The influence of ibandronate treatment on bone density and biochemical bone markers in patients with osteogenesis imperfecta

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Abstract

Osteogenesis imperfecta (OI) is characterized by different signs including increased bone fragility, short stature, blue sclera, abnormal tooth growth and often secondary immobility. No curative therapy has been found for this rare disease up to now, and different pharmacological substances have been tried as treatment for severe forms of OI. Promising results were seen with intravenous bisphosphonates in the treatment of patients with OI. The aim of present study was to show the effect of intravenous ibandronate therapy on bone density and bone metabolism markers. We analyzed the data of 27 patients with the diagnosis of OI who were treated off-label with intravenous ibandronate. Ibandronate was administered by intravenous infusion every three months at a dosage of 0.3-2 mg. Bone turnover markers and bone density were measured before starting therapy and every three months during treatment. Bone density was measured by using an ultrasound imaging system providing an accurate image of the calcaneus and by evaluating broadband ultrasound attenuation (BUA). Twenty-seven patients were treated with intravenous ibandronate during the observation period. 18 were female. The mean age of all patients was 23.9 years ± 19.6 (range 4-63). Seventeen patients were categorized to have OI Type I, 5 patients to have OI Type III and 5 patients to have OI Type IV. There was a statistically significant decrease in total alkaline phosphatase (P<0.0001). We detected also a statistically significant decrease in the ratio urinary deoxypyridinoline/urinary creatinine (P=0.0048) and the ratio urinary pyridinoline/urinary creatinine (P<0.0001) respectively. There was also a statistically significant increase in serum magnesium (P=0.034) and BUA (P=0.0071). No statistically significant changes were seen for total serum calcium (P=0.16), the ratio of urine calcium/urine creatinine (P=0.29), alkaline phosphatase (isoform bone) (P=0.3), procollagen-I-peptide (P=0.5), osteocalcin (P=0.9), serum phosphatase (P=0.71), parathormone (P=0.11) and the ratio urine phosphatase/urine creatinine (P=0.58) Therapy with ibandronate in patients with OI leads to a normalisation of bone turnover markers and increasing bone density. Therefore serum alkaline phosphatase and bone density are possible parameters to monitor bisphosphonate treatment in patients with OI.

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Introduction

Osteogenesis imperfecta (OI) is a heritable skeletal disorder characterized by different signs including increased bone fragility, short stature, blue sclera, abnormal tooth growth and often secondary immobility. A mutation in the *COL1A1* or *COL1A2* gene, encoding the two collagen type I chains [α1(I) and α2(I)] has been detected in individuals with the clinical diagnosis of OI.[1-4]

In 1979, Sillence *et al.* classified the OI into four major groups.[4] This classification is still most often used. Recently additional types (V-XI) have been indentified. They have a similar phenotype as OI type IV but are not associated with a collagen type I mutation.[1,5,6] These types are caused by a mutation in the gene encoding for cartilage-associated protein, *CRTAP;* prolyl-3-hydroxylase 1, *LEPRE1,* and cyclophilin B, *PPIB*.[1,5,6]

No curative therapy has been found for this rare disease up to now, so different pharmacological substances have been tried as treatments for severe forms of OI. Sodium fluoride, anabolic steroids, and magnesium oxide have been tried without any convincing benefit with regard to fracture risk and sustained improvement.[7-11]

A positive effect was seen during the application of growth hormones and (+)-cyanidanol-3.[12,13] A benefit was also seen during treatment with calcitonin, but the pronounced side effects predominated.[14,15]

Over the last two decades promising results were seen with intravenous bisphosphonates in the treatment of patients with OI.[16,17]

Bipshosphonates are stable analogues of pyrophosphates with a complex effect on the bone metabolism. The predominantly inhibitory effect on osteoclasts is due to an inhibition of the farensyl pyrophosphotase synthase, which leads to osteoclast apoptosis,[18-21] which may lead to an increased bone density.

