < 0.05$) healthy subjects, the following markers of bone turnover were assessed: serum osteocalcin (OC), osteoprotegerin (OPG), s-RANKL and urinary deoxypyridinoline - DPD. In addition, a severity of alveolar atrophy using dental panoramic radiograms was evaluated.

Results: Lower serum OC ($p = 0.0004$) and urinary DPD levels ($p = 0.0056$) were observed in the study group compared to controls. In ALMS and BBS patients, serum OC and urinary DPD values negatively correlated with the HOMA-IR index, while a positive correlation between the OC and 25-OHD levels and a negative correlation between s-RANKL and fasting glucose concentrations were found. A significant difference in the incidence of low-grade mandibular atrophy between patients with ALMS and BBS and controls ($p < 0.0001$) was observed.

Conclusions: The identification of bone metabolism disorders in patients with ALMS and BBS syndromes indicates the necessity to provide them with appropriate diagnosis and treatment of these abnormalities.

1. Introduction

Alström syndrome (ALMS) and Bardet-Biedl syndrome (BBS) are rare autosomal recessively inherited disorders with the incidence of 1 per 100,000 and 0.7 per 100,000 cases, respectively (Marshall et al., 2011; Forsythe and Beales, 2013; "Prevalence and incidence of rare diseases :

Abbreviations: 25-OHD, 25-hydroxyvitamin D; ALMS, Alström syndrome; AP, alkaline phosphatase; AST, aspartate transaminase; BBS, Bardet-Biedl syndrome; BMD, bone mineral density; BMI, body mass index; BMI SDS, body mass index standard deviation score; DM, diabetes mellitus; DPD, urinary deoxypyridinoline; DXA, dual energy X-ray absorptiometry; HDL, high-density lipoprotein; HOMA-IR, homeostatic model of insulin resistance assessment; IQR, interquartile range; LDL, low-density lipoprotein; LMS, least mean squares; MCW, mandibular cortical width; MIC, mandibular inferior cortex; Me, median; OC, serum osteocalcin; OPG, osteoprotegerin; PTH, parathyroid hormone; s-RANKL, soluble Receptor Activator of Nuclear factor κB Ligand; T2DM, type 2 diabetes mellitus.

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Bibliographic data. Orphanet Report Series, Rare disease collection. January 2020, Number 1"). Unlike BBS syndrome in which >20 genes variants with even triallelic model of inheritance have been described so far (Forsythe et al., 2018; Katsanis, 2001), ALMS syndrome is caused by up to date over 230 different mutations in one gene only – *ALMS1* located on chromosome 2p13 (Marshall et al., 2011, 2015).

Both ALMS and BBS belong to the ciliopathies. Mutations in the specific genes cause damage to the structures of the primary cilium (non-motile microtubule-based organelle found in most cell types) responsible for the proper functioning of the body's cell signal pathways. It is known that BBS proteins form the BBSome complex and chaperonin complex responsible for vesicular and molecular transport within the primary cilium (Álvarez-Satta et al., 2017). ALMS protein also plays a role in intraciliary transport, endosomal proteins function, cell migration and cell cycle regulation (Marshall et al., 2015). The prevalence of cilium in many organs and the significant role of proteins resulting from the expression of *ALMS* and *BBS* genes in important cellular processes determines the multitude and severity of symptoms observed in patients.

Main clinical symptoms of both syndromes include early-childhood obesity and retinal degeneration leading to blindness. Other common disorders diagnosed in the ALMS and BBS patients are: insulin resistance, type 2 diabetes mellitus (T2DM), kidneys failure, cardiovascular problems (cardiomyopathy or hypertension), liver diseases, hypothyroidism and dental and/or facial development abnormalities and many others. Unlike BBS patients, ALMS patients do not present symptoms of polydactyly or brachy- and syndactyly, as well as an intellectual disability but only show motor development delay and school difficulties (Beales et al., 1999; Forsythe and Beales, 2013; Majumdar et al., 2012; Marshall et al., 2011; Panny et al., 2017; Tahani et al., 2020).

Different time of appearance of individual symptoms, their degree of expression and overlapping of clinical features make it very difficult to establish an accurate and early diagnosis on the basis of clinical observation alone. Therefore, proper diagnosis is also based on genetic testing, and carrying this out quickly is important for further care of patients (Jeziorny et al., 2020; Zmyslowska et al., 2016).

