Digestive and Liver Disease 47 (2015) 652–657

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Alimentary Tract

Seasonal variability of vitamin D and bone metabolism in infliximab-treated paediatric Crohn's disease

Dolóresz Szabó[a], Éva Hosszú[b], András Arató[a], Katalin Eszter Müller[a], Nóra Béres[a], Péter László Lakatos[c], Mária Papp[d], Antal Dezsőfi[a], Attila J. Szabó[a,e], Dániel Szűcs[f], Gabor Veres[a,*]

[a] 1st Department of Pediatrics, Semmelweis University, Budapest, Hungary
[b] 2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary
[c] 1st Department of Medicine, Semmelweis University, Budapest, Hungary
[d] Institute of Internal Medicine, Department of Gastroenterology, University of Debrecen, Clinical Center, Debrecen, Hungary
[e] MTA-SE, Pediatrics and Nephrology Research Group, Budapest, Hungary
[f] Department of Pediatrics and Paediatric Health Care Center, University of Szeged, Faculty of Medicine, Szeged, Hungary

ARTICLE INFO

Article history:
Received 15 October 2014
Accepted 1 May 2015
Available online 19 May 2015

Keywords:
Bone mineral density
Bone markers
Crohn's disease
Infliximab
Paediatric
Vitamin D

ABSTRACT

Background: Paediatric Crohn's disease patients suffer from several complications, including low bone mineral density and inadequate serum levels of 25-hydroxy vitamin D.
Aims: The aim of this prospective study was to address the effect of infliximab therapy on bone metabolism, bone mineral density and vitamin D homeostasis. The seasonal variability of serum vitamin D levels in relation to infliximab treatment was also analysed.
Methods: Serum osteocalcin and beta-crosslaps (markers of bone metabolism), seasonal variability of vitamin D, and bone mineral density were assessed and followed throughout the yearlong treatment regimen of infliximab in 50 consecutive paediatric patients with moderate to severe Crohn's disease.
Results: Bone forming osteocalcin levels were significantly ($p < 0.001$) increased during infliximab therapy. In contrast, no significant changes in beta-crosslaps and vitamin D levels were observed. Vitamin D levels were significantly different when the summer and winter periods were compared at week 0 ($p = 0.039$); however, this difference was not detected after one year of infliximab therapy. Despite the beneficial clinical effect of infliximab, there was no significant change in bone mineral density *Z*-scores after one year of treatment.
Conclusion: Infliximab may beneficially affect bone homeostasis. Moreover, seasonal variability in vitamin D levels observed prior to initiation of infliximab treatment was diminished after one year of treatment.

1. Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) condition affecting the gastrointestinal tract. There is an increasing incidence of CD in childhood, which has thus contributed to an increased number of affected children [1,2]. Chronic intestinal inflammation leads to low bone mineral density (BMD), a frequent complication in both children and adults with CD [3,4]. It has been hypothesized that low BMD in CD occurs either because of the disease itself or due to the therapeutics used. Abnormalities in BMD, which can be evaluated by densitometry, can also be analysed by biochemical markers of bone turnover such as osteocalcin, a marker of bone formation, and β-isomerized C-terminal telopeptide fragments of collagen type I (beta-CrossLaps or bCL), an indicator of bone resorption [5].

In addition to inflammation, several other mechanisms have been implicated in CD-related reduction of BMD, such as malabsorption and malnutrition, genetics, drug therapy (e.g. corticosteroids), low dietary intake of essential vitamins (e.g. 25-hydroxy vitamin D), and a lack of physical activity. Release of chronic pro-inflammatory cytokines plays a pivotal role in the regulation and maintenance of bone health. Tumour necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and IL-6β stimulate bone resorption through a balanced modulation of osteoclast and osteoblast activation and differentiation [6,7]. While previous studies have

* Corresponding author at: 1st Department of Pediatrics, Semmelweis University, 53 Bókay Street, 1083 Budapest, Hungary. Tel.: +36 1 3343743; fax: +36 1 3036077.
E-mail address: veres.gabor@med.semmelweis-univ.hu (G. Veres).

http://dx.doi.org/10.1016/j.dld.2015.05.006
1590-8658/

implicated corticosteroids in impaired BMD [8], other treatments have been shown to have a beneficial effect on bone through regulation of pro-inflammatory cytokines. Infliximab (IFX), a chimeric monoclonal antibody therapeutic targeted against TNF-α, may improve bone mineralization [9]. Several different mechanisms contribute to the positive effects of IFX. While IFX-mediated reduction of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, represent an important mode of action [10,11], the IFX-induced reduction in gut inflammation also promotes improved absorption of critical bone nutrients such as vitamin D and calcium. Moreover, an improvement in patient health leads to increased physical activity, which may also lead to an increase bone formation [6].