Many previous studies focused on the results after treatment with pamidronate on bone density, bone turnover markers and fracture rate in patients with osteogenesis imperfect,[22-29] but only a few studies have measured the effects of intravenous ibandronate in children and adults with osteogenesis imperfect.[30]

The aim of present study was to show the effect of intravenous ibandronate therapy on bone density and bone metabolism markers in patients with osteogenesis imperfecta. We also wanted to prove the hypothesis that there are higher levels of bone metabolism markers in these patients, indicating an increased bone turnover.

Materials and Methods

We analyzed the data of 27 patients with the diagnosis of OI who were treated off-label with an intravenous ibandronate therapy between January 2002 and December 2007 at the Technical University of Munich Children's Hospital (Germany). All patients and their parents signed a declaration accepting the unlicensed (off-label) use of ibandronate for the treatment of osteogenesis imperfecta.

Inclusion criteria were a complete patient history and a continuous duration of treatment over at least 18 months. Demographic data of the different patients is demonstrated in Table 1.

Treatment protocol

Ibandronate was administered by intravenous infusion every three months at a dosage of 0.3-2 mg (0.016 mg/kg - 0.2 mg/kg body weight). There was a dosage increase in the adult patients during treatment, whereas the children's dosage was constant during the duration of treatment.

The observation period was at least 18 months.

Bone markers

The following bone turnover markers were measured before starting ibandronate therapy and every three months during treatment (reference intervals):

- Serum calcium (2.2-2.6 mmol/L)
- Serum phosphate (2.5-4.5 mg/dL)
- Ratio urinary calcium/urinary creatinine (<0.5)
- Serum magnesium (0.65-1.1 mmol/L)
- Serum total alkaline phosphatase (60-180 UL)
- Serum alkaline phosphatase isoform bone (4-21 U/L)
- Parathormone (15-65 pg/mL)
- Serum Prokollagen-1-Peptide (m: 50-170 ug/mL; f: 38-202 ug/mL)
- Ratio urinary deoxypyridinoline/urinary creatinine (3-12 nmol/mmol)
- Ratio urinary pyridinoline/urinary creatinine (19-51 nmol/mmol)
- Ratio urinary phosphate/urinary creatinine [<2.7 mmol/mmol (age>14)]
- Osteocalcin (9-42 ng/mL)

Bone density

Bone density was measured using an ultrasound imaging system providing an accurate image of the calcaneus and by evaluating broadband ultrasound attenuation (UBIS 5000; DMS group - Mauguio, France). The broadband ultrasound attenuation (BUA) was evaluated by positioning a circle in the region of interest which was located in the dorsal part of the calcaneus (Figure 1).

Statistical analysis

Statistical analysis was done using R 2.13.2 (R Foundation for Statistical Computing, Vienna, Austria) with packages *nlme* and *aod*.[31,32] Data are presented as mean ± standard deviation (range). To analyze the longitudinal data we used a mixed effects model. To accommodate for various kinds of non-linearity in the longitudinal trends we used B-splines in the mixed model analysis. All analyses were done using a 0.05 level of significance.

Study registration and informed consent

This study protocol was reviewed and approved by the local ethics committee of the

Table 1. Demographic data of all patients.