The presence of obesity diagnosed already within the first years of life in all patients with ALMS and BBS syndromes in combination with insulin resistance and subsequent development of hyperglycemia and diabetes can predispose to the occurrence of further metabolic disorders. According to many studies, bone metabolism abnormalities and osteoporosis are also mentioned among these potential disorders (Fintini et al., 2020; Halade et al., 2011; Hou et al., 2020; Montalvany-Antonucci et al., 2018a, 2018b). In addition, the presence of primary cilia in bone cells and their involvement in bone development and metabolism, as well as the contribution of selected proteins of the BBS complex in ossification disorders, prompt the search for potential bone metabolism disorders in patients with ALMS and BBS (Barba et al., 2018; Kaku and Komatsu, 2017). It is known that bone metabolism can be assessed by the routine laboratory biochemical tests including vitamin D level and recognized markers of bone turnover such as: serum levels of osteocalcin (OC), soluble Receptor Activator of Nuclear factor κB Ligand (s-RANKL) and its antagonist osteoprotegerin (OPG) and urine concentration of pyridinoline (DPD) (Boyce and Xing, 2007; Delmas et al., 1991; Seibel, 2005; Zoch et al., 2016). Furthermore, in the search for new indicators of bone metabolism, it should be mentioned that some reports suggest that alveolar bone loss may precede skeletal atrophy and therefore pantomographic imaging should be considered as a screening test in the patients (Božič and Hren, 2006; Tounta, 2017). However, there are no studies on the relationship between generalized bone lesions and alveolar atrophy in children. This seems particularly relevant in pediatric patients with genetically determined bone lesions.

The aim of the study was to evaluate markers of bone metabolism turnover and their relation to metabolic disorders in patients with ALMS and BBS syndromes. In addition, the presence and severity of mandibular atrophy was assessed in patients in the study and control groups.

2. Material and methods

2.1. Patients

The study protocol was approved by the University Bioethics Committee at the Medical University in Lodz, Poland (RNN/343/17/KE and RNN/267/18/KE). Patients and/or their parents gave written informed consent for participation in the study.

The study group consisted of 18 patients with genetically confirmed ALMS or BBS syndromes (aged 5–29 years) including 11 ALMS and 7 BBS patients referred to the Department of Pediatrics, Diabetology, Endocrinology and Nephrology of Medical University of Lodz, Poland. The control group included 42 age-matched ($p < 0.05$) healthy subjects (median age = 13 years (IQR25%–75 %: 10–16); M/F: 14/28; BMI Me = 19.5 kg/m^2 (IQR25%–75 %: 17.1–21.1) without overweight/obesity, glucose metabolism disorders, chronic kidney disease and cirrhosis. None of the study participants had end-stage renal insufficiency (only 4 patients of the study group had stage II chronic kidney disease (CKD) with the lowest eGFR level according to the CKD-EPI creatinine formula of 73 mL/min/1.73 m^2), a history of frequent bone fractures and no medical history of systematic steroids intake. All chronic diseases such as hypogonadism, hypothyroidism and diabetes mellitus were in a compensated state at the time of the study.

2.2. Methods

An analysis of each patient included clinical measurements and laboratory tests such as: lipids profile, OGTT – oral glucose tolerance test, HbA1c and fasting C-peptide level, insulin resistance based on HOMA-IR–homeostatic model of insulin resistance assessment, kidney function parameters – serum creatinine and/or cystatin C levels and liver functions parameters. The BMI (body mass index) was defined as body weight divided by the square of body height (kg/m^2). Then, the *Z*-score for the body mass index standard deviation score (BMI SDS) was calculated by LMS (least mean squares) method according to Cole et al. (Cole, 2000). Detailed characteristics of the study group is shown in Tables 1 and 2.

Next, routine biochemical bone metabolism parameters such as: serum levels of 25-hydroxyvitamin D (25-OHD), alkaline phosphatase (AP), parathyroid hormone (PTH), calcium and phosphorus) and bone turnover markers - serum OC, OPG and s-RANKL concentrations and urine DPD levels were measured. In both patients and controls, serum and urine samples were collected from fasting subjects in the morning, frozen and then stored at −80 °C for further analysis.

Serum OC level was evaluated by the N-MID Osteocalcin test using an electrochemiluminescence method (ECLIA) (Roche Diagnostics GmbH, Germany), while serum OPG and s-RANKL levels were assessed by the ELISA method (respectively, BI-20403, BIOMEDICA, Austria and BI-20462, BIOMEDICA, Austria). Urine DPD level was calculated in a single morning urine sample using a chemiluminescence method (Siemens, UK).

In addition, in both groups, an assessment of the condition of the masticatory organ was performed, by standard intraoral examination and using a dental panoramic radiograph (Gendex, PA, USA). On the basis of the pantomographic images, the IC/IM resorption index was calculated, indicating the degree of alveolar bone loss according to Wical and Swoope in Ortman's modification (Ortman et al., 1989; Wical and Swoope, 1974). The IC/IM ratio was measured as the numerical ratio of two sections: IC - the distance from the lower edge of the mandible to the upper border of the alveolar part, assessed in the line of the chin opening, and IM - the distance from the lower edge of the mandible to the lower edge of the chin opening (Fig. 1). The interpretation of the IC/IM score was as follows: IC/IM >3 - no alveolar atrophy; IC/IM from 3 to 2.34 - small atrophy; IC/IM from 1.67 to 2.33 - moderate atrophy and IC/IM < 1.66 - severe atrophy. In addition, the mandibular cortical width (MCW) was measured, as previously described (Ledgerton

Table 1
Detailed clinical and genetic characteristics of the study group.