Vitamin D is an essential nutrient required for proper bone mineralization. Vitamin D has also been shown to regulate the immune system due to its indispensable role in the development of self-tolerance. Studies in animal and in vitro models have shown that vitamin D functions in the formation of a normal immune response and enhancement of cytokine production [12,13]. Recent studies have revealed a higher prevalence of vitamin D deficiency in adults and children with IBD [3,14]. The benefits of vitamin D indicate that it may be an additional treatment option in CD. An earlier study, however, demonstrated conflicting results when CD patients with low BMD were treated with calcium and vitamin D [15].

Vitamin D levels fluctuate with the different seasons of the year. The late winter months are associated with lower vitamin D levels both in healthy individuals and IBD patients [13,16,17]. The effect of seasons on vitamin D levels in patients receiving IFX therapy, however, has not yet been studied.

The primary aim of this study was to evaluate the effect of IFX treatment on clinical parameters, bone metabolism, bone mineral density, and vitamin D homeostasis in moderate to severe paediatric CD patients. We also provide a characterization of the seasonal variability of vitamin D levels at baseline and follow-up in our patient population.

2. Patients and methods

2.1. Patients

Our prospective study was performed in the 1st Department of Paediatrics of Semmelweis University from January 2009 to December 31, 2013. Fifty children with moderate to severe CD who were resistant to conventional therapy (azathioprine, systemic steroids) and had a Paediatric Crohn's Disease Activity Index (PCDAI) >30 were included in the analysis of vitamin D level, bone markers and BMD changes during the yearlong IFX therapy. To monitor the seasonal changes of serum vitamin D levels, an additional 25 children with CD who were treated with IFX were included (only the vitamin D serum level was available).

Inclusion criteria were resistance to conventional therapy for at least three months following their diagnosis, a PCDAI score higher than 30 when IFX therapy was initiated, and vitamin D supplementation for at least three months prior to IFX therapy. Administration of azothiprine or steroids was not grounds for exclusion.

Control group. A control group containing 34 children with CD in clinical and IFX-free (PCDAI≤12.5) remission was also included in this study. Vitamin D serum level was measured in every patient from the control group. Additionally, 14 patients were also evaluated for osteocalcin and bCL levels. In the patients analysed for osteocalcin and bCL levels, steroid treatment was not allowed as a form of remission induction.

Written, informed consent was obtained from parents prior to study enrollment. The study was approved by the Semmelweis University Regional and Institutional Committee and Research Ethics.

2.2. Methods

Vitamin D and calcium supplementation. During treatment, patients (IFX and control groups) received 1000 U/day vitamin D and 500 mg/day calcium supplements.

Patients in the IFX treatment group received a 5 mg/kg intravenous infusion of IFX as an induction therapy at week 0, 2 and 6, and as a maintenance therapy at every following 8th week, based on the guidelines for IFX treatment in paediatric CD [18].

IMPACT-III and PCDAI. To measure health-related quality of life (QoL) the IMPACT-III questionnaire – developed by Otley et al. and validated in Hungary – was administered to patients. IMPACT-III is a self-reported questionnaire consisting of 35 queries. On a five point Likert scale, children indicated the extent to which they belong to each query by specific aspects of their health condition. IMPACT-III scores range from 35 to 175, with a higher score indicative of a better QoL [19,20]. In addition, PCDAI was evaluated and followed over time [21].

Bone markers and vitamin D. Levels of serum osteocalcin and bCL, markers of bone metabolism and bone turnover respectively, and 25-hydroxicholecalciferol (vitamin D) quantity were measured at week 0, week 6, week 30 and week 53. Blood samples were collected by venepuncture into a vacutainer tube with no additive, and the serums were processed before the IFX treatment started. Samples for the vitamin D analysis were protected from sunlight and were post-processed within a few hours of collection from the patient. A standard consensus regarding the normal range of vitamin D levels has not yet been established. Using the recent guidelines established by the Endocrine Society in 2011, vitamin D deficiency was defined as ≤20 ng/mL while vitamin D insufficiency was defined as 21–29 ng/mL [16,22].

Based on the date of the first IFX therapy, seasonal variability of vitamin D level was measured. The summer period was designated as the 21st of March through 22nd of September while the rest of the year was designated the winter period.

