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Effects of zoledronic acid on vertebral shape of children and adolescents with osteogenesis imperfecta

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ABSTRACT

Vertebral compression fracture (VCF) is a common and severe complication of osteogenesis imperfecta (OI). We prospectively observe the changes of vertebral shape during zoledronic acid (ZOL) treatment and assess influence factors of VCF in OI children. 32 children with VCF and 10 children without VCF (NVCF) were included and given ZOL treatment for 2 years, who were matched in age and gender. Control group included 17 treatment naïve OI patients with VCF who were matched in age, gender and clinical severity to 17 patients in VCF group received ZOL treatment for 1 year (as ZOL treated group). We performed quantitative vertebral morphometry and calculated concavity index (mh/ph), height-length ratio (ah/LL, mh/LL, ph/LL) and projection area (PA) of vertebrae from T4 to L4 before and after treatment. At baseline, patients in VCF group had significantly lower PA, mh/ph, ah/LL, mh/LL and ph/LL than patients in NVCF group ($P < 0.01$). PA, mh/ph, ah/LL, mh/ LL and ph/LL of patients with VCF were raised by (35.2 ± 19.5)%, (22.9 ± 15.1)%, (19.6 ± 13.9)%, (33.6 ± 25.5)%, and (8.1 ± 8.8)% ($P < 0.01$) after 1-year treatment of ZOL, and were increased by (71.8 ± 28.2)%, (42.8 ± 21.8)%, (35.1 ± 20.6)%, (65.4 ± 43.2)%, and (12.5 ± 11.4)% after 2-year treatment of ZOL ($P < 0.01$). Compared to control group, mh/ph, ah/LL and mh/LL were significantly higher ($P < 0.01$) in ZOL treated group. LS-BMD and its increase were positively correlated to vertebral height and PA at baseline and the improvement of vertebral height and PA after ZOL treatment, respectively. In conclusion, the compressive vertebrae of OI children could be effectively reshaped during ZOL treatment. Low LS-BMD was an independent risk factor for VCF and its increase was positively correlated to the improvement in vertebral shape after ZOL treatment.

1. Introduction

Osteogenesis imperfecta (OI) is a rare heritable bone disorder with an incidence of 1/15,000–20,000 neonates, which is mainly caused by mutations in genes involving synthesis of type I collagen or regulating osteoblast functions [1,2]. The most common clinical manifestations include reduced bone mineral density (BMD) and increased bone fragility, which result in frequent fractures and progressive bone deformities [3]. In addition, OI often leads to a variety of extra-skeletal manifestations, including dentinogenesis imperfecta (DI), ligamentous laxity, blue sclera and hearing impairment [3]. The most commonly used OI subgroup classification, Sillence classification, characterized OI patients into 4 groups: type I is the mildest, type II is lethal during perinatal period, type III is the most severe type in survivors and type IV is the intermediate type between types I and III [4].

Vertebral compression fracture (VCF) is a common and severe complication of OI [1], which not only induces scoliosis, but also may lead to impairment of cardiorespiratory function, even result in death [5–7]. However, VCF is under recognized in OI patients. It remains unclear to improve the shape of vertebrae. Bisphosphonates (BPs), synthetic analogues of inorganic pyrophosphate, increased BMD and reduced fracture risk of OI patients by inhibiting osteoclastic activities [8,9]. Small-sample studies indicated that pamidronate and neridronate help to reshape the compressive vertebrae of OI children [10–13]. Recently, zoledronic acid (ZOL), a third-generation BPs, has been widely used to treat bone fragility of OI [14]. However, it is unclear whether

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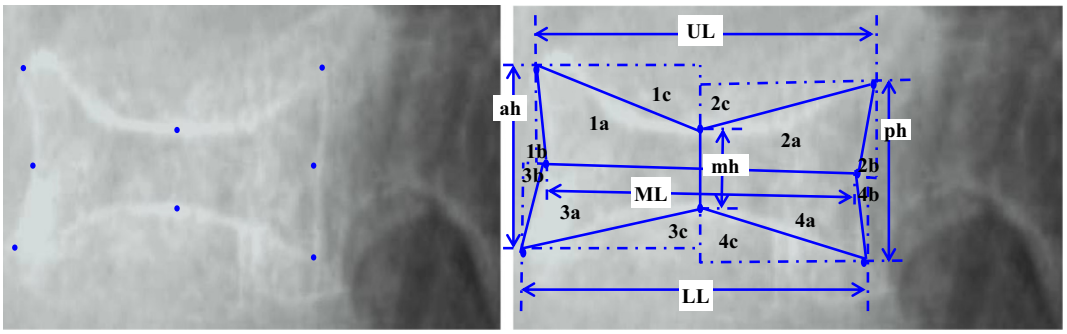