Patient ID	Gene/ Phenotype OMIM No.	Gene/ Locus MIM No.	Sex (F/ M)	Age (years)	Ophtalmologic abnormalities	Polydactyly	Obesity	Learning problems	Renal impairment	Other manifestation
BBS No. 1	*BBS10* #615987	610148	M	21	Yes	Yes	Yes	No	Yes	insulin resistance, bronchial asthma, cryptorchidism, dyslipidemia, scoliosis, cardiovascular problems
BBS No. 2	*BBS8* #615985	608132	M	5	Yes	Yes	Yes	Yes	Yes	insulin resistance, dyslipidemia, autism, cardiovascular problems, hearing loss
BBS No. 3	*BBS9* #615986	607968	F	7	Yes	Yes	Yes	Yes	Yes	insulin resistance, dyslipidemia, hyperglycaemia
BBS No. 4	*BBS10* #615987	610148	M	15	Yes	Yes	Yes	No	Yes	dyslipidemia, hypospadias, cardiovascular problems
BBS No. 5	*BBS9* #615986	607968	F	10	Yes	No	Yes	Yes	No	insulin resistance, dyslipidemia, hyperglycaemia
BBS No. 6	*BBS6* #605231	604896	M	6	No	Yes	No	No	Yes	–
BBS No. 7	*BBS2* (#615981) *BBS8* (#615985) *BBS10* (#615987)	606151 608132 610148	M	17	No	No	Yes	Yes	Yes	insulin resistance, hepatic steatosis, dyslipidemia,diabetes mellitus, hearing loss, scoliosis
ALMS No. 1	*ALMS1* (#203800)	606844	M	19	Yes	No	Yes	Yes	No	insulin resistance hypothyroidism, dyslipidemia, bronchial asthma, hearing loss
ALMS No. 2	*ALMS1* (#203800)	606844	M	17	Yes	No	Yes	Yes	No	insulin resistance, diabetes mellitus, hypothyroidism, bronchial asthma, dyslipidemia, hearing loss
ALMS No. 3	*ALMS1* (#203800)	606844	M	23	Yes	No	Yes	Yes	No	insulin resistance hypothyroidism, hypogonadism, dyslipidemia, hearing loss
ALMS No. 4	*ALMS1* (#203800)	606844	M	19	Yes	No	Yes	Yes	No	insulin resistance dyslipidemia, hearing loss
ALMS No. 5	*ALMS1* (#203800)	606844	F	20	Yes	No	Yes	No	Yes	insulin resistance, diabetes mellitus, dyslipidemia, cardiovascular problems, hearing loss
ALMS No. 6	*ALMS1* (#203800)	606844	M	29	Yes	No	Yes	No	No	insulin resistance, diabetes mellitus, bronchial asthma, hypogonadism, dyslipidemia, cardiovascular problems, hearing loss
ALMS No. 7	*ALMS1* (#203800)	606844	F	25	Yes	No	Yes	No	Yes	insulin resistance, diabetes mellitus, hypothyroidism, dyslipidemia, cardiovascular problems, hearing loss
ALMS No. 8	*ALMS1* (#203800)	606844	M	6	Yes	No	Yes	No	No	insulin resistance bronchial asthma, dyslipidemia, hearing loss
ALMS No. 9	*ALMS1* (#203800)	606844	M	24	Yes	No	Yes	Yes	No	insulin resistance, diabetes mellitus, pituitary adenoma, dyslipidemia, hearing loss
ALMS No. 10	*ALMS1* (#203800)	606844	F	9	Yes	No	Yes	Yes	No	insulin resistance dyslipidemia, hearing loss
ALMS No. 11	*ALMS1* (#203800)	606844	M	16	Yes	No	Yes	Yes	No	gynaecomasty, dyslipidemia, hearing loss

et al., 1999) (Fig. 1), which is interpreted as a predictor of reduced bone mineral density (BMD) when it is <3–4 mm (Karayianni et al., 2007; White et al., 2005). To reduce the error of the method, all measurements were taken twice, 2 weeks apart, by two independent researchers, experienced radiologist and dentist, separately for the right and left side of the mandible and given in millimeters (mm). The intra-observer variability of IC/IM and MCW was 0.1 mm. The results were then averaged.

The mandibular inferior cortex (MIC) index was also assessed according to Klemetti et al. (Klemetti and Kolmakow, 1997). The MIC index allowed determining the outline of the lower edge of the mandibular compact lamina bilaterally, distal to the chin opening. Class 1 (c1) represents an even and smooth inner rim of the lamina propria, class 2 (c2) shows crescent-shaped defects on the inner rim of the lamina propria, while class 3 (c3) expresses a clearly ragged mandibular lamina propria, with prominent intraosseous defects.