Procedures. Immunoassay analyses were carried out with electrochemiluminescence immunoassay (ECLIA) methods (Elecsys N-MID Osteocalcin and Elecsys beta-CrossLaps, Roche®) and with a chemiluminescence immunoassay (25-OH Vitamin D, LIAISON®, DiaSorin), which were performed according to the manufacturer's instructions.

Whole-body and lumbar spine Dual-energy X-ray absorptiometry (DEXA) (type: QDR Discovery, model: Discovery A (S/N 83638), HOLOGIC, Inc., USA) scans were used primarily to evaluate BMD status before IFX treatment and at the conclusion of one year of IFX treatment. All scans were performed by the same operator. The scan provided measurements of lumbar and whole-body area BMD (g/cm^2). *Z*-scores were calculated by subtracting the standard deviation of measured BMD from the expected BMD of individuals of the same age and sex. A *Z*-score <−2.0 was reported as reduced bone mineral mass [23]. Whole-body DEXA measurements served as a readout for cortical bone health, whereas lumbar spine DEXA served as an assessment of trabecular bone.

2.3. Statistics

All analyses were performed using SPSS 22 (IBM®, Somers, NY) statistical software. Based on the results of Kolmogorow–Smirnov and Shapiro–Wilk normality tests, the PCDAI, IMPACT-III, bone markers and vitamin D data sets followed a non-normal distribution. The BMD data showed a normal distribution. To determine significance, the Friedman test was used as a non-parametric test and a paired *t*-test was used as a parametric probe. A *p*-value of <0.05 was set as the threshold for statistical significance. Pairwise comparisons were performed with a Bonferroni correction for

Table 1
Characteristics of the study population.

Total number of patients	50
Male gender (%)	19 (38)
Mean age; years (±SD)	14.8 (±2.4)
Mean disease duration: years (±SD)	2.1 (±2.0)
Medical treatment when IFX therapy was introduced; *n* (%)	
Azathioprine + 5-ASA + steroid + antibiotic	3 (6)
Azathioprine + 5-ASA + steroid	9 (18) (4 methilprednisolone, 5 budesonide)
Azathioprine + 5-ASA + antibiotic	3 (6)
Azathioprine + 5-ASA	24 (48)
Azathioprine + steroid	1 (2)
Azathioprine + antibiotic	1 (2)
5-ASA + antibiotic	2 (4)
5-ASA + steroid	2 (4)
Azathioprine	2 (4)
5-ASA	3 (6)

5-ASA: 5-aminosalicylic acid, IFX: infliximab.

multiple comparisons. A p-value of 0.01 was considered statistically significant.

To determine the correlation between the laboratory and clinical parameters, the Spearman correlation coefficient was calculated.

3. Results

3.1. Patient data

The IFX group included 50 children (38% males, mean age 14.8 ± 2.4 years, range 8–18.6). The mean elapsed time since diagnosis was 2.1 ± 2.1 years (Table 1).

The control group included 34 children (55.8% males, mean age 14.5 ± 3.6 years). The mean elapsed time since diagnosis was 3.15 ± 2.88 years. In the control group, none of the patients received IFX therapy, and were all treated with azathioprine and 5-ASA the time of measurement; three patients also received systemic steroids.

With regard to seasonal variability, 36 initiated treatment in the winter (42.8%, mean age 14.6 ± 3 years) while 39 patients began their treatment during the summer (46.4%, mean age 14.7 ± 2.4 years).

3.2. Effect of IFX on PCDAI and IMPACT-III

PCDAI scores were assessed to monitor the efficacy of IFX induction and maintenance therapy. PCDAI decreased significantly ($p < 0.001$) during the treatment period when compared to the initial scores. The median PCDAI score was 35.0 (25th percentile [pc-25] 30; 75th percentile [pc-75] 42.5) at week 0 and decreased to 10.0 (pc-25, -75: 1.3, 20) by week 6 ($p < 0.0001$). During maintenance therapy, the PCDAI was 10.0 (pc-25, -75: 5, 20) and 5.0 (pc-25, -75: 0, 25) at week 30 and week 53, respectively ($p < 0.0001$). When compared with the baseline, all changes in the IFX group were significant.

After one year of IFX therapy, 33/50 of the IFX-treated children were in steroid-free remission (66%, PCDAI ≤ 12.5).