Patient	Sex	Age	OI-Type	Total calcium	Serum phosphate	Urinary calcium/ creatinine	Magnesium	Total alkaline phosphatase	Alkaline phosphatase (isoform bone)	Parathormone	Procollagen-1-Peptide	Urinary deoxypyridinoline/ creatinine	Urinary pyridinoline/ creatinine	Urinary phosphate/ creatinine	Osteocalcin
1	m	12	4	x	x	x	x	x						x	
2	f	7	4	x	x	x	x	x		x	x			x	
3	f	10	4	x	x		x	x	x	x			x		x
4	f	59	4	x	x	x	x	x	x			x	x		
5	f	50	4	x	x		x	x	x			x	x		x
6	f	13	1	x	x		x	x							
7	m	39	1	x	x		x	x	x			x	x		x
8	f	48	1	x	x		x	x	x		x			x	
9	m	17	1	x	x	x	x	x			x			x	
10	m	16	1	x	x	x	x	x						x	
11	m	63	1	x	x		x	x	x			x	x		x
12	m	14	1	x	x		x	x		x					
13	f	33	1	x	x		x	x							
14	f	4	1	x	x		x	x			x				
15	f	60	1	x	x	x	x	x	x			x	x	x	x
16	f	5	1	x	x			x							
17	f	34	1	x	x	x	x	x	x						
18	f	5	1	x	x	x	x	x		x				x	
19	f	4	1	x	x	x	x	x			x			x	
20	f	48	1	x	x		x	x	x	x				x	
21	m	36	1	x	x	x	x	x		x					
22	f	34	1	x	x	x	x	x		x					
23	m	5	3	x	x		x	x		x					
24	f	7	3	x	x	x	x	x		x	x			x	
25	f	6	3	x	x	x	x	x		x	x	x	x	x	
26	f	6	3	x		x	x								
27	m	11	3	x	x	x	x	x		x				x	

OI, Osteogenesis imperfect; f, female; m, male; x, bone turnover marker was measured at the beginning of the therapy.

Table 2. Levels of bone turnover markers at the beginning of therapy.

Bone turnover marker	Material	N	Initial values (before therapy)	Reference interval
Total calcium	Serum	27	2.3±0.1 (2.1-2.5)	2.2-2.6 mmoL/L
Serum phosphate	Serum	25	4.1±0.8 (2.8-5.4)	2.5-4.5 mg/dL
Urinary calcium/creatinine	Urine	15	1.2±0.6 (0.3-2.5)	<0.5 mmoL/mmoL
Magnesium	Serum	26	0.8±0.1 (0.7-1.0)	0.65-1.1 mmoL/L
Total alkaline phosphatase	Serum	26	288.6±195.7 (76-827)	60-180 U/L
Alkaline phosphatase (isoform bone)	Serum	9	8.9±3.5 (5-15)	4-21 U/L
Parathormone	Serum	11	3.7±3.5 (0.1-13.0)	15-65 pg/mL
Prokollagen-1-Peptide	Serum	6	91.9±115.3 (21.7-322.4)	m: 50-170 ug/mL; f: 38-202 ug/mL
Urinary deoxypyridinoline/ creatinine	Urine	6	31.3±32.9 (7.5-96.0)	3-12 nmoL/mmoL
Urinary pyridinoline/creatinine	Urine	7	189.2±210. 4 (72.3-661)	19-51 nmoL/mmoL
Urinary phosphate/creatinine	Urine	12	1.5±3.6 (0.1-12.8)	<2.2 mmoL/mmoL
Osteocalcin	Serum	5	11.0±8.1 (3.3-20.9)	9-42 ng/mL

N, number of cases included.

University of Tuebingen (719/2011A). Written informed consent was obtained from all patients or their parents for the unlicensed use of intravenous ibandronate.

Results

Twenty-seven patients were treated with intravenous ibandronat during the observation period: 18 were female. The mean age of all patients was 23.9 years±19.6 (range 4-63). Seventeen patients were categorized to have OI type I, 5 patients to have OI type III and 5 patients to have OI type IV. Table 1 is marking the different patients where bone turnover markers have been analysed before starting the therapy.

The mean values of bone turnover markers at the beginning of the therapy are demonstrated in Table 2.

Two patients (7%) were under the range for reference intervals of serum calcium levels at the beginning of the treatment. 8 patients (30%) were upper the range of reference intervals for serum phosphatase and 14 patients (60%) demonstrated increase levels of urinary calcium. In regard to magnesium serum levels, all patients were in the range for reference intervals. There was an increased serum total alkaline phosphatase upper the reference seen in 15 patients (57%) and one (10%) increased value for bone alkaline phosphatase. The values for parathormone were under the lower limit in 16 patients (84%) and 2 female patients (22%) were under the lower limit for procollagen-I-petide. Increased levels for urine deoxypyridinolin were seen in 8 patients (80%) and 9 patients (75%) demonstrated elevated urine pyridinolin levels. 3 patients (60%) were under the lower limit for serum osteocalcin levels.