Fig. 1. Method of quantitative vertebral morphometry on lateral spine radiographs.
ah: anterior height of vertebra, mh: middle height of vertebra in the middle of the endplates, ph: posterior height of vertebra, UL: upper length, ML: middle length represents the distance between midpoints of anterior and posterior line, LL: lower length.
concavity index = mh/ph, anterior height ratio = ah/LL, mid-height ratio = mh/LL, posterior height ratio = ph/LL.
Lateral projection area = Area 1 + Area 2 + Area 3 + Area 4.
Area 1 = 1a − 1b − 1c = [(ah/2) × (UL/2)] − [((ah/2) × ((UL − ML)/2))/2] − [((UL/2) × ((ah − mh)/2))/2].
Area 2 = 2a − 2b − 2c = [(ph/2) × (UL/2)] − [((ph/2) × ((UL − ML)/2))/2] − [((UL/2) × ((ph − mh)/2))/2].
Area 3 = 3a − 3b − 3c = [(LL/2) × (ah/2)] − [((ah/2) × ((LL − ML)/2))/2] − [((LL/2) × ((ah − mh)/2))/2].
Area 4 = 4a − 4b − 4c = [(LL/2) × (ph/2)] − [((ph/2) × ((LL − ML)/2))/2] − [((LL/2) × ((ph − mh)/2))/2].

the vertebral shape of OI children can be improved during ZOL treatment.

Therefore, we prospectively observe the effects of ZOL infusion on vertebral shape in children and adolescents with OI through a quantitative vertebral morphometry. We also investigate the influencing factors of vertebral shape in OI children.

2. Methods

2.1. Study participants

42 children and adolescents (3–15 years old) were diagnosed with OI in the department of endocrinology of Peking Union Medical College Hospital (PUMCH) from October 2014 to March 2018.

Patients with a family history of OI were diagnosed with OI if they had more than one non-traumatic fracture with an age- and sex-adjusted BMD Z-scores ≤ -1.0 at lumbar spine or femoral neck or had an age- and sex-adjusted BMD Z-scores ≤ -2.0 at lumbar spine or femoral neck. For patients without a family history of non-traumatic fracture, diagnosis of OI was made if they had more than one non-traumatic fracture and at least a kind of extra-skeletal manifestations [8,15]; or with a genetic diagnosis of OI. Patients were excluded if they had other metabolic bone disease or received a previous treatment of BPs or had a prior spine surgery or recently received drugs that affected bone metabolism, such as glucocorticoids, anti-epileptics agents or eGFR < 35 ml/min or aspartate aminotransferase/alanine aminotransferase higher than twofold of the normal upper limit or intolerance to BPs.

Patients were divided into type I, III, or IV OI according to the Sillence classification. As OI type II was perinatal lethal, no patients with this type were included in this study.

2.2. Study procedures

This was a 2-year, prospective, open label study. According to the lateral spine radiographs, 32 patients were classified as VCF group and 10 patients were matched in age and gender as non-VCF (NVCF) group. As the dosage, frequency and course of ZOL treatment were still controversial and OI was more serious than primary osteoporosis, we chose intravenous treatment of ZOL 5 mg (Aclasta®, Novartis Pharma Schweiz AG, Switzerland) annually to treat these patients. Our previous study had confirmed the safety of the dosage [15]. If fracture occurred during treatment period, ZOL would be held until the fracture was healed. All patients received calcium (300-600 mg/day) plus vitamin D3 (62.5–125 IU/day) (Caltrate D, Wyeth Pharmaceuticals, USA) and calcitriol (0.25 μg/2 days) (Rocaltrol, R.P. Scherer GmbH & Co.KG, Germany) were supplemented to all patients. If hypocalcemia occurred after ZOL treatment, calcitriol would be increased to 0.25 μg daily till the serum calcium level recovered to normal. The flow chart of this study was shown in Supplemental Fig. 1.

This study was approved by the Scientific Ethic Committee of PUMCH. All participants and their legal guardians provided written informed consent before they participated in this study.

2.3. Assessment of effects of ZOL on vertebral shape

The effects of ZOL on vertebral shape were assessed by two ways. We longitudinally compared the vertebral shape at baseline and after ZOL treatment. In addition, a control group included 17 treatment naïve OI patients with VCF who were matched in age, gender and clinical severity to 17 patients in VCF group received ZOL treatment for 1 year (as ZOL treated group). A cross-sectional comparison of vertebral shape was conducted between ZOL treated group and control group.

With the morphometry developed, vertebral shape could be quantitatively measured [16]. We used the vertebral morphometry to evaluate the shape from the 4th thoracic vertebra (T4) to the 4th lumbar vertebra (L4) on lateral radiographs [10]. 8 points were labeled at the silhouette of each vertebra, including the 4 corners of the vertebral body and 4 midpoints of the endplates. Basing on these points, anterior, posterior and mid-height (ah, ph, mh) as well as upper, lower and mid-length (UL, LL, ML) were measured using the DICOM system of PUMCH with a precision of 0.1 mm. Each point-to-point distance was measured 3 times and then averaged. All measurements were performed by the same investigator. The coefficient of variation among the three measurements was 0.01–0.05. To assess the degree of vertebrae compression, the concavity index was calculated as mh/ph and the height to length ratios were calculated as ah/LL, mh/LL and ph/LL. To evaluate the growth of the vertebrae, the projection area of vertebra was calculated as the surface area of the polygons that were defined by the 8 measurement points (Fig. 1) [10]. VCF was defined when a vertebra was compressed > 20% [17].

2.4. Height and weight measurements

Patients' height and weight were measured by Harpenden stadiometer (Seritex Inc., East Rutherford, NJ, USA) and RGZ-120 weighing scale (Xiheng, Wuxi, China). For patients unable to stand, the height was replaced by length in supine position. If the lower limbs were not equal, the longer one would be measured. The age- and sex-specific Z

scores of height and weight were calculated on the basis of reference data from the Chinese National Centers for Disease Control and Prevention [18].

