Acta Med. Okayama, 2016
Vol. 70, No. 6, pp. 435-439

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Urinary Cross-linked N-terminal Telopeptide of Type I Collagen Levels of Infants with Osteogenesis Imperfecta and Healthy Infants

Miho Yamashita[a], Kosei Hasegawa[a]*, Yousuke Higuchi[a], Takayuki Miyai[a], Ayumi Okada[b], Hiroyuki Tanaka[c], and Hirokazu Tsukahara[b]

Department of Pediatrics, [a]Okayama University Hospital, and [b]Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan, [c]Department of Pediatrics, Okayama Saiseikai General Hospital, Okayama 700-8511, Japan

The urinary cross-linked N-terminal telopeptide of type I collagen (uNTx) levels in infantile osteogenesis imperfecta (OI) have not been well studied. Here we investigated the levels of uNTx in infants with OI and healthy infants. We collected spot urine samples from 30 infants with OI (male/female, 14/16; Sillence classification, I/II/III/IV: 15/3/6/6; age, 5.2 ± 4.4 months) and 120 healthy infants (male/female, 75/45; age, 5.1 ± 4.1 months) for the measurement of uNTx levels. The uNTx levels of the OI infants were significantly lower than those of the healthy infants (mean ± SD, 1,363.7 ± 530.1 vs. 2,622.2 ± 1,202.6 nmol BCE/mmol Cr; $p < 0.001$). The uNTx levels of the infants with type I OI were significantly lower than those of the age-matched healthy infants, although an overlap was observed between the 2 groups. Among the 1-month-old infants, the uNTx levels of the infants with types I, III or IV OI were significantly lower than those of the healthy infants, without overlap (1,622.5 ± 235.8 vs. 3,781.0 ± 1,027.1 nmol BCE/mmol Cr; $p < 0.001$). These results indicate that uNTx levels are significantly lower in infants with OI than in healthy infants, and they suggest that uNTx might be useful as a reference for diagnosing OI.

Key words: bone resorption marker, bone turnover, bone mass

Osteogenesis imperfecta (OI) is a heritable bone disorder that is manifested as various degrees of bone fragility. A widely used clinical classification of OI was proposed by Sillence in 1979 [1] and revised by van Dijk and Sillence in 2014 [2]. Most cases of OI are caused by genetic mutation of the type I collagen gene [3,4]. Type I collagen molecules are connected to one another by pyridinoline cross-links that contribute to increased strength and bone flexibility. The N-terminal telopeptide of type I collagen (NTx) is a peptide that includes a pyridinoline cross-link. NTx is excreted during bone resorption, and the NTx levels in serum and urine can be measured. Urinary NTx (uNTx) reflects the state of bone resorption in various conditions, such as postmenopausal osteoporosis and bone metastasis of cancer [5-7]. Urinary NTx levels in healthy children and adolescents are higher than those in adults and change according to the growth rate during childhood [8,9].

In an earlier study we compared the uNTx levels of children with OI with those of healthy children, and

Received March 30, 2016; accepted May 26, 2016.
*Corresponding author. Phone:+81-86-235-7249; Fax:+81-86-221-4745
E-mail:haseyan@md.okayama-u.ac.jp (K. Hasegawa)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

our findings revealed that the uNTx levels of the children with OI tended to be lower than those of the healthy children, especially at a younger age [10]. This finding conflicts with those of other studies in children and adult patients with OI, which reported that bone resorption markers in OI are normal or slightly elevated compared with healthy age-matched controls [11–13].

In contrast to pediatric and adult patients with OI, the uNTx levels of infant OI patients have not been clarified. In the present study we analyzed the uNTx levels of infants with OI and healthy infants to determine whether the measurement of uNTx—which is done in a noninvasive examination—makes it possible to differentiate infants with OI from healthy infants.

Subjects and Methods

Subjects. Thirty infants with OI were included in this study. For controls, 120 healthy infants who presented to a hospital in Okayama (latitude, 34° north) on the mainland of Japan, which had 2,028.2 h of daylight per year in 2011, were also included. The subjects' characteristics (age, sex, and OI type) are summarized in Table 1. The distribution of ages was comparable between the groups ($p = 0.85$).