IMPACT-III scores were similarly improved after IFX treatment with significant differences at each monitoring time point ($p < 0.0001$). The median IMPACT-III score was 116.5 (pc-25, -75: 106.5, 131) at baseline and increased to 142 (pc-25, -75: 130, 149) by week 6 ($p < 0.0001$). During the maintenance therapy period, the IMPACT-III score was 140 (pc-25, -75: 129.5, 156.75) and 146 (pc-25, -75: 127, 153.75) at week 30 and week 53, respectively ($p < 0.0001$).

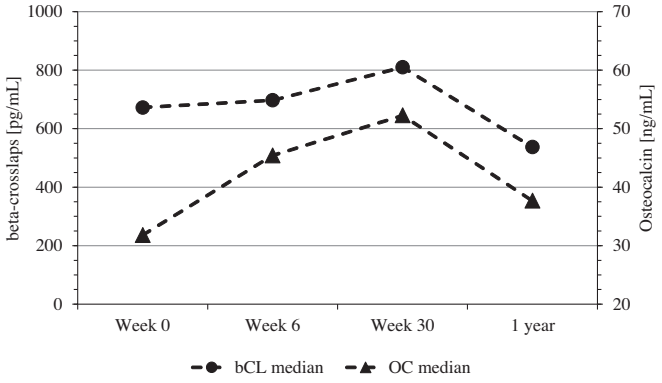

Fig. 1. Development of beta-crosslaps and osteocalcin medians during infliximab therapy course. bCL: beta-crosslaps; OC: osteocalcin.

3.3. Effect of IFX on bone markers

After IFX therapy, serum osteocalcin levels increased significantly ($\chi^2 = 18.61$, $p < 0.001$). A post hoc analysis revealed a significant change in the osteocalcin level from week 0 to week 6 (median 45.43; $p < 0.004$) and to week 30 (median 52.26; $p = 0.001$), but not from week 0 to week 53 (median 37.67). While significant changes in osteocalcin levels were observed, we did not observe significant changes in the serum bCL levels ($p = 0.105$) at any point during the yearlong IFX treatment (Table 2 and Fig. 1).

When the osteocalcin and bCL levels of patients in the control group were compared with those of IFX-treated patients, we did not observe significant differences at the beginning (osteocalcin: $p = 0.6$; bCL: $p = 0.08$) or end of the yearlong IFX treatment regimen (osteocalcin: $p = 0.66$; bCL: $p = 0.32$), including only those patients who were in remission (Table 2).

3.4. Vitamin D levels and seasonal variability of vitamin D

After one year of IFX treatment, there were no significant differences in the vitamin D level when the initial serum level prior to treatment was compared to the endpoint serum level ($p = 0.099$; Table 2).

The ratio of vitamin D deficiency to vitamin D insufficiency was improved after the course of IFX therapy. The proportion of patients with a vitamin D deficiency decreased from 57.4% at the onset of the study to 40.0% after one year of IFX. When the study began, 18% of patients had a serum vitamin D level >29 ng/mL, and this percentage increased to 22% by week 53 (Fig. 2). In the control group, 25% of patients had a vitamin D deficiency and 21% had vitamin D level >29 ng/mL (Table 2). When vitamin D levels were compared

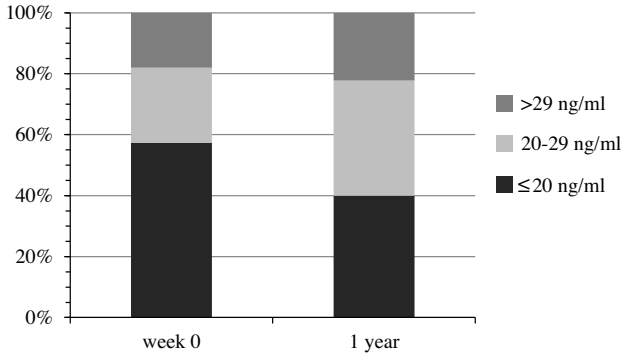

Fig. 2. Stratifications of vitamin D levels at baseline and after one year of infliximab therapy in paediatric patients with moderate to severe Crohn's disease.

Table 2
Median scores of serum osteocalcin, beta-crosslaps and vitamin D, and the significance results of a Friedman analysis.