The development of the different bone turnover markers during treatment with ibandronat is demonstrated in Figures 2-4.

The development of broadband ultrasound attenuation under therapy is shown in Figure 5.

There was a statically significant decrease in total alkaline phosphatase (P<0.001). We detected also a statistically significant decrease in the ratio urinary deoxypyridinoline/urinary creatinine (P<0.001) and the ratio urinary pyridinoline/urinary creatinine (P<0.001). There was also a statistically significant increase in serum magnesium (P=0.034) and BUA (P=0.007).

No statistical significant changes were seen for total serum calcium (P=0.158), the ratio urine calcium/urine creatinine (P=0.292), alkaline phosphatase (isoform bone) (P=0.296), procollagen-I-peptide (P=0.503), osteocalcin (P=0.903), serum phosphatase (P=0.712), parathormone (P=0.107) and the ratio urine phosphatase/urine creatinine (P=0.587).

Discussion

Osteogenesis imperfecta is a hereditary disorder that results from a genetic defect in the synthesis of type-I-collagen. This genetic defect results in a typical phenotype with increased bone fragility, short stature, blue sclera and abnormal tooth growth.[1-4] Sillence *et al*.[4] classified the OI into four different types ranging from a mild form to a lethal form, according to their appearance. OI type I is a mild form with only a few fractures in childhood, blue sclera and a good mobility. OI type II is the most serve form with multiple perinatal fractures and normally lethal course. Types III and IV are accompanied by increased bone fragility, with or without blue sclera and are clinically difficult to treat because of multiple deformities and a high rate of immobility.[4,33]

Bisphosphonates with their influence on bone turnover have been established in osteoporotic disorders and in the treatment of osteogenesis imperfecta. The benefits of bisphosphonate treatment, especially with pamidronate, have been demonstrated in several studies. There was an increase in bone density, a decreased fracture rate, a normalization of bone turnover markers and a subjective reduction of bone pain seen with an improvement in mobility.[26-29,34-37]

In the present study we retrospectively analyzed the data of 27 patients who were treated with intravenous ibandronate for at least 1.5 years. To our knowledge only one previous study also focused on the benefits of this kind of bisphosphonate in children with osteogenesis imperfect.[30]

Bone alkaline phosphatase and osteocalcin have been concluded to be the most valuable bone markers for bone formation, whereas urinary deoxypyridinoline (DPD) and the cross-linked telopeptides of type-I-collagen are most valuable markers for bone resorption.[38] One of the main problems of the present study is that the bone turnover markers were not compared with an age-gender-matched group of healthy children. Most children with OI are smaller and lighter when compared with children of the same age and a correlation between the levels of bone turnover markers and body size and weight has been reported.[39] Because of these

findings and because of the mean age of our patients, we did not compare initial bone turnover markers with the few and still incompletely published paediatric reference intervals.[38,40,41]

Nevertheless, we evaluated bone turnover markers before starting therapy with ibandronate and monitored changes during treatment. Most patients demonstrated elevated serum and urinary markers for bone formation and bone resorption, which implies that there is an increased turnover in patients with OI. We were able to detect statistically significant changes with a normalisation of the different bone markers during the therapy. There was decrease in total alkaline phosphatase (bone formation), in the ratio of urinary deoxypyridinoline/urinary creatinine (bone resorption) and the ratio of urinary pyridinoline/creatinine (bone resorption). In our opinion, these markers are suitable to detect the benefits of ibandronate therapy. These findings support the results by Aström *et al*., where serum alkaline phosphatase (ALP) and urinary DPD were also seen as most informative in the observation of treatment benefit, although they used pamidronate in their study.[25] However Braga *et al.* reported serum bone ALP, urinary DPD and collagen-type I Ntelopeptides (NTX) as being the best for clinical separation.[42] On the other hand there might be the possibility of a type I failure while detecting a decrease in the ratio of urinary deoxypyridinoline/urinary creatinine and the ratio of urinary pyridinoline/creatinine caused by the small sample size.