All 30 OI patients were clinically diagnosed with OI based on clinical features, such as frequent bone fractures, family history, dentinogenesis imperfecta, and blue sclera. All but one of the infants with OI suffered from one or more bone fractures 2–4 weeks before the collection of urine samples. All of the infants with type II OI had experienced multiple fractures before and after birth, and they received mechanical ventilation. All 120 of the healthy infants were born at term and had an appropriate weight for gestational age. Most of the healthy infants and infants with OI, except for the type II OI patients, were breast-fed. None of the subjects showed apparent renal impairment. Informed consent was obtained from the parents or guardians of all infants included in this study. This study was approved by the Ethics Committee of Okayama University Hospital (approval no. 1454) and conducted according to the Declaration of Helsinki.

Urine sample collection and measurement of uNTx. Spot urine samples were collected from each infant after informed consent was obtained from the parent/guardian. Urine samples were collected from the healthy infants at a random time during a medical checkup performed in the months from July to September (summer to early autumn) 2011. The urine samples were collected from the infants with OI at a random time before the first pamidronate infusion during any of the seasons: spring (March to May): $n = 6$, summer (June to August): $n = 7$, autumn (September to November): $n = 9$, and winter (December to February): $n = 8$. All urine samples were stored at −20 ℃ until analysis. The levels of uNTx were measured by an enzyme-linked immunosorbent assay (ELISA) at the Life Science Institute (Tokyo, Japan) using the Osteomark® NTx Urine Assay (Alere Scarborough, Scarborough, ME, USA). The inter- and intra-assay coefficients of variation were below 7–10%. All uNTx assay results were corrected by the creatinine level in the urine (nmol BCE/mmol creatinine).

Statistics. All values are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using the software program JMP 9.02 (SAS Institute, Cary, NC, USA). A *t*-test was conducted to evaluate the differences in values between the healthy infants and OI infants. Differences were considered significant at $p < 0.05$.

Table 1 Characteristics of the study subjects

Subjects	Healthy infants	Infants with osteogenesis imperfecta				
		All	Type I	Type II	Type III	Type IV
Number	120	30	15	3	6	6
Age (month*)	5.06 ± 4.14	5.23 ± 4.41	6.93 ± 4.31	1.00 ± 0.00	3.33 ± 4.41	5.00 ± 4.15
Sex (m/f)	(75/45)	(14/16)	(7/8)	(2/1)	(2/4)	(3/3)

*Data areas mean ± SD.

Results

Levels of uNTx in the healthy infants and infants with OI. The uNTx levels of the healthy infants and infants with OI are shown in Fig. 1. The uNTx levels of the healthy infants tended to be higher at a younger age. The comparison of the uNTx levels between the 2 groups revealed that the uNTx levels were significantly lower in the infants with OI compared to the age-matched healthy infants ($1,363.7 \pm 530.1$ vs. $2,622.2 \pm 1,202.6$; $p < 0.001$). We re-analyzed the data, excluding the infants with type II OI from the all-OI group, taking into consideration the effect of the extremely serious condition of type II OI on bone turnover. When the 3 patients with type II OI were excluded (age of healthy infants: 5.1 ± 4.1 months; OI: 5.7 ± 4.4 months, $p = 0.47$), the uNTx levels were still significantly lower in the infants with OI compared to the healthy infants ($1,444.0 \pm 488.1$ vs. $2,622.2 \pm 1,202.6$; $p < 0.001$).

Comparison of uNTx levels in type I OI infants and age-matched healthy infants. Because infants with type I OI, the mildest form of OI, show only mild clinical features, it is sometimes difficult to diagnose OI clinically. We therefore compared the uNTx levels of the 15 infants with type I OI with those of the 79 age-matched healthy infants (age of type I patients 6.9 ± 4.3 months; healthy infants 6.8 ± 4.0 months; $p = 0.92$) to determine whether the measurement of the uNTx level could differentiate infants with type I OI from healthy infants.

The results of the comparison demonstrated that the uNTx levels of the infants with type I OI were significantly lower than those of the age-matched healthy infants, but an overlap of values was observed between the 2 groups ($1,504.6 \pm 553.8$ vs. $2,218.2 \pm 949.0$; $p = 0.006$), and it was difficult to observe any clear differentiation by uNTx level between the infants with type I OI and the healthy infants.