2.3. Statistical analysis

Verification of the normality of distribution was carried out using the Shapiro-Wilk test. Categorical variables were presented as numbers with appropriate percentages and continuous variables as medians (Me) with interquartile range (IQR: 25 %–75 %). The analyses were performed using the non-parametric Mann-Whitney test. Spearman's correlation was calculated for evaluation of the relations between clinical parameters and the bone turnover markers. Pearson's chi-square test was used to compare nominal values. Results with p-values <0.05 were considered as statistically significant. Analyses were performed using Statistica 13.1 PL software (Statsoft, Tulsa, OK, USA).

Table 2
Clinical parameters in the study group.

Parameter	Me (IQR 25 %–75 %) or %
Gender	Male - 13/18 (72 %) Female – 5/18 (28 %)
Age (years)	17.0 (9.0–21)
BMI (kg/m2)	30.0 (27.2–34.0)
BMI SDS	2.94 (2.14–3.66)
Fasting glucose (mg/dl)	84 (76–101)
Insulin (IU/l)	11.1 (7.5–27.8)
HbA1c (%)	5.7 (5.4–6.7)
HOMA-IR	2.33 (1.57–6.21)
Fasting C-peptide (ng/ml)	3.78 (1.89–7.34)
Serum creatinine (mg/dl)	0.85 (0.76–0.98)
Serum cystatin C (mg/l)	0.99 (0.74–1.24)
Aspartate transaminase AST (U/l)	34 (25–40)
Lipids profile:	
Total cholesterol (mg/dl)	174 (163–209)
Low-density lipoprotein (LDL) (mg/dl)	124 (111–161)
High-density lipoprotein (HDL) (mg/dl)	39 (32–45)
Triglycerides (mg/dl)	140 (105–228)
Diagnosed glucose metabolism disorders based on the OGTT test:	
Impaired glucose tolerance (IGT)	2/18 (11.1 %)
Diabetes mellitus (DM)	6/18 (33.3 %)

BMI - body mass index; OGTT - oral glucose tolerance test; HOMA-IR - homeostatic model of insulin resistance assessment.

3. Results

Significantly lower serum OC ($p = 0.0004$) and urinary DPD ($p = 0.0056$) levels were observed in patients with ALMS and BBS syndromes as compared to controls. There was also a trend towards lower s-RANKL values in the study group in comparison to the controls ($p = 0.0512$), whereas OPG values remained similar in both groups ($p = 0.5067$) (Table 3).

Among the assessed routine biochemical parameters of bone metabolism, only the values of alkaline phosphatase were lower in the patients in the study group than in controls ($p = 0.0244$), while other parameters did not differ significantly between the groups ($p > 0.05$) (Table 3). With the exception of alkaline phosphatase levels ($p = 0.0073$), the ALMS and BBS patient subgroups did not differ significantly ($p > 0.05$) in the parameters studied.

Next, in the study group the correlations between bone metabolism markers and laboratory test results such as: indicators of glucose tolerance (HbA1c, fasting glucose, fasting C-peptide), insulin resistance based on HOMA-IR value as well as 25-OHD, AP, PTH, calcium and phosphorus levels in serum blood were evaluated.

In patients from the study group, serum osteocalcin and urinary DPD values negatively correlated with the HOMA-IR index (respectively: $p = 0.0004$ and $p = 0.0316$) (Fig. 2a and b; Table 4). Also urinary DPD levels positively correlated both with serum alkaline phosphatase ($p = 0.0030$) and phosphorus levels ($p = 0.0473$). There was also a positive correlation between serum OC and both 25-OHD ($p = 0.0242$) (Fig. 2c; Table 4), fasting C-peptide level ($p = 0.0436$) (Table 4) and alkaline phosphatase levels ($p = 0.0001$) (Table 4). A tendency towards negative correlations between serum OC and both HbA1c ($p = 0.0728$) and fasting glucose levels ($p = 0.0501$) (Table 4) was also found. In addition, a negative correlation between s-RANKL and fasting glucose levels ($p = 0.0211$) (Fig. 2d; Table 4) and a positive correlation between s-RANKL and alkaline phosphatase level ($p = 0.0491$) were noted. Other parameters of clinical course did not correlate with markers of bone turnover in the study group ($p > 0.05$) (Table 4).

In the control group, there was a positive correlation between serum OC levels and alkaline phosphatase levels ($R = 0.7961$; $p = 0.0001$) and phosphorus levels ($R = 0.5457$; $p = 0.0007$). Urinary DPD values

Table 3
Comparison of bone metabolism markers between the study and control groups. p values <0.05 are indicated in bold.