2.5. Biochemical indexes measurements

Serum levels of alanine aminotransferase (ALT), and creatinine (Cr), calcium (Ca), phosphate (P), alkaline phosphatase (ALP, a bone formation marker) were measured by automated analyzers (ADVIA1800, Siemens, Germany). We used an automated electrochemiluminescence system (E170, Roche Diagnostics, Switzerland) to detect serum levels of beta cross-linked carboxy-terminal telopeptide of type I collagen (β-CTX, a bone resorption marker), 25-hydroxyvitamin D (25OHD), and intact parathyroid hormone (PTH). All parameters were measured in the central clinical laboratory of PUMCH.

2.6. BMD measurements

BMD at the lumbar spine (LS) and femur neck (FN) were measured by dual energy X-ray absorptiometry using software for children (DXA, Lunar Prodigy Advance, GE Healthcare, USA). BMD Z scores were calculated on the basis of BMD data from age- and gender-matched normal Chinese children [19,20].

2.7. Fracture and bone deformity assessment

The total number of clinical fractures (non-vertebral fractures or symptomatic VCF) in the patients' medical history was recorded on each visit, radiological evidence and the statement of legal guardians. The frequency of clinical fracture was calculated as number of clinical fractures/disease course. Bone deformity included limb bending, thoracic deformity, scoliosis and pelvic deformity. Scoliosis was evaluated by X-ray plain film and classified as mild, moderate and severe degree according to Cobb angle as 10°–25°, 25°–50° and > 50°, respectively [21].

2.8. Evaluation of adverse events during ZOL treatment

Adverse events were recorded at each visit. Serious adverse events included osteonecrosis of the jaw, atypical femoral fracture, delayed fracture healing and cardiovascular-related events (such as arrhythmias), which were judged on the basis of clinical manifestation and confirmed by radiological evidence. Other relevant adverse events included acute-phase reactions, hypocalcemia, hypophosphatemia and hepatic or renal function abnormity, which were evaluated by clinical symptom and biochemical data. New fractures were also recorded, including new clinical fractures and VCF. New VCF was defined as new fracture in previously normal vertebrae or the worsening of pre-existing fractures.

2.9. Genotype detection of OI

To evaluate the relationship between vertebral shape and genotype of OI, we detected pathogenic mutations using a panel for next generation sequencing (NGS) (Illumina HiSeq2000 platform, Illumina, Inc., San Diego, CA), which covered 20 candidate genes of OI (*COL1A1*, *COL1A2*, *IFITM5*, *SERPINF1*, *CRTAP*, *P3H1*, *PPIB*, *SERPINH1*, *FKBP10*, *BMP1*, *PLOD2*, *SP7*, *TMEM38B*, *WNT1*, *CREB3H1*, *SPARC*, *PLS3*, *P4HB*, *SEC24D*, *MBTPS2*). The experimental procedures followed a previously described protocol [22]. The mutations identified by NGS were further confirmed by Sanger sequence.

2.10. Statistical analysis

Continuous data of normal distribution (including age, height, weight, biochemical markers, BMD, vertebral projection area, concavity index and height to length ratios) were expressed as mean ± standard deviation (SD). Continuous data of abnormal distribution (including levels of 25OHD and PTH, number of fractures) were expressed as median (quartiles). Categorical data were expressed as the number or percentage (%). Independent sample *t*-test, rank-sum test and chi-square test were utilized to analyze continuous variables and categorical variables in cross-sectional comparison. The vertebral projection area, concavity index and height to length ratios were compared between different groups by covariance analysis adjusted by age, gender and height. Paired samples t-test was used to longitudinally compare the difference of continuous variables between baseline and after treatment. Multiple linear regressions were applied to test the influence factors of vertebral shape at baseline and after treatment.

SPSS software version 23.0 (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. The statistical significance was indicated as two-tailed $P < 0.05$.

3. Results

3.1. Baseline characteristics

Among the 42 patients with OI, 23 (54.8%) were diagnosed with type I OI, 9 were type III OI ($n = 9$, 21.4%) and 10 were diagnosed with type IV ($n = 10$, 23.8%). Gene mutation spectrum of these patients was as follow: *COL1A1* ($n = 26$, 61.9%), *COL1A2* ($n = 7$, 16.7%), *IFITM5* (n = 2, 4.8%), *WNT1* (n = 1, 2.4%) and no mutation detected ($n = 6$, 14.3%). 32 patients were classified into VCF group and 10 patients were matched in NVCF group. No significant differences were found in age, gender, severity of OI, genotype, height and weight, bone turnover markers level, LS-BMD, FN-BMD, the number of clinical fractures, frequency of clinical fracture, percentage of bone deformity and scoliosis between these two groups (Table 1).

Due to some thoracic vertebrae were unclear on the X-ray film, we totally measured shape of 388 vertebrae in 32 patients with VCF and 107 vertebrae in 10 patients without VCF at baseline. Multiple VCFs was common, even all vertebrae could be involved (Supplemental Fig. 2). In NVCF group, mh/ph was approximate to 1.0 and ah/LL, mh/LL, ph/LL was approximate to 0.7 among all vertebrae. The average projection area, mh/ph, ah/LL, mh/LL and ph/LL of T4-L4 in VCF group were significantly lower than NVCF group, after adjusted for age, gender and height (Table 1). Comparison of vertebral morphometry at baseline between these two groups was shown in Supplemental Fig. 3.