We did not detect an increased bone ALP or statistically significant changes in bone ALP which might be caused by a type II failure because of the small sample size.

Bone densitometry in infants and especially in patients with a pathological bone structure

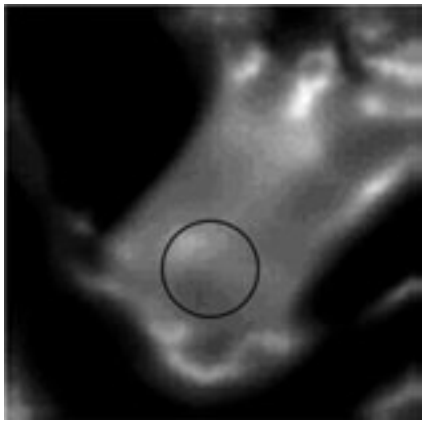

Figure 1. Ultrasound imagine created by UBIS 5000. The circle marks the region of interest for measuring broadband ultrasound attenuation.

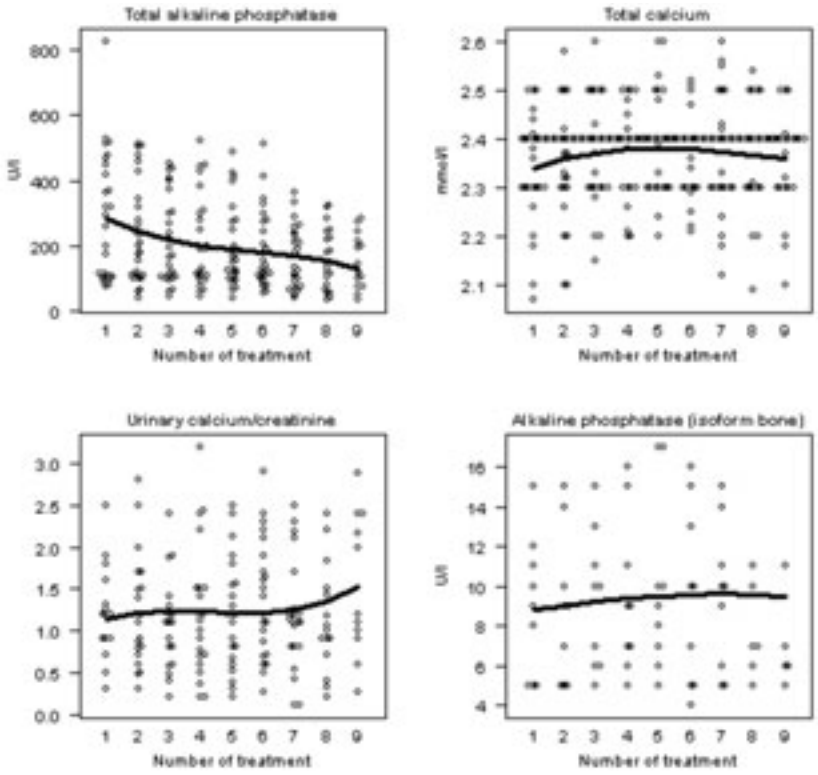

Figure 2. Changes of bone turnover markers during ibandronate therapy.

