Original Article

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Assessment of Bone Metabolism and Bone Mineral Density in Children with Helicobacter *pylori* Infection

*Helicobacter **pylori** Enfeksiyonu Olan Çocuklarda Kemik Metabolizması ve Kemik Mineral Dansitesinin Değerlendirilmesi*

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ABSTRACT

Objective: *Gastrointestinal system diseases may have negative impact on bone metabolism. Bone metabolism and bone mineral density in children with Helicobacter pylori (H. pylori) infection were evaluated in this study.*
Method: *A total of 100 children (mean age: 13.69±2.44 years, M/F:0.66) with chronic gastritis were divided into two groups according to the presence of H. pylori infection and tested for biochemical parameters such as calcium, phosphorus, magnesium, alkaline phosphatase, parathyroid hormone and vitamin D. Bone mineral density was measured at lumbar spine in all of the patients by dual-energy x-ray absorptiometry (DXA).*
Results: *Forty-eight of 72 patients with H. pylori and 16 of 28 patients without H. pylori had low vitamin D levels (p=0.35). The other biochemical parameters were within normal limits in both groups. Bone mineral density was measured as -0.16±2.25 g/cm^2 in H. pylori-positive patients and as -0.08±2.62 g/cm^2 in H. pylori-negative patients (p=0.87). Only 2 patients with H. pylori and 1 without H. pylori had BMD z scores below -2.5 (p=1.00).*
Conclusion: *No significant difference was observed in biochemical parameters of bone metabolism and bone mineral density between H.pylori-positive and -negative children.*

Keywords: *children, bone mineral density, calcium, H.pylori, vitamin d*

ÖZ

Amaç: *Gastrointestinal sistemi tutan hastalıklar kemik metabolizması üzerine negatif etki gösterebilir. Bu çalışmada Helicobacter pylori enfeksiyonu olan çocuklarda kemik metabolizması ve kemik mineral dansitesi değerlendirilmiştir.*
Yöntem: *1-18 yaş arası kronik gastriti olan 100 çocuk hasta (ort yaş:13.69±2.44 yıl, E/K:0.66) Helicobacter pylori enfeksiyonuna göre iki gruba ayrıldı. Hastalar kalsiyum, fosfor, magnezyum, alkalenfosfataz, paratiroid hormon ve D vitamini düzeyleri gibi biyokimyasal parametreler açısından test edildi. Kemik mineral dansitesidual x-ışınıabsorbsiyometri (DXA) tekniği ile lomber omurgada ölçüldü.*
Bulgular: *72 Helicobacter pylori enfeksiyonu olan hastanın 48'inde ve 28 Helicobacter pylori enfeksiyonu olmayan hastanın 16'ında D vitamini düzeyi düşük saptandı (p=0.35). Diğer biyokimyasal parametreler her iki grupta da normal tespit edildi. Kemik mineral dansitesi Helicobacter pylori enfeksiyonu olan hastalarda 0.16±2.25 g/cm^2 ve olmayanlarda -0.08±2.62 g/cm^2 ölçüldü (p=0.87). Helicobacter pylori enfeksiyonu olan 2 hastada ve olmayan 1 hastada kemik mineral dansitesi z skoru -2.5 değerinin altında saptandı (p=1.00).*
Sonuç: *Kemik metabolimasının biyokimyasal parametreleri ve kemik mineral dansitesi açısından Helicobacter pylori enfeksiyonu olan ve olmayan çocuklar arasında anlamlı fark saptanmadı.*

Anahtar kelimeler: *çocuk, kemik mineral dansitesi, kalsiyum, H.pylori, d vitamini*

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INTRODUCTION

Helicobacter pylori (H.pylori) infection, one of the most common gastrointestinal infections worldwide, colonizes human gastric mucosa during early childhood persisting throughout life and may lead to chronic gastritis, gastric mucosal atrophy, peptic ulcer, and gastric cancer. H. pylori infection triggers or aggravates a systemic inflammatory response which affects not only the digestive tract, but may also involve extraintestinal tissues and/or organs [1]. The proposed mechanisms that have been suggested to explain the extra-intestinal manifestations are: atrophic gastritis, enhancement in vascular permeability during the gastric infection, the release of inflammatory mediators, systemic immune response and molecular mimicry [1].