	Number of patients	Week 0 Median (pc-25, -75)	Week 6 Median (pc-25, -75)	Week 30 Median (pc-25, -75)	Week 53 Median (pc-25, -75)	Significance (Friedman's)	Control group Median (pc-25, -75)
Osteocalcin (ng/mL)	$n = 33$	31.82 (19.8–52.2)	45.43 (31.2–63.9)	52.26 (34.7–78.9)	37.67 (22.7–59.7)	$p < 0.0001$	56.9 (38.2–96.7)
Beta-crosslaps (pg/mL)	$n = 39$	674 (514–1046)	723 (442–1163)	821 (497–1205)	554 (407–863)	$p = 0.105$	258 (235–640)
Vitamin-D (ng/mL)	$n = 39$	18.30 (12.4–25)	22.50 (18.3–26.7)	20.10 (14.9–24.2)	21.95 (15.8–27.4)	$p = 0.099$	24.2 (20.2–27.6)

pc-25, -75 = percentile 25 and percentile 75.

Table 3
Effect of infliximab therapy on bone mineral density in children with Crohn's disease.

	Week 0 Mean (±SD)	1 year Mean (±SD)	Significance $n = 29$
Lumbar 2-4 *Z*-score	−0.779 (±1.233)	−0.728 (±1.566)	$p = 0.985$
Body BMD *Z*-score	−0.827 (±0.137)	−0.872 (±0.138)	$p = 0.155$

BMD: bone mineral density.

between the control and IFX patients in remission, no significant differences were observed at the study onset ($p = 0.093$) or study conclusion ($p = 0.042$).

Seasonal variability was assessed to determine if vitamin D levels were influenced by the winter or summer period. Indeed, significant differences in the serum level of vitamin D were observed between the summer and winter period at week 0 ($p = 0.039$). After one year of IFX therapy, however, this significance was no longer apparent ($p = 0.426$). The median vitamin D values were 16.5 ng/mL (pc-25, -75: 10.3, 20.77) and 20.6 ng/mL (pc-25, -75: 14.15, 27) at week 0, and 18.1 ng/mL (pc-25, -75: 14.85, 24.7) and 23.6 ng/mL (pc-25, -75: 17, 28.1) at week 53 in the winter and summer groups, respectively. At the beginning of the study, 69% of patients were severely deficient and 19% of patients were insufficient in the winter period group, while the 48.6% of patients were deficient and 28.6% were insufficient in the summer period group. We observed an improved ratio of the vitamin D deficiency and insufficiency in both groups after one year of IFX treatment (Fig. 3).

3.5. Effects of IFX on bone mineral density

Prior to initiation of IFX treatment, 18.3% of the children in the IFX group had a reduced lumbar BMD *Z*-score (BMD *Z*-score < −2.0). In 29/50 cases, repeated DEXA results were available. When this subset of patients was analysed, there were no significant changes in BMD *Z*-scores over time (lumbar and total body BMD *Z*-scores were $p = 0.985$, and $p = 0.155$, respectively) (Table 3).

3.6. Results of the correlation analysis

A Spearman correlation analysis indicated a weak negative correlation between the vitamin D level and the PCDAI at week 0 ($\rho = -0.303$) and at week 53 ($\rho = -0.26$), and between the vitamin D and osteocalcin levels at week 0 ($\rho = -0.241$) and week 6 ($\rho = -0.315$).

4. Discussion

In this study, we confirmed the beneficial effect of a yearlong IFX therapy course on the clinical status (decreased PCDAI, improved IMPACT-III), and thus QoL, of paediatric patients with moderate to severe CD. Despite favourable clinical changes, we did not observe significant improvement in the BMD *Z*-score after one year of treatment. Similarly, there were no significant differences in serum bCL levels or vitamin D levels. Treatment with IFX, however, was associated with a significant increase in serum levels of osteocalcin.

The goal of this study was to determine whether bone biomarker levels and BMD were affected by one year of IFX therapy in children with moderate to severe CD. Osteocalcin, a marker of bone osteoblast activity, improved significantly over the course of IFX therapy, with the exception of week 53. Serum bCL levels did not change significantly. While decreases in disease activity (lower PCDAI score) could give rise to favourable changes in bone metabolism, our correlation analysis did not support this relationship in the present study. When compared with the control group, there were no significant differences in bone marker levels between the two therapy types (IFX vs. standard). Future studies should