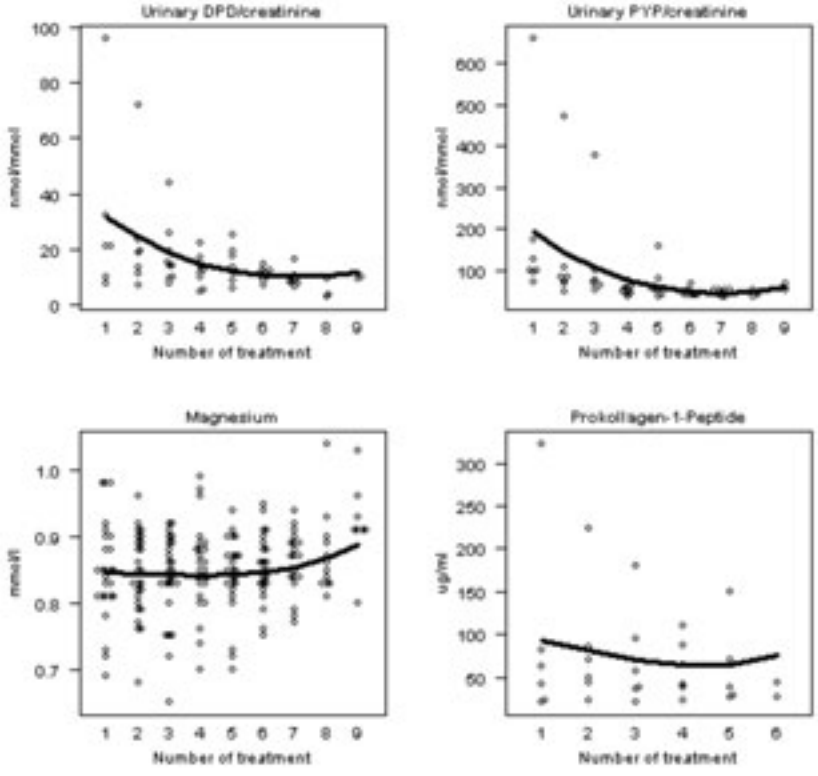

Figure 3. Changes of bone turnover markers during ibandronate therapy (DPD, deoxypyridinoline; PYP, pyridinoline).

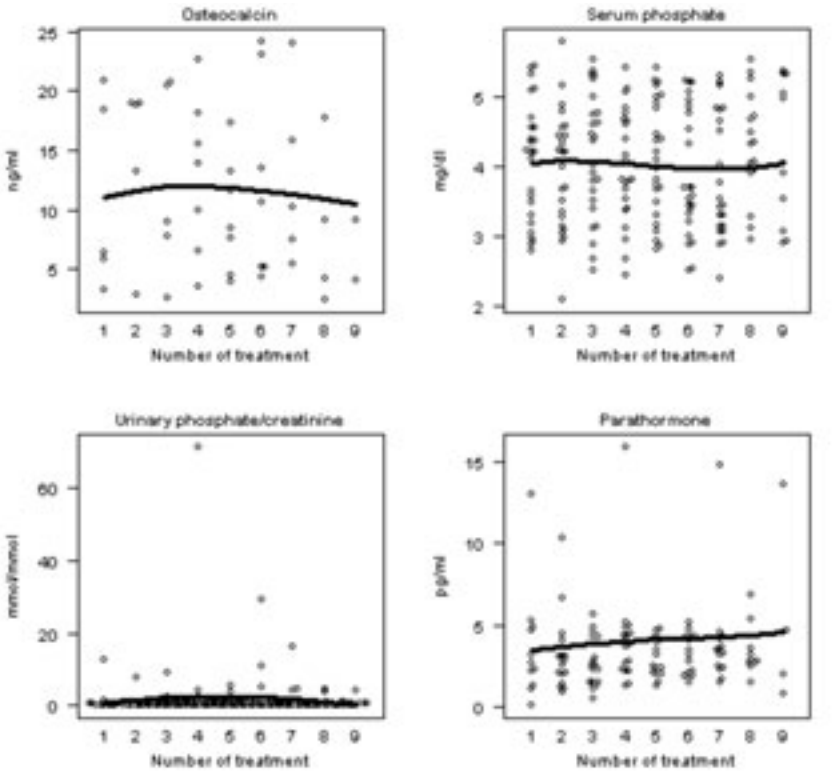

Figure 4. Changes of bone turnover markers during ibandronate therapy (PTH: parathormone).