Parameter	Study group Me (IQR 25–75)	Control group Me (IQR 25–75)	p value
Osteocalcin (ng/ml)	36.8 (26.4–72.6)	97.7 (47.8–132.3)	**0.0004**
OPG (pmol/l)	3.4 (2.94–4.78)	3.52 (2.77–4.25)	0.5068
sRANKL (pmol/l)	0.44 (0.24–0.66)	0.54 (0.44–0.66)	0.0512
OPG/sRANKL ratio	7.87 (5.13–15.55)	5.54 (4.07–8.65)	0.1084
DPD (nmol/nmol)	8.83 (5.5–17.1)	17.7 (11.2–23.9)	**0.0056**
Alkaline phosphatase (AP) (U/l)	88 (51–133)	172 (81–230)	**0.0244**
25-hydroxyvitamin D (25-OHD) (ng/ml)	27.8 (12.2–33.7)	22.0 (16.3–28.2)	0.3171
Parathyroid hormone (PTH) (pg/ml)	42.0 (27.4–50.3)	37.8 (28.0–49.2)	0.7936
Calcium (mg/dl)	10.0 (9.7–10.4)	9.8 (9.6–10.1)	0.0822
Phosphorus (mg/dl)	4.2 (3.6–4.4)	4.3 (3.7–4.7)	0.1891

s-RANKL - soluble Receptor Activator of Nuclear factor κB Ligand; OPG - osteoprotegerin; DPD - urine concentration of pyridinoline.

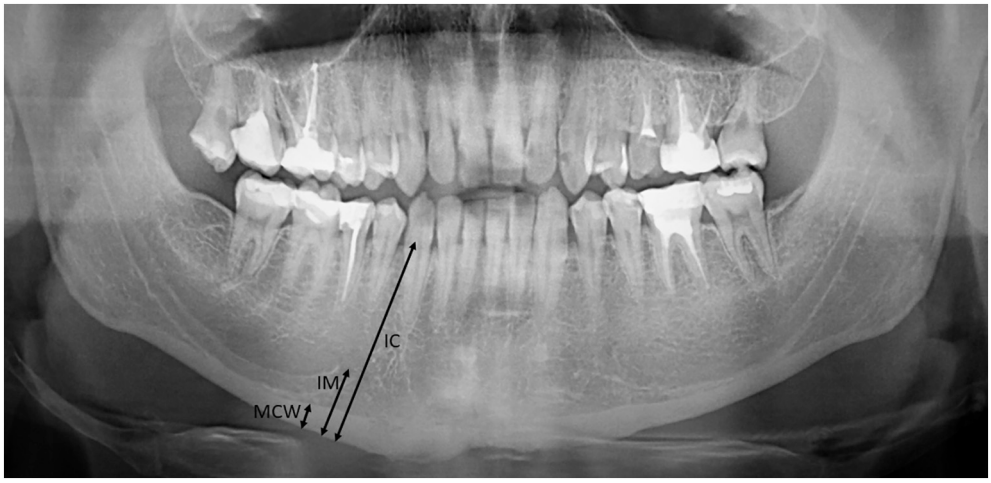

Fig. 1. Panoramic dental radiograph of the mandibular bone with assessment of the IC/IM resorption index and MCW index.
IC - the distance from the lower edge of the mandible to the upper border of the alveolar part, assessed in the line of the chin opening; IM - the distance from the lower edge of the mandible to the lower edge of the chin opening; MCW - mandibular cortical width.