3.2. Longitudinal comparison between baseline and after ZOL treatment

There were 28 patients in VCF group completed 1-year follow-up (Supplemental Fig. 1). After treated with ZOL for 1 year, serum β-CTX levels were significantly decreased, and LS-BMD, FN-BMD and their Z score were obviously increased in VCF group ($P < 0.001$) (Table 2). The height and height-Z score were also significantly increased, indicating that the ZOL treatment could lead to catch-growth ($P = 0.020$). We measured the shape of 344 vertebrae in 28 patients with VCF at 1-year follow-up, and the proportion of compressed vertebrae was decreased from 63.4% to 58.7% after 1-year of ZOL treatment, but the difference was not statistically significant. The average of mh/ph, ah/LL, mh/LL, ph/LL and projection area were significantly increased by (22.9 ± 15.1)%, (19.6 ± 13.9)%, (33.6 ± 25.5)%, (8.1 ± 8.8)% and (35.2 ± 19.5)% after 1-year of ZOL treatment (Table 2). There were 16 patients in VCF group completed 2-year follow-up (Supplemental Fig. 1). We measured the shape of 197 vertebrae in 16 patients with VCF at 2-year follow-up, and the proportion of compressed vertebrae was reduced from 72.6% to 67.0% after two years of ZOL treatment, but the difference was not statistically significant. The average of mh/ph, ah/LL, mh/LL, ph/LL and projection area were significantly increased by (42.8 ± 21.8)%, (35.1 ± 20.6)%, (65.4 ± 43.2)%, (12.5 ± 11.4)% and (71.8 ± 28.2)% after two years

Table 1
Baseline clinical characteristics and vertebral morphometry of OI patients.

	VCF (n = 32)	NVCF (n = 10)	P	Reference range
Boys/girls	22/10	7/3	0.941	/
Age (years)	8.8 ± 3.7	8.9 ± 4.2	0.968	/
Sillence classification (I/III/IV)	18/7/7	6/2/2	0.842	/
COL1A1/COL2A2/IFITM5/WNT1	19/5/2/1	7/2/0/0	0.428	/
Height	127.1 ± 22.0	126.3 ± 23.5	0.919	/
Height-Z score	−0.8 ± 1.6	−1.1 ± 1.8	0.543	/
Weight	31.0 ± 13.6	31.2 ± 15.5	0.957	/
Weight-Z score	0.2 ± 1.4	−0.1 ± 1.3	0.535	/
ALT (IU/L)	17 ± 11	12 ± 3	0.123	7–50
Cr (μmol/L)	32 ± 9	34 ± 11	0.622	18–88
Ca (mmol/L)	2.46 ± 0.08	2.45 ± 0.04	0.843	2.13–2.70
P (mmol/L)	1.63 ± 0.19	1.64 ± 0.11	0.931	1–6 years old: 1.10–1.87; 7–11 years old: 0.97–1.77; 12–17 years old: 0.81–1.53;
ALP (IU/L)	292 ± 67	269 ± 46	0.336	42–390
β-CTX (ng/ml)	0.886 ± 0.360	0.940 ± 0.360	0.685	/
25OHD (ng/ml)	22.5 (15.7, 27.7)	17.0 (11.9, 26.2)	0.301	Deficiency: < 20; Insufficiency: 20–30; Sufficiency: ≥30
PTH (pg/ml)	21.3 (9.7, 37.0)	19.4 (9.7, 33.2)	0.973	12–68
LS-BMD (g/cm^2)	0.385 ± 0.161	0.485 ± 0.205	0.122	/
LS-BMD Z score	−3.1 ± 2.2	−2.2 ± 1.9	0.237	/
FN-BMD (g/cm^2)	0.447 ± 0.142	0.449 ± 0.208	0.974	/
FN-BMD Z score	−3.5 ± 2.0	−3.4 ± 2.1	0.918	/
Number of clinical fractures	4.0 (2.0, 7.0)	5.0 (3.0, 7.0)	0.673	/
Frequency of clinical fracture (per year)	0.8 (0.5, 1.5)	0.9 (0.5, 2.6)	0.731	/
Bone deformity (n (%))	12 (37.5%)	2 (20.0%)	0.311	/
Scoliosis (n (%))	7 (21.9%)	1 (10.0%)	0.410	/
Average PA of T4-L4 (mm^2)	280.7 ± 143.7	368.2 ± 190.8	0.006*	/
Average mh/ph of T4-L4 (mm^2)	0.63 ± 0.16	0.99 ± 0.01	< 0.001*	/
Average ah/LL of T4-L4	0.44 ± 0.10	0.70 ± 0.09	< 0.001*	/
Average mh/LL of T4-L4	0.38 ± 0.14	0.70 ± 0.08	< 0.001*	/
Average ph/LL of T4-L4	0.59 ± 0.09	0.71 ± 0.08	< 0.001*	/

OI: Osteogenesis imperfecta, VCF: vertebral compressive fractures, NVCF: non vertebral compressive fracture, ALT: alanine aminotransferase, Cr: creatinine, Ca: Serum calcium, P: Serum phosphate, ALP: alkaline phosphatase, β-CTX: β-isomerized carboxy-telopeptide of type I collagen, 25OHD: 25 hydroxy-vitamin D, PTH: parathyroid hormone, BMD: bone mineral density, LS: lumbar spine, FN: femoral neck, PA: projection area.