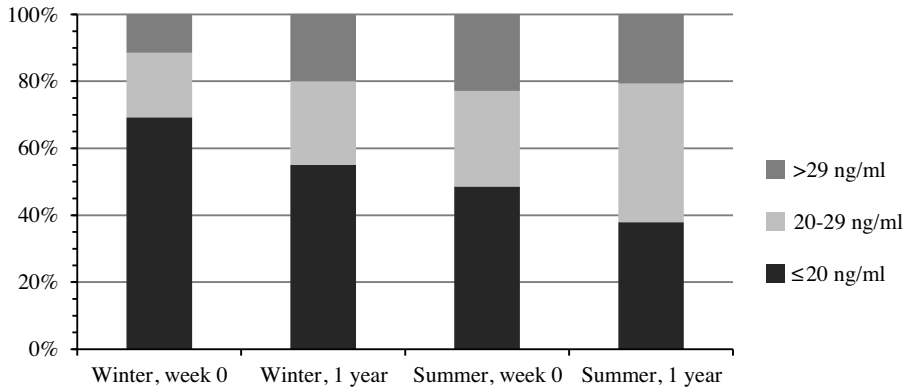

Fig. 3. Stratifications of vitamin D level at baseline and after one year of infliximab treatment with regard to season of treatment initiation.

investigate how BMD, osteocalcin and bCL are affected by "deep remission" to understand the relationship of bone health to CD. In adult CD patients, Miheller and colleagues also observed a significant increase in osteocalcin levels after IFX therapy [24]. Similar to our study, no significant changes in bCL levels were observed [24]. In a subset of the REACH study, bone formation markers (serum bone specific alkaline phosphatase and N-terminal propeptide of type 1 collagen) and resorption (urine C-telopeptide of collagen cross-links and deoxypyrodinoline) changed significantly, though this result included only the induction therapy period and no long-term data [25]. While a recent study reported significant changes in the anthropometry quality of children throughout a one year IFX course, they did not observe changes in biomarkers of bone metabolism of BMD [26].

In our study, BMD *Z*-scores were not significantly different after one year of IFX therapy. A reduction in bone mineral mass per age group was detected in 18% of patients at the onset of the study. In an Italian study, Paganelli and colleagues reported that 31.4% of the CD patients in the study had a low BMD (Z-score ≤ -2) [7]. Another population-based study in children and adolescents with IBD showed that almost 50% of patients had decreased BMD (Z-score ≤ -1), while approximately 25% of the study population had a low BMD (Z-score ≤ -2) [27].

Our results, as well as data from the literature, highlight the importance of routine patient follow up and improvement of bone metabolism, since improvement of BMD cannot be assumed. Because peak bone mass is achieved during childhood, finding an optimal treatment to maintain/improve bone health is essential in paediatric CD patients.

Because vitamin D plays roles in immune system regulation in addition to its roles in bone health and homeostasis, monitoring vitamin D levels should be an important aspect in the treatment of CD [28,29]. Thus, ensuring patients have adequate serum vitamin D levels may further increase the efficacy of treatment in IBD, as a previous study revealed [30]. A recently published review reported that low vitamin D levels were a global problem in all age groups [16]. In a survey from the United States, 35.8% paediatric IBD patients had a serum vitamin D concentration ≤15 ng/mL [13]. Similarly, Levin et al. reported that 19% of children with IBD had a vitamin D deficiency while 38% had an insufficiency [31]. In our study, 57% and 25% of the patients had vitamin D deficiency and insufficiency, respectively, prior to IFX therapy initiation. Our results have a higher incidence of deficiency and insufficiency due to the threshold selected for our study versus the threshold applied to the previous studies [22].

Little data is currently available on the correlation between IFX and vitamin D levels. The study from Pichler and colleagues reported a significant increase in vitamin D level as result of IFX therapy [26]. Yet a recent study found that 1,25-dihydroxy vitamin D serum concentrations were increased without concomitant changes in 25-hydroxy vitamin D after IFX induction therapy. The stable 25-hydroxy vitamin D levels indicate that the increases in 1,25-hydroxy vitamin D levels were not due to improved gut absorption of vitamin D but rather may be due to the suppressive effect of inflammation on renal activation of vitamin D [32].

When IFX-treated CD patients in remission were compared with the control group, clinical remission did not significantly improve serum vitamin D levels. Future studies should investigate whether patients in 'deep remission' show different correlations with vitamin D levels.

The suggested vitamin D intake in healthy children is 600 U/day. The Endocrine Society recommends a dose of 2000 U/day for at least six weeks in patients with a deficiency. In the present study, patients received a 1000 U/day vitamin D supplementation for ≥15 months. In future studies, a higher dose (2000 U/day) might be recommended, though in inflammatory conditions such as IBD the vitamin D level is influenced by several mechanisms (e.g. intestinal absorption). Thus, the effect of an increased dose on serum vitamin D levels is difficult to predict.