Levels of uNTx at 1 month old. Lastly, we analyzed the difference in uNTx levels between the OI and healthy infants at 1 month old. The results are shown in Fig. 2. For the reason described above, we also excluded the infants with type II OI from this analysis. The uNTx levels of the 1-month-old OI infants were significantly lower than those of the same-age healthy infants ($1,622.5 \pm 235.8$ vs. 3,781.0

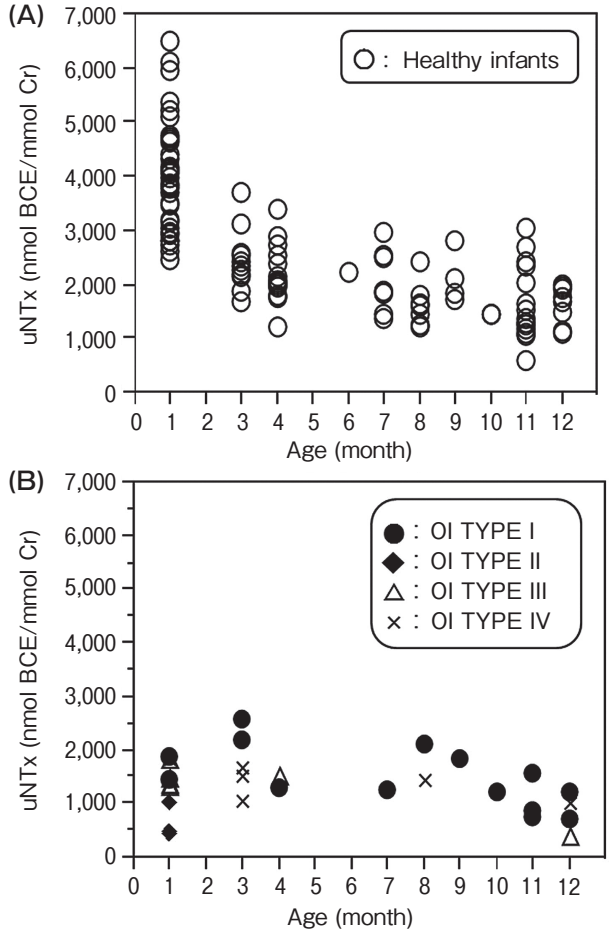

Fig. 1 Relationship between age and uNTx levels from 1 to 12 months, in the 120 healthy infants (A) ($y = 2,746.9619 - 157.49x + 50.01\ [x - 5.0667]^2 - 5.2099\ [x - 5.07]^3$, $r = -0.70$, $p < 0.001$) and the 30 infants with osteogenesis imperfecta (OI) (B).

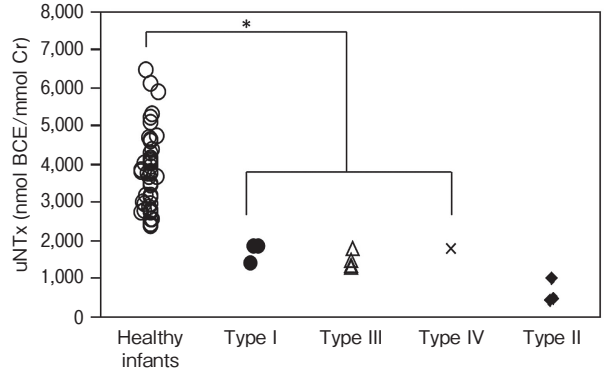

Fig. 2 Urinary NTx levels in 1-month-old OI infants (total = 11; type I, $n = 3$; type II, $n = 3$; type IV, $n = 4$; type III, $n = 1$) and 1-month-old healthy infants ($n = 45$). $^*p < 0.001$.

$\pm 1{,}027.1$; $p < 0.001$). No overlap in uNTx levels was observed between these 2 groups.

To analyze whether the body size of infants at 1 month old affects uNTx levels, we analyzed the relation between uNTx levels and body height and between uNTx levels and body weight at each sample collection. No significant relationship between uNTx levels and height or body weight was demonstrated in the 44 healthy infants and in the OI infants (Fig. 3A, B).

Discussion

The results of the present study demonstrated for the first time that the uNTx levels of infants with OI are significantly lower compared to those of healthy infants. uNTx reflects the amount of excreted pyridinoline cross-links, as well as the state of bone resorption. The total amount of pyridinoline cross-links per one collagen molecule in the bone of OI patients was reported to be equal to that of control bone [14]. Our present findings suggest that the lower uNTx levels in infants with OI are attributable to their lower bone mass.