* P values were adjusted for age, gender and height.

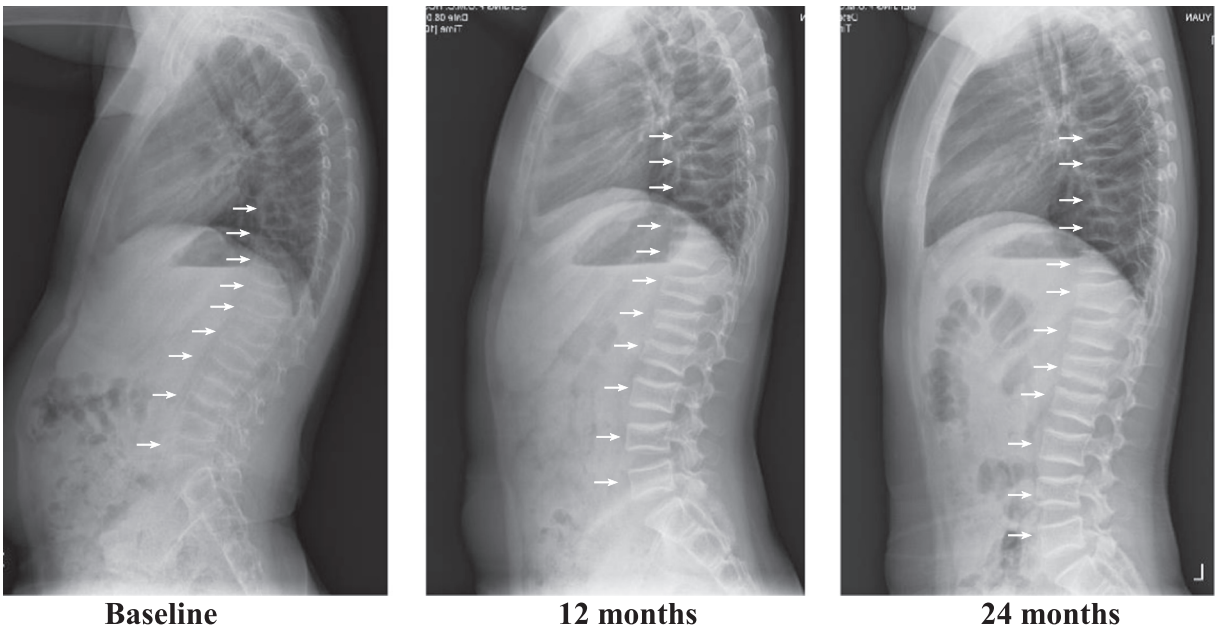

Fig. 2. Lateral spine radiographs of an OI children with VCF at baseline and after ZOL treatment.
The compressed vertebrae of OI children were effectively reshaped after ZOL treatment.
ZOL: zoledronic acid.

Table 2
Comparison of changes of clinical characteristics and vertebral morphometry between baseline and after 6–36 months of treatment with ZOL.

	VCF (n = 28)			VCF (n = 16)			NVCF (n = 9)		
	Baseline	After 1 year treatment	P	Baseline	After 2 year treatment	P	Baseline	After 1 year treatment	P
Boys/girls	19/9	19/9	–	12/4	12/4	–	6/3	6/3	–
Age (years)	9.0 ± 3.8	10.0 ± 3.8	–	8.8 ± 3.9	10.8 ± 3.9	–	8.9 ± 4.2	9.9 ± 4.2	–
Height	125.8 ± 24.9	133.6 ± 24.0	< 0.001	122.1 ± 20.2	136.0 ± 19.4	< 0.001	124.7 ± 24.3	131.0 ± 23.8	< 0.001
Height-Z score	−1.3 ± 2.6	−1.0 ± 2.8	0.020	−1.3 ± 1.9	−0.8 ± 1.8	< 0.001	−1.1 ± 1.9	−1.0 ± 1.9	0.624
Weight	31.0 ± 13.8	35.3 ± 16.2	< 0.001	29.0 ± 11.5	37.0 ± 16.5	0.001	29.3 ± 15.1	32.6 ± 16.6	0.004
Weight-Z score	0.0 ± 1.4	0.1 ± 1.3	0.816	0.2 ± 1.6	0.1 ± 1.4	0.795	−0.2 ± 1.4	−0.2 ± 1.2	0.952
ALP (IU/L)	293 ± 67	263 ± 89	0.066	290 ± 74	216 ± 64	< 0.001	267 ± 48	235 ± 78	0.159
β-CTX (ng/ml)	0.922 ± 0.328	0.611 ± 0.243	< 0.001	0.882 ± 0.375	0.642 ± 0.236	0.004	0.966 ± 0.403	0.689 ± 0.301	0.031
LS-BMD (g/cm^2)	0.385 ± 0.175	0.643 ± 0.128	< 0.001	0.326 ± 0.106	0.716 ± 0.114	< 0.001	0.477 ± 0.216	0.658 ± 0.202	< 0.001
LS-BMD Z score	−3.2 ± 2.3	−0.3 ± 1.3	< 0.001	−3.9 ± 1.8	0.2 ± 1.6	< 0.001	−2.3 ± 1.9	−0.2 ± 1.7	0.004
FN-BMD (g/cm^2)	0.462 ± 0.121	0.588 ± 0.145	< 0.001	0.419 ± 0.154	0.628 ± 0.142	< 0.001	0.444 ± 0.221	0.636 ± 0.178	< 0.001
FN-BMD Z score	−3.4 ± 1.9	−1.7 ± 1.6	< 0.001	−3.7 ± 2.1	−1.5 ± 1.9	< 0.001	−3.5 ± 2.2	−1.2 ± 1.7	0.007
VCF (%)	63.4%	58.7%	0.211	72.6%	67.0%	0.228	/	/	/
Average PA (mm^2)	285.8 ± 150.1	377.4 ± 182.9	< 0.001	243.0 ± 97.0	404.2 ± 143.5	< 0.001	363.2 ± 201.7	400.9 ± 205.9	< 0.001
Average mh/ph	0.63 ± 0.16	0.75 ± 0.13	< 0.001	0.56 ± 0.15	0.77 ± 0.11	< 0.001	0.99 ± 0.01	0.99 ± 0.01	0.600
Average ah/LL	0.44 ± 0.10	0.52 ± 0.09	< 0.001	0.40 ± 0.10	0.52 ± 0.08	< 0.001	0.70 ± 0.09	0.72 ± 0.09	0.065
Average mh/LL	0.38 ± 0.15	0.48 ± 0.13	< 0.001	0.31 ± 0.12	0.47 ± 0.10	< 0.001	0.70 ± 0.09	0.72 ± 0.08	0.075
Average ph/LL	0.59 ± 0.09	0.63 ± 0.08	< 0.001	0.54 ± 0.08	0.61 ± 0.07	< 0.001	0.71 ± 0.09	0.72 ± 0.09	0.106