In childhood, osteoporosis is generally secondary to chronic diseases such as intestinal inflammatory disease, cystic fibrosis, hepatobiliary disease and anorexia nervosa which interfere with the reabsorption of nutrients, arthritis due to immobility, thyroid disorders, hormonal factors or complication of treatments [2,3].

There are limited data regarding the association between H.pylori infection and osteoporosis in children and adults.In the present study, we aimed to evaluate bone metabolism and bone mineral density (BMD) in children with chronic gastritis and determine its association with H.pylori infection.

MATERIAL and METHODS

A total of 100 children who referred for endoscopy with dyspeptic symptoms (mostly recurrent abdominal pain) suggestive of organic disease and followed as chronic gastritis between 2014 and 2016 at division of pediatric gastroenterology were evaluated prospectively. Exclusion criteria were treatment with antisecretory, antimicrobial, or anti-inflammatory medication for the 3 months preceding the endoscopy.The patients with previous H.pylori eradication and who had diseases that affect bone metabolism such as inflammatory bowel disease, malignancies, chronic kidney disease, diabetes mellitus,hypo/hyperthyroidism, hypo/hyperparathyroid disorders, hypogonadism, malignancies, anorexia nervosa, gastrointestinal disorders with malabsorption, collagen diseases,and were given current or previous treatment with glucocorticoids, thyroid/parathyroid drugs, anticonvulsants, vitamin D, calcium and bisphosphonate were also excluded.The control group was not selected from healthy children because bone mineral measurements would not be ethical in healthy children.Informed consents were taken from all of the parents before procedures.

Diagnosis of H.pylori infection was based on at least two of the three methods; histological examination, culture and rapid urease test. From indicated number of patients, biopsy specimens were systematically taken from the duodenum (n=2), gastric antrum (n=2), and gastric body (n=2). A modified Giemsa stain was used for identification of H. pylori, and gastritis was evaluated according to the updated Sydney scoring system. H. pylori density was scored by using visual analogue scales described in the updated Sydney scoring system on a four-point scale (0, normal/absent; 1,mild; 2, moderate; and 3, marked) [4].

The levels of calcium, phosphorus, magnesium, parathyroid hormone (PTH) and total alkaline phosphatase (ALP) were studied with a Roche/Hitachi Modular PP automated clinical chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). 25-hydroxy vitamin D (25-OH-D) was measured by liquid chromatography–mass spectrometry (LC-MS/MS).

According to Lawson Wilkins Pediatric Endocrine Society Drug and Therapeutics Committee vitamin D status was defined as follows: 25(OH) vitamin D < 20 ng/mL, hypovitaminosis D ; 5–20 ng/ mL, vitamin D insufficiency,<15 ng/ mL, vitamin D deficiency and <5ng/mL, severe vitamin D deficiency [5].

Body mass index (BMI) was calculated as body weight (kg) divided by the square of body height in meters (kg/m^2). Overweight is defined as a BMI at or above the 85th percentile and below the 95th percentile for children and teens of the same age and sex. Obesity is defined as a BMI at or above the 95th percentile and underweight as BMI less than the 5th percentile.

Bone mineral density (BMD) of the lumbar vertebrae 2-4 (L2-4) was measured by dual-energy x-ray absorptiometry (DXA) using a Discovery A (HOLOGIC, Bedford, Massachusetts, USA) densitometer BMD between -1 and -2.5 SD was defined as osteopenia and if BMD <-2.5SD as osteoporosis [6].

This study was performed in accordance with the principles of Declaration of Helsinki.The study was approved by hospital ethics committee (09.17. 2019/1349).

Statistical Analysis

Statistical analysis were performed using the NCSS (NumberCruncher Statistical System) 2007&PASS 2008 Statistical Software (Utah, U.S.A). All results were expressed as the mean ± SD. Statistical comparisons were made using the unpaired Student's t tests. The analysis was conducted using Fisher's exact test and chi-square test to analyze qualitative variables. A value of $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the patients was 13.69±2.44 years (range 8-17years) and male:female ratio was 0.66. The patients were admitted with the complaint of abdominal pain (80%) , inactive (27%), mild (15%) and moderate (58%) chronic gastritis. Only two of H. pylori-positive patients had obesity, whereas none of the patients had malnutrition. The demographic and clinical characteristics of the patients are presented in Table 1.