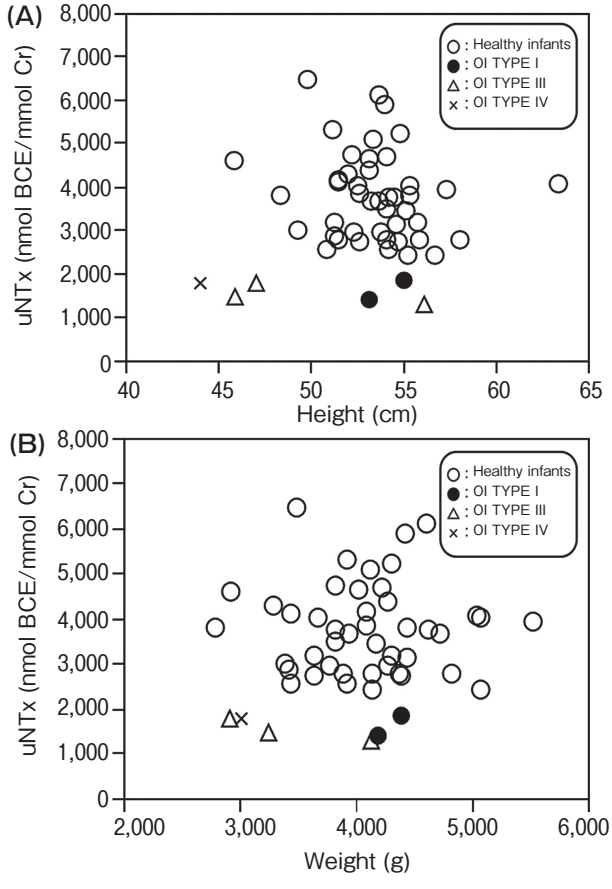

Fig. 3 Relationship between uNTx levels and the body height and body weight of 1-month-old healthy controls ($n = 44$) and infants with OI ($n = 6$). In the healthy infants, there was no significant relationship between uNTx levels and body height (**A**), ($r = -0.15$, $p = 0.32$) or body weight (**B**), ($r = -0.008$, $p = 0.96$). Also in the OI infants, no significant relationship between uNTX levels and body height (**A**) ($r = -0.12$, $p = 0.40$) or body weight (**B**) ($r = 0.06$, $p = 0.65$) was observed.

We collected urine samples from the healthy infants in summer to early autumn in order to minimize the effects of vitamin D deficiency on bone turnover. Most of the infants with OI and healthy infants in our study were breast-fed. Breast-fed infants have lower serum 25-hydroxy vitamin D concentrations and significantly increased intact parathyroid hormone levels compared to formula- or mixed-fed infants [15]. In summer, the prevalence of vitamin D deficiency is reduced and bone resorption is decreased compared with the other seasons. Although uNTx levels are thought to be decreased in healthy infants, we observed that the uNTx levels of the present study's OI infants, from whom urine was collected across all four seasons, were significantly lower than those of the healthy infants.

We also observed that, among the 1-month-old subjects, the uNTx levels of the infants with OI were significantly lower than those of the healthy infants, with no overlap of the NTx levels between the 2 groups. We attribute this finding to the difference in bone mass between the 2 groups and to the highest bone turnover state at this age [16]. Although the number of 1-month-old infants with OI that we analyzed in this study was small, the uNTx levels at 1 month old might become a good marker for diagnosing OI.

The limitations of our study are as follows. (1) The effects of bone fractures on the uNTx level are not known, and (2) we were unable to collect blood samples from the subjects at the same time of urine sample collection and thus we could not measure serum 25(OH) vitamin D levels, parathyroid hormone levels or bone formation markers. The uNTX level is affected by vitamin D status, parathyroid hormone levels and bone formation status [17]. To determine whether the uNTX level in a 1-month-old patient can be used as a diagnostic marker of OI, these problems

must be addressed.

In conclusion, we observed that the uNTx levels of infants with OI were significantly lower than those of healthy infants. While additional studies are necessary, our present findings suggest that uNTx might be useful as a reference for diagnosing OI.