OI: Osteogenesis imperfecta, VCF: vertebral compressive fractures, NVCF: non vertebral compressive fracture, vs: versus, ALP: alkaline phosphatase, β-CTX: β-isomerized carboxy-telopeptide of type I collagen, BMD: bone mineral density, LS: lumbar spine, FN: femoral neck, PA: projection area.

of ZOL treatment (Table 2). These results indicated that the compressive vertebrae of OI children could be significantly reshaped during ZOL treatment (Fig. 2).

9 patients in NVCF group completed 1-year follow-up (Supplemental Fig. 1), who also had significant increases in height, weight, LS-BMD, FN-BMD and the Z scores, decreases in β-CTX levels after ZOL treatment ($P < 0.05$) (Table 2). The projection area of vertebrae was also increased after ZOL treatment. But no change in mh/ph, ah/LL, mh/LL and ph/LL after ZOL treatment was found in NVCF group (Table 2). Due to the small sample size, we did not analyze the data after 2 years of ZOL treatment in NVCF group. Comparison of vertebral morphometry in NVCF group between baseline and after treatment was shown in Supplemental Fig. 4.

3.3. Cross-sectional comparison between ZOL treated and control groups

Compared to treatment naïve patients, the level of β-CTX was lower; BMDs and their Z scores were higher in the ZOL treated group. The mh/ph, ah/LL and mh/LL were prominently higher in ZOL treated group than the control group after adjusted for age, gender and height ($P < 0.01$). Although there was no significantly statistical difference, the average of projection area and ph/LL were prone to be higher in ZOL treated group than the control group (Table 3). These results supported that compared to the same age, gender and clinical severity treatment naïve patients, the compressive vertebrae of OI children could be improved after ZOL treatment.

3.4. Safety of ZOL treatment

During ZOL treatment, no necrosis of the jaw, atypical femoral fracture, delayed fracture healing and arrhythmia was observed. 2 patients in VCF group had worsening of L1 and L3 compression fracture during the first year of ZOL treatment. The percentage of new non-vertebral fractures was 16.5% and 20.0% in VCF and NVCF group, respectively. No hepatic or renal function abnormity was found during ZOL treatment. Acute-phase reactions were the most common side effects of ZOL treatment. All patients presented fever within one to three days after the first infusion of ZOL with the highest body temperature of 38.0–40.0 °C. The serum levels of ALT, Cr, Ca, P, ALP and β-CTX were measured in 10 patients within the first three day after the first infusion of ZOL, and mild hypocalcemia and hypophosphatemia were found in these patients (Supplemental Table 1). After we increased the dose of calcitriol as 0.25 μg daily, hypocalcemia and hypophosphatemia were improved.

Table 3
Comparisons between ZOL treated group and treatment naïve control group matched for age, gender and clinical severity.