When patient vitamin D levels were analysed with respect to therapy initiation season, patients who initiated IFX during the summer months had a significantly higher vitamin D level. At baseline, 22% of patients that initiated treatment during the summer season had vitamin D serum levels >29 ng/mL, as compared to 11.5% of patients who began treatment in the winter months. Severe vitamin D deficiency was observed in more than 69% of patients in the winter group and nearly 50% of patients in the summer group at the onset of treatment. While vitamin D levels did not change significantly after one year of IFX, the ratio of vitamin D deficiency (<20 ng/mL) decreased regardless of the season. These findings suggest that IFX may reduce the seasonal variability of vitamin D levels during treatment. In the winter group, vitamin D levels did not decrease, and we observed an increasing trend during IFX treatment. Although significant long term improvement in vitamin D levels was not observed after IFX administration, decreases in vitamin D levels during the winter season did not occur, though the nature of the relationship between vitamin D and IFX is not yet understood. Previous studies have also reported an effect of seasonality of vitamin D levels in IBD. McCarthy and colleagues found lower vitamin D concentrations in CD patients than in control subjects during both seasons. Moreover, lower vitamin D concentrations were observed during late winter compared to late summer both in CD patients and healthy controls. The authors also found that patients receiving vitamin D supplementation did not have seasonal variation [17]. In contrast, other studies have not identified a significant association between vitamin D level and seasonality in patients with ulcerative colitis or IBD [33].

In summary, IFX therapy demonstrated a clear clinical benefit in paediatric patients with moderate to severe CD. Moreover, the addition of IFX therapy improved bone health initially, though this beneficial effect was diminished after one year of treatment. Moreover, despite supplemental administration of the vitamin D and calcium, low vitamin D levels in CD patients persisted. Seasonal variability of vitamin D levels was observed prior to IFX initiation but these differences were no longer apparent after one year of IFX treatment. We did not observe an exclusive influence of IFX therapy on bone metabolism and vitamin D levels.

Conflict of interest

None declared.

Acknowledgements

This study was supported by OTKA K108688, OTKA K100909, OTKA K105530, OTKA K108655, OTKA PD105361, KMR_12-1-2012-0074, and LP008/2014.