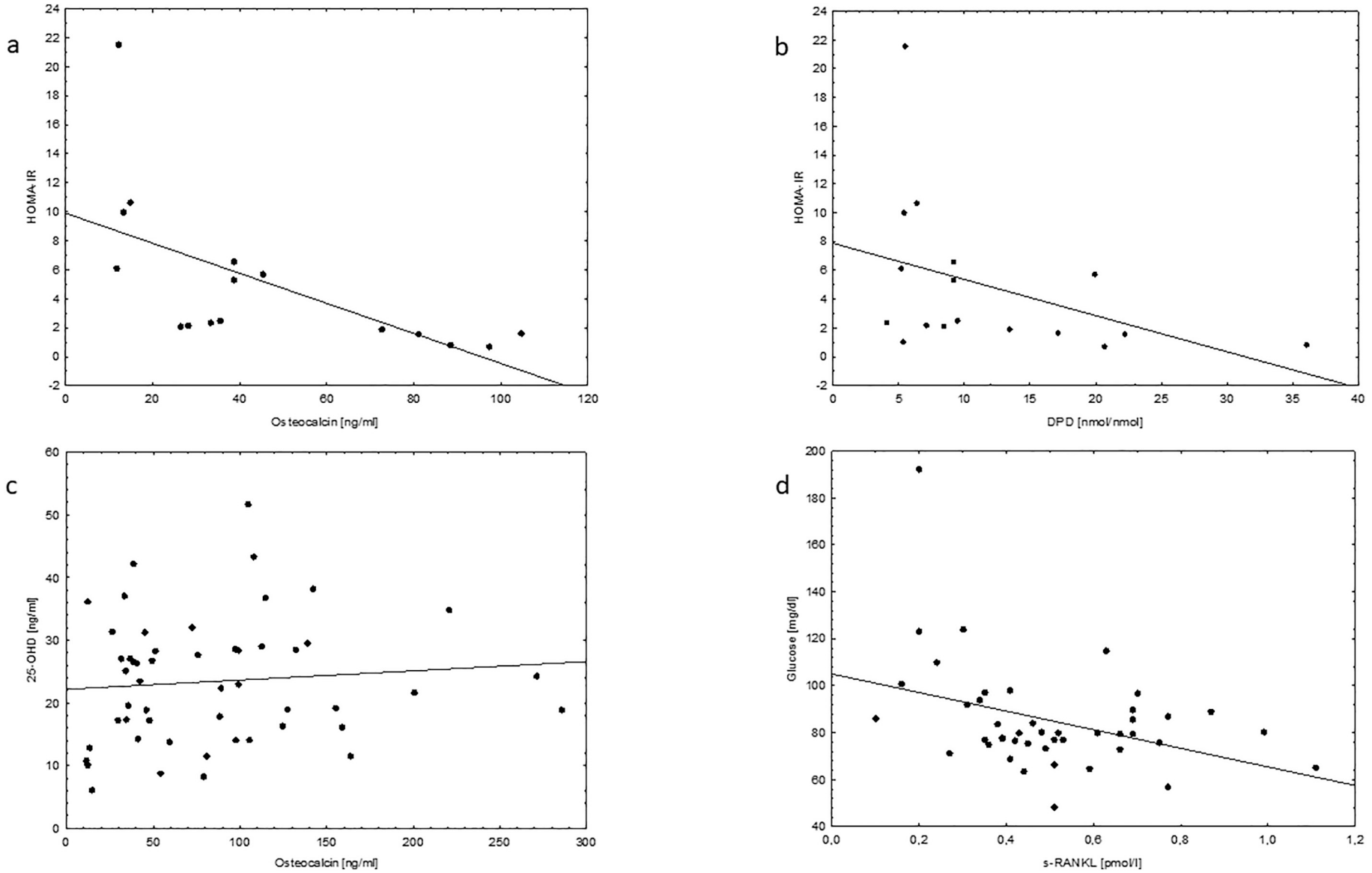

Fig. 2. Correlations between metabolism turnover markers and laboratory test results in the study group: a. HOMA-IR vs. osteocalcin (R = -0.7785; p = 0.0004); b. HOMA-IR vs. DPD (R = -0.5221; p = 0.0316); c. 25-OHD vs. osteocalcin (*R* = 0.5773; p = 0.0242); d. fasting glucose level vs. sRANKL (R = -0.5537; p = 0.0211).
s-RANKL - soluble Receptor Activator of Nuclear factor κB Ligand; DPD - urine concentration of pyridinoline; 25-OHD - 25-hydroxyvitamin D; HOMA-IR - homeostatic model of insulin resistance assessment.

Table 4
Correlations between bone turnover markers and routine biochemical parameters of bone metabolism. Values $p < 0.05$ are indicated in bold.

Parameters	R	*p* value
OPG vs. HbA1c	−0.0093	0.9708
OPG vs. HOMA-IR	−0.1619	0.5348
OPG vs. fasting C-peptide level	−0.3128	0.2563
OPG vs. fasting glucose level	0.0209	0.9367
OPG vs. 25-OHD	0.3444	0.1915
OPG vs. AP	0.2833	0.2706
OPG vs. PTH	0.0368	0.8885
OPG vs. calcium	−0.0144	0.9593
OPG vs. phosphorus	0.2392	0.3905
s-RANKL vs. HbA1c	−0.3012	0.2245
s-RANKL vs. HOMA-IR	−0.1705	0.5128
s-RANKL vs. fasting C-peptide level	−0.1467	0.6019
s-RANKL vs. fasting glucose level	**−0.5537**	**0.0211**
s-RANKL vs. 25-OHD	0.1370	0.6130
s-RANKL vs. AP	**0.4837**	**0.0491**
s-RANKL vs. PTH	0.3033	0.2367
s-RANKL vs. calcium	−0.2114	0.4495
s-RANKL vs. phosphorus	0.0045	0.9873
OPG/RANKL vs. HbA1c	0.1581	0.5308
OPG/RANKL vs. HOMA-IR	−0.0490	0.8518
OPG/RANKL vs. fasting C-peptide level	−0.12	0.6570
OPG/RANKL vs. fasting glucose level	0.3066	0.2314
OPG/RANKL vs. 25-OHD	0.1934	0.4899
OPG/sRANKL vs. AP	−0.1936	0.4565
OPG/sRANKL vs. PTH	0.1059	0.6963
OPG/sRANKL vs. calcium	0.1262	0.6539
OPG/sRANKL vs. phosphorus	0.1934	0.4899
DPD vs. HbA1c	−0.1220	0.6297
DPD vs. HOMA-IR	**−0.5221**	**0.0316**
DPD vs. fasting C-peptide level	−0.3000	0.2773
DPD vs. fasting glucose level	−0.2048	0.4304
DPD vs. 25-OHD	0.1558	0.5643
DPD vs. AP	**0.6740**	**0.0030**
DPD vs. PTH	0.0147	0.9553
DPD vs. calcium	−0.2940	0.2876
DPD vs. phosphorus	**0.5193**	**0.0473**
Osteocalcin vs. HbA1c	−0.4459	0.0728
Osteocalcin vs. HOMA-IR	**−0.7785**	**0.0004**
Osteocalcin vs. fasting C-peptide level	**−0.5457**	**0.0436**
Osteocalcin vs. fasting glucose level	−0.4971	0.0501
Osteocalcin vs. 25-OHD	**0.5773**	**0.0242**
Osteocalcin vs. AP	**0.8344**	**0.0001**
Osteocalcin vs. PTH	0.3664	0.1627
Osteocalcin vs. calcium	−0.3394	0.2159
Osteocalcin vs. phosphorus	0.3241	0.2582