	ZOL group(n = 17)	Control group(n = 17)	P
Boys/girls	10/7	10/7	–
Age (years)	8.9 ± 3.7	8.9 ± 3.7	–
Sillence classification (I/III/IV)	13/0/4	13/0/4	–
COL1A1/IFITM5	12/2	15/0	–
Height	132.1 ± 21.6	132.5 ± 20.9	0.949
Height-Z score	−0.3 ± 1.4	−0.2 ± 1.4	0.833
Weight	33.1 ± 16.1	33.1 ± 14.0	0.996
Weight-Z score	0.3 ± 1.4	0.4 ± 1.2	0.644
ALP (IU/L)	274 ± 78	318 ± 79	0.116
β-CTX (ng/ml)	0.608 ± 0.200	1.052 ± 0.381	< 0.001
LS-BMD (g/cm^2)	0.662 ± 0.168	0.414 ± 0.093	< 0.001
LS-BMD Z score	0.1 ± 1.8	−2.7 ± 1.1	< 0.001
FN-BMD (g/cm^2)	0.625 ± 0.104	0.502 ± 0.092	0.001
FN-BMD Z score	−1.1 ± 1.3	−2.6 ± 1.2	0.001
Proportion of compressed vertebrae	55.5%	61.9%	0.189
Average PA of T4-L4 (mm^2)	373.0 ± 208.9	314.9 ± 158.7	0.106*
Average mh/ph of T4-L4	0.79 ± 0.11	0.62 ± 0.12	< 0.001*
Average ah/LL of T4-L4	0.53 ± 0.09	0.46 ± 0.10	0.009*
Average mh/LL of T4-L4	0.52 ± 0.12	0.39 ± 0.12	0.001*
Average ph/LL of T4-L4	0.66 ± 0.07	0.61 ± 0.08	0.059*

OI: Osteogenesis imperfecta, VCF: vertebral compressive fractures, NVCF: non vertebral compressive fracture, AR: autosome recessive gene including *WNT1* and *PLOD2*, ALT: alanine aminotransferase, Cr: creatinine, Ca: Serum calcium, P: Serum phosphate, ALP: alkaline phosphatase, β-CTX: β-isomerized carboxy-telopeptide of type I collagen, 25OHD: 25 hydroxy-vitamin D, PTH: parathyroid hormone, BMD: bone mineral density, LS: lumbar spine, FN: femoral neck, PA: projection area.

* P values were adjusted for age, gender and height.

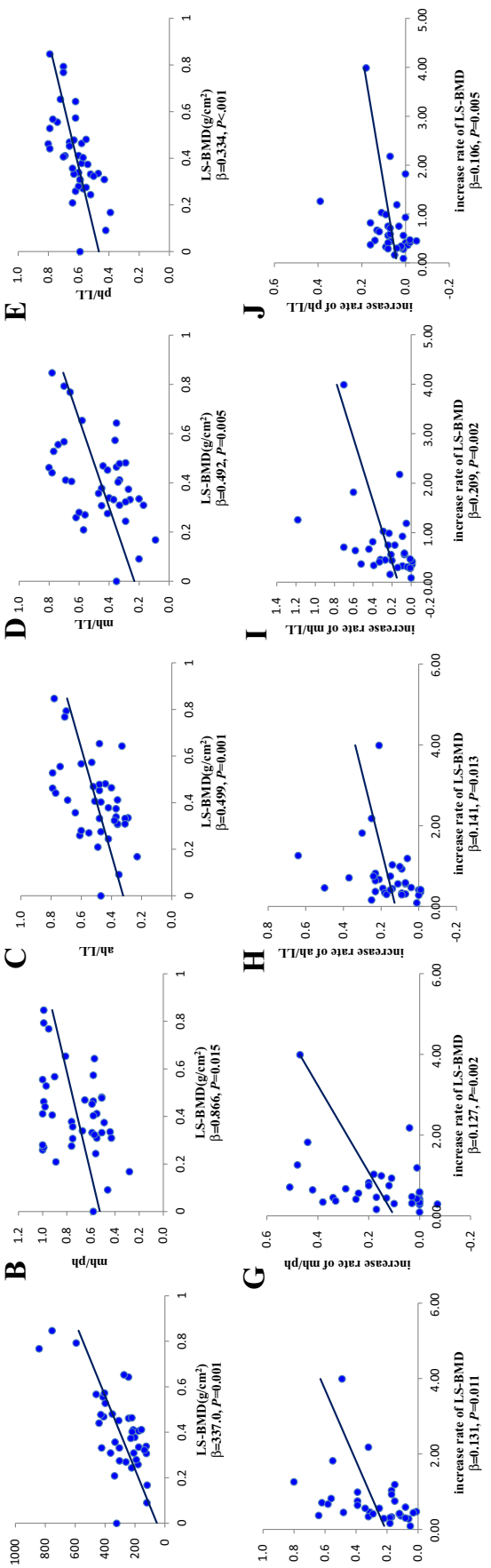

Fig. 3. Influence factors of vertebral shape at baseline and after ZOL treatment for 1 year.
A–E. Relationship between LS-BMD and PA, mh/ph, ah/LL, mh/LL, ph/LL at baseline.
F–J. Relationship between increase rate of LS-BMD and increase rate of PA, mh/ph, ah/LL, mh/LL, ph/LL after ZOL treatment for 1 year.
β means un-standard coefficients, which was adjusted for age, gender, clinical severity, height, weight, ALP, β-CTX, LS-BMD, FN-BMD, number of fractures at baseline and adjusted for age, gender, clinical severity, growth rate, the decrease rate of ALP, the decrease rate of β-CTX, the increase rate of LS-BMD, the increase rate of FN-BMD after ZOL treatment for 1 year.
ZOL: zoledronic acid, ALP: alkaline phosphatase, β-CTX: β-isomerized carboxy-telopeptide of type I collagen, BMD: bone mineral density, LS: lumbar spine, FN: femoral neck.