References

[1] Muller KE, Lakatos PL, Arato A, et al. Incidence, Paris classification, and follow-up in a nationwide incident cohort of paediatric patients with inflammatory bowel disease. Journal of Pediatric Gastroenterology and Nutrition 2013;57:576–82.
[2] Lovasz BD, Lakatos L, Horvath A, et al. Incidence rates and disease course of paediatric inflammatory bowel diseases in Western Hungary between 1977 and 2011. Digestive and Liver Disease 2014;46:405–11.
[3] El-Matary W, Sikora S, Spady D. Bone mineral density, vitamin D, and disease activity in children newly diagnosed with inflammatory bowel disease. Digestive Diseases and Sciences 2011;56:825–9.
[4] Sylvester FA, Wyzga N, Hyams JS, et al. Natural history of bone metabolism and bone mineral density in children with inflammatory bowel disease. Inflammatory Bowel Diseases 2007;13:42–50.
[5] Risteli L, Risteli J. Biochemical markers of bone metabolism. Annals of Medicine 1993;25:385–93.
[6] Veerappan SG, O'Morain CA, Daly JS, et al. Review article: the effects of anti-tumour necrosis factor-alpha on bone metabolism in inflammatory bowel disease. Alimentary Pharmacology and Therapeutics 2011;33:1261–72.
[7] Paganelli M, Albanese C, Borrelli O, et al. Inflammation is the main determinant of low bone mineral density in paediatric inflammatory bowel disease. Inflammatory Bowel Diseases 2007;13:416–23.
[8] Tsampalieros A, Lam CK, Spencer JC, et al. Long-term inflammation and glucocorticoid therapy impair skeletal modeling during growth in childhood Crohn disease. Journal of Clinical Endocrinology and Metabolism 2013;98:3438–45.
[9] Hyams J, Walters TD, Crandall W, et al. Safety and efficacy of maintenance infliximab therapy for moderate-to-severe Crohn's disease in children: REACH open-label extension. Current Medical Research and Opinion 2011;27:651–62.
[10] Danese S. Mechanisms of action of infliximab in inflammatory bowel disease: an anti-inflammatory multitasker. Digestive and Liver Disease 2008;40(Suppl. 2):S225–8.
[11] Armuzzi A, De Pascalis B, Fedeli P, et al. Infliximab in Crohn's disease: early and long-term treatment. Digestive and Liver Disease 2008;40(Suppl. 2):S271–9.
[12] Iijima H, Shinzaki S, Takehara T. The importance of vitamins D and K for the bone health and immune function in inflammatory bowel disease. Current Opinion in Clinical Nutrition and Metabolic Care 2012;15:635–40.
[13] Pappa HM, Grand RJ, Gordon CM. Report on the vitamin D status of adult and paediatric patients with inflammatory bowel disease and its significance for bone health and disease. Inflammatory Bowel Diseases 2006;12:1162–74.
[14] Jorgensen SP, Hvas CL, Agnholt J, et al. Active Crohn's disease is associated with low vitamin D levels. Journal of Crohn's and Colitis 2013;7:e407–13.
[15] Bakker SF, Dik VK, Witte BI, et al. Increase in bone mineral density in strictly treated Crohn's disease patients with concomitant calcium and vitamin D supplementation. Journal of Crohn's and Colitis 2013;7:377–84.
[16] Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? Journal of Steroid Biochemistry and Molecular Biology 2013. pii:S0960-0760(13)00233-1.
[17] McCarthy D, Duggan P, O'Brien M, et al. Seasonality of vitamin D status and bone turnover in patients with Crohn's disease. Alimentary Pharmacology and Therapeutics 2005;21:1073–83.
[18] Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132:863–73, quiz 1165–6.
[19] Otley A, Smith C, Nicholas D, et al. The IMPACT questionnaire: a valid measure of health-related quality of life in paediatric inflammatory bowel disease. Journal of Pediatric Gastroenterology and Nutrition 2002;35:557–63.
[20] Szabo D, Kokonyei G, Arato A, et al. Autoregressive cross-lagged models of IMPACT-III and Paediatric Crohn's Disease Activity indexes during one year infliximab therapy in paediatric patients with Crohn's disease. Journal of Crohn's and Colitis 2014;8:747–55.
[21] Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a paediatric Crohn's disease activity index. Journal of Pediatric Gastroenterology and Nutrition 1991;12:439–47.
[22] Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism 2011;96:1911–30.
[23] Sylvester FA. IBD and skeletal health: children are not small adults! Inflammatory Bowel Diseases 2005;11:1020–3.
[24] Miheller P, Muzes G, Racz K, et al. Changes of OPG and RANKL concentrations in Crohn's disease after infliximab therapy. Inflammatory Bowel Diseases 2007;13:1379–84.
[25] Thayu M, Leonard MB, Hyams JS, et al. Improvement in biomarkers of bone formation during infliximab therapy in paediatric Crohn's disease: results of the REACH study. Clinical Gastroenterology and Hepatology 2008;6:1378–84.
[26] Pichler J, Hanslik A, Dietrich Huber W, et al. Paediatric patients with inflammatory bowel disease who received infliximab experienced improved growth and bone health. Acta Paediatrica 2014;103:e69–75.
[27] Schmidt S, Mellstrom D, Norjavaara E, et al. Low bone mineral density in children and adolescents with inflammatory bowel disease: a population-based study from Western Sweden. Inflammatory Bowel Diseases 2009;15:1844–50.
[28] Garg M, Lubel JS, Sparrow MP, et al. Review article: vitamin D and inflammatory bowel disease – established concepts and future directions. Alimentary Pharmacology and Therapeutics 2012;36:324–44.
[29] Ooi JH, Chen J, Cantorna MT. Vitamin D regulation of immune function in the gut: why do T cells have vitamin D receptors? Molecular Aspects of Medicine 2012;33:77–82.
[30] Nicholson I, Dalzell AM, El-Matary W. Vitamin D as a therapy for colitis: a systematic review. Journal of Crohn's and Colitis 2012;6:405–11.
[31] Levin AD, Wadhera V, Leach ST, et al. Vitamin D deficiency in children with inflammatory bowel disease. Digestive Diseases and Sciences 2011;56:830–6.
[32] Augustine MV, Leonard MB, Thayu M, et al. Changes in vitamin D-related mineral metabolism following induction with anti-tumor necrosis factor-alpha therapy in Crohn's disease. Journal of Clinical Endocrinology and Metabolism 2014:jc20133846.
[33] Blanck S, Aberra F. Vitamin d deficiency is associated with ulcerative colitis disease activity. Digestive Diseases and Sciences 2013;58:1698–702.