s-RANKL - soluble Receptor Activator of Nuclear factor κB Ligand; OPG - osteoprotegerin; DPD - urine concentration of pyridinoline; 25-OHD - 25-hydroxyvitamin D; AP - Alkaline phosphatase; PTH - parathyroid hormone; HOMA-IR - homeostatic model of insulin resistance assessment.

correlated positively with phosphorus and alkaline phosphatase levels ($R = 0.6383$; p = 0.0001 and $R = 0.7653$; p = 0.0001, respectively). Also in the control group, there was a trend towards a positive correlation between OPG and 25-OHD levels ($R = 0.2893$; $p = 0.0702$), s-RANKL and alkaline phosphatase levels ($R = 0.3141$; $p = 0.0548$) and OC and calcium levels ($R = 0.3023$; $p = 0.0823$). No other correlations between clinical course parameters and bone turnover markers were observed in the control group ($p > 0.05$).

In assessing the degree of mandibular alveolar atrophy, it was observed that the IC/IM index differed significantly between the study and control groups, both for the right and left sides: Me = 3.0 (IQR 2.91–3.09) vs. Me = 3.09 (IQR 3.09–3.20); $p = 0.0059$ and Me = 3.0 (IQR 2.92–3.10) vs. Me = 3.10 (IQR 3.00–3.12); $p = 0.0337$; respectively.

There was also a significant difference in the presence of small-degree mandibular atrophy between patients with ALMS and BBS syndromes and healthy controls (88.9 % vs. 5.5 %; $p < 0.0001$). No moderate or severe atrophy of the mandible was noted in either group.

A trend towards lower MCW values in the study group compared to controls was also found, both on the right and left side of the mandible: Me = 4.6 (IQR 4.12–5.12) vs. Me = 5.0 (IQR 4.50–5.75); $p = 0.0857$ and Me = 4.6 (IQR 4.00–5.00) vs. Me = 5.0 (IQR 4.50–5.25); $p = 0.1462$; respectively. However, the MIC index did not differ significantly between the groups ($p = 0.4526$). Class 1 (c1) for MIC index was observed in the study and control groups in 75 % and 83.3 % of subjects, respectively, class 2 (c2) in 6.3 % and 10 % and class 3 (c3) in 18.7 % and 6.7 % of individuals.

4. Discussion

For the first time, bone turnover markers and biochemical indicators of bone metabolism were evaluated collectively in patients with ALMS and BBS syndromes and compared to controls, as well as their relation to parameters of glucose tolerance and insulin sensitivity.

Lower serum osteocalcin, urinary DPD levels and a trend towards lower s-RANKL were found in patients with BBS and ALMS syndromes compared to healthy individuals, indicating impaired bone turnover in these patients. Moreover, negative correlations were observed between serum OC and urinary DPD and the insulin resistance marker, HOMA-IR, as well as between s-RANKL and fasting glucose level, and a positive correlation of OC with 25-OHD. This may show a link between bone metabolism disturbances and the increase in insulin resistance and progression of hyperglycemia observed in ALMS and BBS patients.

Other studies have also confirmed the negative association of osteocalcin levels with fasting glucose levels, insulin resistance as measured by the HOMA-IR index and central obesity in pediatric patients (El-Dorry et al., 2015; Kim et al., 2014; Reinehr and Roth, 2010; Wang et al., 2014; Zoch et al., 2016). Bador et al. found a negative correlation between OC and fasting glucose and HOMA-IR, but did not observe that OC correlated with waist circumference and BMI index (Bador et al., 2016). Furthermore, Starup-Linde et al. observed a negative correlation between OC levels and HbA1c suggesting that reduced bone turnover may be a consequence of a chronic hyperglycemic state (Starup-Linde et al., 2014). Some recent studies have also shown that patients with diabetes have lower levels of serum OC compared to non-diabetic subjects (Starup-Linde et al., 2014; Valentini et al., 2020). Other studies also highlighted the protective effect of osteocalcin on the development of metabolic syndrome and the reduction of cardiovascular risk (Magni et al., 2016).

According to Çakatay et al. osteocalcin levels in the T2DM group were significantly lower than in the healthy group, with no difference found in urinary DPD levels, indicating that the diabetic state is rather associated with reduced bone formation, while bone resorption remains unchanged (Çakatay et al., 1998). Another study also found a lack of difference in urinary DPD values between non-obese and obese children (El-Dorry et al., 2015).