3.5. Influence factors of vertebral shape at baseline and after ZOL treatment

Multiple linear regressions analysis revealed that LS-BMD was positively correlated to projection area (β = 337.0, $P = 0.001$), mh/ph (β = 0.866, $P = 0.015$), ah/LL (β = 0.499, $P = 0.001$), mh/LL (β = 0.492, $P = 0.005$) and ph/LL (β = 0.334, $P < 0.001$) at baseline in 42 OI patients after adjustment by age, gender, clinical severity, height, weight, ALP, β-CTX, FN-BMD and number of fractures (Fig. 3A–E).

After one year of ZOL treatment, the increase rate of LS-BMD was positively correlated to the increase degree of projection area (β = 0.131, $P = 0.011$), mh/ph (β = 0.127, $P = 0.002$), ah/LL (β = 0.141, $P = 0.013$), mh/LL (β = 0.209, $P = 0.002$) and ph/LL (β = 0.106, $P = 0.005$) after adjustment by age, gender, clinical severity, growth rate, the decrease rate of ALP and of β-CTX and the increase rate of FN-BMD (Fig. 3F–G). As the limited quantity of patients completed the two years of ZOL treatment, multiple variates analysis was not completed.

4. Discussion

To our knowledge, this was the first prospective study to quantitatively evaluate the effects of ZOL treatment on vertebral shape of children and adolescents with OI. The results indicated that the compressive vertebrae of OI children could be effectively reshaped after ZOL treatment. Low LS-BMD was the independent risk factor of VCFs in OI patients and the increase in LS-BMD was positively correlated to the improvement of vertebral shape during treatment.

VCF was a common and severe complication of OI patients, but it had been neglected for a long period. Approximately 30% of type I OI and 79% of types III/IV OI children had multiple VCFs [23]. VCF not only led to scoliosis, but also impaired the quality of life of patients, and even led to impairment of cardiorespiratory, one of the main causes of mortality in OI patients [7]. Previous studies did not accurately evaluate VCF in OI patients by quantitative assessment [15,24]. We applied the vertebral morphometry to quantitatively measure the deformity and compression of vertebrae, which improved the diagnostic accuracy of VCF in OI patients.

The risk factors of VCF in OI were unclear. In this study, we found that low LS-BMD was an independent risk factor of VCF at baseline. Moreover, a retrospective study revealed that with age increasing, VCF was prone to occur and could be aggravated if no effective treatment was given [11]. Another risk factor of VCF was the clinical severity of OI, and types III/IV OI patients had a higher proportion of VCF than OI type I patients [11]. The effect of genotype on VCF occurrence was still unclear, which needed to be explored in future.

BPs was widely used to treat bone fragility of OI [25]. As a third-generation BP, ZOL had high binding affinity for hydroxyapatite and played strong inhibiting roles on bone resorption, which could be administered annually [26]. Considering the severity of OI and pharmacoeconomic factors, we chose a relative high dose of ZOL to treat OI patients. The safety was good in OI children in this study and our previous study [15]. ZOL could increase BMD, reduce fracture incidence, and with well tolerance in children and adolescents with OI [15]. In the present study, we confirmed that the compressive vertebrae of OI children could be significantly reshaped after ZOL treatment. We also found that the increase of LS-BMD was an important determinant of vertebral reshaping, which was similar to a previously study [27]. The effect of other intravenous BPs on vertebral shape was also reported. Pamidronate was demonstrated to improve the vertebral shape in children with OI type I, III, IV, VI (with *SERPINF1* mutation) and VII (carrying *CRTAP* mutation) [10–12,27–33]. Infusion of neridronate had the same effect on vertebral shape of OI children [10,13]. We also observed that the compressed vertebrae of OI children could be reshaped during ibandronate treatment [34]. Although oral BPs also could inhibit bone resorption, increase BMD and reduce fracture incidence in children and adolescents with OI [35], it seemed to have a limited effect on vertebral shape [36–38].

We also found the height-Z score was considerably increased in VCF group after ZOL treatment, indicating catch-growth. This phenomenon was also observed in children and adolescents with OI after treatment of pamidronate [27]. This beneficial effect may be partially attributed to the vertebral reshape after the infusions of BPs. In addition, BPs could increase metaphyseal bone mass, and reduce bone fracture incidence, which were also beneficial to catch-growth of children [39].

There were several limitations of our study. The measurement of vertebral shape might be influenced by scoliosis in some patients. The sample size was relatively small which may limit our ability to elucidate the relationship between vertebral shape and genotype of OI. The duration of ZOL treatment was relatively short. We would continue to follow our patients for a longer period to confirm the effects of ZOL on vertebral shape.

In conclusion, through quantitatively assessment of the vertebral morphology, we verified that the compressive vertebrae of children and adolescents with OI could be effectively reshaped during ZOL treatment. Low LS-BMD was an independent risk factor for the occurrence of VCF and the increase in LS-BMD was positively correlated to the improvement in vertebral shape after ZOL treatment.

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Declaration of Competing Interest

The authors state that they have no conflicts of interest.

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Author contributions

L.J.L. analyzed data and wrote the manuscript. W.B.Z. and D.C.Z. contributed to data collection. W.Y., O.W., Y.J., W.B.X. contributed to review the manuscript. M.L. contributed to the conception and design of the research, acquisition and interpretation of the data, and revised the manuscript.

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