Acknowledgments. This study was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan (23791177), and by the Foundation for Growth Science, Japan.

References

1. Sillence DO, Senn A and Danks DM: Genetic heterogeneity in osteogenesis imperfecta. J Med Genet (1979) 16: 101–116.
2. van Dijk FS and Sillence DO: Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A (2014) 164A: 1470–1481.
3. Chu ML, Williams CJ, Pepe G, Hirsch JL, Prockop DJ and Ramirez F: Internal deletion in a collagen gene in a perinatal lethal form of osteogenesis imperfecta. Nature (1983) 304: 78–80.
4. van Dijk FS, Byers PH, Dalgleish R, Malfait F, Maugeri A, Rohrbach M, Symonens EA and Pals G: EMQN best practice guidelines for the laboratory diagnosis of osteogenesis imperfecta. Eur J Hum Genet (2012) 20: 11–19.
5. Randall AG, Kent GN, Garcia-Webb P, Bhagat CI, Pearce DJ, Gutteridge DH, Prince RL, Stewart G, Stuckey B, Will RK, Price RI and Ward L: Comparison of biochemical markers of bone turnover in Paget disease treated with pamidronate and a proposed model for the relationships between measurements of the different forms of pyridinoline cross-links. J Bone Miner Res (1996) 11: 1176–1184.
6. Kamiya N, Suzuki H, Endo T, Yano M, Naoi M, Nishimi D, Kawamura K, Imamoto T and Ichikawa T: Clinical usefulness of bone markers in prostate cancer with bone metastasis. Int J Urol (2012) 19: 968–979.
7. Tella SH and Gallagher JC: Prevention and treatment of postmenopausal osteoporosis. J Steroid Biochem Mol Biol (2014) 142: 155–170.
8. Mora S, Prinster C, Proverbio MC, Bellini A, de Poli SC, Weber G, Abbiati G and Chiumello G: Urinary markers of bone turnover in healthy children and adolescents: age-related changes and effect of puberty. Calcif Tissue Int (1998) 63: 369–374.
9. Sato J, Hasegawa K, Tanaka H and Morishima T: Urinary N-telepeptides of type I collagen in healthy children. Pediatr Int (2010) 52: 398–401.
10. Hasegawa K, Kataoka K, Inoue M, Seino Y, Morishima T and Tanaka H: Impaired pyridinoline cross-link formation in patients with osteogenesis imperfecta. J Bone Miner Metab (2008) 26: 394–399.
11. Braga V, Gatti D, Rossini M, Colapietro F, Battaglia E, Viapiana O and Adami S: Bone turnover markers in patients with osteogenesis imperfecta. Bone (2004) 34: 1013–1016.
12. Garnero P, Schott AM, Prockop D and Chevrel G: Bone turnover and type I collagen C-telopeptide isomerization in adult osteogenesis imperfecta: associations with collagen gene mutations. Bone (2009) 44: 461–466.
13. Brenner RE, Vetter U, Bollen AM, Morike M and Eyre DR: Bone resorption assessed by immunoassay of urinary cross-linked collagen peptides in patients with osteogenesis imperfecta. J Bone Miner Res (1994) 9: 993–997.
14. Bank RA, Tekoppele JM, Janus GJ, Wassen MH, Pruijs HE, Van der Sluijs HA and Sakkers RJ: Pyridinium cross-links in bone of patients with osteogenesis imperfecta: evidence of a normal intrafibrillar collagen packing. J Bone Miner Res (2000) 15: 1330–1336.
15. Yorifuji J, Yorifuji T, Tachibana K, Nagai S, Kawai M, Momoi T, Nagasaka H, Hatayama H and Nakahata T: Craniotabes in normal newborns: the earliest sign of subclinical vitamin D deficiency. J Clin Endocrinol Metab (2008) 93: 1784–1788.
16. Lapillonne A, Travers R, DiMaio M, Salle BL and Glorieux FH: Urinary excretion of cross-linked N-telopeptides of type 1 collagen to assess bone resorption in infants from birth to 1 year of age. Pediatrics (2002) 110: 105–109.
17. Baroncelli GI, Bertelloni S, Ceccarelli C, Amato V and Saggese G: Bone turnover in children with vitamin D deficiency rickets before and during treatment. Acta Paediatr (2000) 89: 513–518.