In our study, no differences in osteoprotegerin levels were noticed between groups, while other studies have shown a reduction in OPG levels in obese patients as compared to non-obese subjects and a correlation between OPG and insulin resistance as measured by HOMA-IR index (Ashley et al., 2011; Gannagé-Yared et al., 2008; Ugur-Altun et al., 2005). However, some research found no difference between OPG concentrations and BMI index (Gannagé-Yared et al., 2006, 2008; Holecki et al., 2007).

In the search for a link between OPG and obesity, the contribution of insulin sensitivity of peripheral tissues and the role of adiponectin whose levels negatively correlate with body fat, have been highlighted (Ashley et al., 2011). On the other hand, adiponectin concentration is known to correlate positively with the OPG/RANKL ratio and negatively with bone density (Ostrowska et al., 1998, 2015). Ostrowska et al. observed that increased adiponectin levels resulted in a rise in the OPG/RANKL ratio in obese postmenopausal women and a decrease in OPG and s-RANKL levels (Ostrowska et al., 1998, 2015). In contrast to these studies, Asley et al. showed that s-RANKL levels did not differ between obese and non-obese individuals (Ashley et al., 2011). However, other

research in diabetic patients highlighted significantly higher OPG levels than in non-diabetic subjects and its positive correlation with HbA1c levels (Valentini et al., 2020).

With the growing interest of researchers in rare genetic syndromes, we have found so far two papers that have looked at bone disorders in patients with these ultra-rare ciliopathies. Tahani et al. showed that patients with ALMS tend to have increased bone mineral density (BMD) as measured by dual energy X-ray absorptiometry (DXA) study (Tahani et al., 2021). However, similar to the results of Han et al. these differences were no longer marked when compared with the BMI-matched group (Han et al., 2018).

A novelty of the presented study is also the use of radiological index evaluating the atrophy of the mandibular bone as one of the parameters suggesting the presence of bone metabolism disturbances in patients with ALMS and BBS syndromes. It has already been confirmed in animal models that a diet leading to obesity promotes bone resorption concerning both systemic and alveolar bone loss (Montalvany-Antonucci et al., 2018a, 2018b; Shu et al., 2015). Our study shows a significant prevalence of low-grade mandibular alveolar atrophy in patients with ALMS and BBS compared to controls, which in the context of the abnormal bone turnover markers found indicates the need for multispecialty care for these patients. Furthermore, the MCW index, which is an indicator of bone mineral density, was also evaluated. So far, it has been used as a marker in the prediction of osteoporosis, but mainly in adults, as studies have focused on perimenopausal women (Karayianni et al., 2007; White et al., 2005). In our study, we only observed a trend towards lower MCW values in the study group compared to healthy children. It is worth emphasizing the dual function of bone, not only mechanical but also endocrine. It seems that in children and adolescents from our study group, in whom obesity is a very early and predominant symptom in both studied genetic syndromes, metabolic disorders play a key role in the development of the observed bone changes at this stage of the disease. It is also not known whether these changes are due to altered biology of their bones, although the absence of significant abnormalities in the qualitative assessment of the mandible was indirectly confirmed by similar MIC index values in the study and control groups. However, it is important to continue follow-up of these patients because of the risk of bone abnormalities worsening with disease progression and the addition of age-related disorders.

Our study has some limitations. Due to the low frequency of ALMS and BBS syndromes in Caucasian population our study has a small group size. This might be caused by the lack of correct diagnosis in some patients with early obesity and associated subsequent symptoms. Another limitation is not performing DXA examination in our patients, which could be a significant complement to the observed abnormalities despite the absence of frequent bone fractures in the medical history. It is also worth mentioning that the control group was not matched for gender and pubertal status.

Thus, abnormalities in selected markers of bone turnover were found in patients with ALMS and BBS syndromes compared with age-matched controls. This was also confirmed by a small degree alveolar atrophy of the mandible, observed much more frequently in the study group. It seems that the imbalance between bone formation and loss in this group of patients may be determined by obesity with concomitant insulin resistance and impaired glucose metabolism.

5. Conclusions

In conclusion, our study is the first attempt to identify bone metabolism disorders in patients with ALMS and BBS. The variety of observed disorders in patients with the syndromes indicates the necessity to provide them with proper diagnosis and treatment of these conditions.

Human studies statement

All procedures followed were in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Data accessibility statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Krzysztof Jeziorny: Methodology, Data curation, Writing – original draft. **Ewa Zmyslowska-Polakowska:** Methodology, Investigation, Writing – original draft. **Krystyna Wyka:** Investigation. **Aleksandra Pyziak-Skupień:** Software, Validation, Visualization. **Maciej Borowiec:** Methodology, Validation. **Agnieszka Szadkowska:** Data curation, Methodology. **Agnieszka Zmysłowska:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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