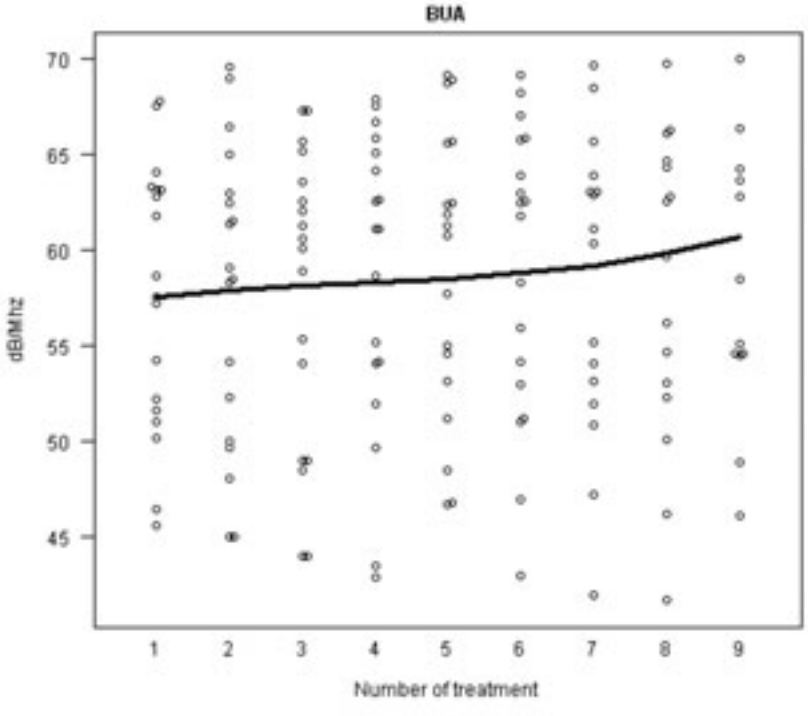

Figure 5. Changes of broadband ultrasound attenuation during ibandronate therapy.

such as in patients with OI is difficult. DXA is the most commonly used method and a lot of studies have shown an increase in bone mineral density during the therapy with bisphosphonates especially with pamidronate.[28,29,34,35]

To our knowledge this is the first study using BUA for monitoring bone density during bisphosphonate treatment. We used BUA for measurement of bone density to avoid radiation exposure during follow-up especially in children and to minimize measuring time. Different studies have demonstrated the high sensitivity of this technique when measuring bone density.[43-45] We were able to show a statistically significant increase in bone density, which is consistent with previous studies.[28,29,34,35]

We note several limitations of the present study. The most important limitation was that we performed a retrospective analysis of patient data. Therefore we analyzed a very heterogeneous patient collective especially in regard to the age. For the same reason there was a lack of information about different bone turnover markers such as osteocalcin. Most patients were tested for the most important bone turnover markers during therapy, such as alkaline phosphatase, deoxypyridinoline or pyridinoline and we were therefore unable to perform a statistical analysis of most parameters retrospectively. On the other hand in our opinion the patients which have been included into the study represented there age-group sufficiently.

Secondly we did not analyse clinical features such as bone fracture rate, mobility, pain during the treatment with ibandronate and have not differentiated between OI Type I/III/IV when analyzing the effects of ibandronate. Further studies should focus on these topics.

Thirdly, we analysed bone density by using broadband ultrasound attenuation and the region of interest was plotted free hand, so the interobserver quality seems to be low. However, this was only performed by one observer (author II) in a standard manner so that there was a high conformity.

Conclusions

Finally, therapy with ibandronate in patients with OI leads to a normalisation of bone turnover markers and increase in bone density. Therefore serum alkaline phosphatase and bone density are possible parameters to monitor bisphosphonate treatment in patients with OI. There seems also to be a decrease in the ratio of urinary deoxypyridinoline/urinary creatinine and the ratio of urinary pyridinoline/creatinine but because of the small sample size these parameters could only be suggested as possible parameters to monitor therapy.

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