Table 1. The clinical characteristics of the patients.

Age, y, mean ± SD	13.69±2.44
Gender (M/F)	0.66 (40/60)
Duration of disease, y, mean ± SD	1.86±1.31
Clinical presentation	
Abdominal pain	80 (80%)
Flatulans	64 (64%)
Nausea	60 (60%)
Regurgitation	49 (49%)
Endoscopic findings	
Erosive oesophagitis	100 (100%)
Nodular gastritis	26 (26%)
Duodenal ulcer	11 (11%)
Eosinophilic oesophagitis	1 (1%)
Polyp	1 (1%)
Type of chronic gastritis	
Inactive	27 (27%)
Mild	15 (15%)
Moderate	58 (58%)
H.pyloridensity	
0 (normal, no bacteria)	28 (28%)
1 (mild)	21 (21%)
2 (moderate)	27 (27%)
3 (marked)	24 (24%)

Forty-eight patients out of 72 patients with and 16 of 28 patients without H. pylori had low vitamin D levels (p=0.35). Thirteen H. pylori-positive patients and 9 H. pylori-negative patients had vitamin D insufficiency,whereas 9 H. pylori-positive patients and 2 H. pylori-negative patients had severe vitamin D deficiency (Table 2).

Table 2. It showes comparison of preopertive and postoperative patients' VAS. A statistically significant decline in pain control was detected in both groups.

	H.pylori-positive patients (n=72)	H.pylori-negative patients (n=28)	P
Age	13.64±2.53	13.82±2.35	0.74
Gender (male/female)	0.56 (26/46)	1.0 (14/14)	0.25
Height (cm)	154.6±16.6	152.3±9.2	0.49
Z score for height corrected for age and sex	0.68±0.93	0.49±1.04	0.9
Weight(kg)	55±22.32	52.65±19.5	0.62
Z score for weight corrected for age and sex	1.29±1.05	0.93±1.1	0.84
Body mass index (kg/m²)	25.39±5.53	24.56±3.58	0.46
Laboratory findings			
ALP (U/L)	168.79±81.8	161.78±78.4	0.69
Calcium (mg/dL)	9.47±0.36	9.5±0.41	0.71
Phosphorus (mg/dL)	4.17±0.46	4.37±0.59	0.07
Magnesium (mg/dL)	2.01±0.30	1.95±0.18	0.32
PTH (pg/mL)	48.91±23.4	43.36±16	0.25
25-OH-D (ng/mL)	16.07±9.53	19.68±8.51	0.08
<5	3.6±0.9 (n=9)	2.5±0.7 (n=2)	0.15
5-15	9.9±2.6 (n=26)	9.6±2.4 (n=5)	0.81
15-20	16.7±1.4 (n=13)	17.5±1.9 (n=9)	0.26
≥ 20	27.3±6.3 (n=24)	27±5.2 (n=12)	0.88
Ferritin (ng/ml)	24.74±10	26.22±12.8	0.54
Vitamin B12 (pg/mL)	267.38±95	26.22±12.8	0.56
Folic acid (ng/mL)	7.96±2.1	7.75±1.9	0.64
Bone mineral density (g/cm²)	-0.16±2.25	-0.08±2.62	0.87
< -2.5	-10±9.89 (n=2)	-13 (n=1)	1.00
<-1 and>-2.5	-1.37±0.35 (n=19)	-1.22±0.17 (n=4)	0.35

ALP: alkaline phosphatase
PTH= parathormone
25-OH-D= 25-hydroxyvitamin D
P<0.05 is statistically significant

There were no significant differences in terms of other biochemical parameters and in mean BMD z scores between H. pylori-positive and H. pylori-negative children (p> 0.05) (Table 2). Only 2 (2.7%) H. Pylori-positive and 1(3.5%) H. pylori negative patient had BMD z scores below -2.5 (p=1.00). Nineteen (26.3%) H. pylori –positive and 4 (14.2%) H. pylori –negative patients had osteopenia (p=0.35). Significant difference was observed between patients with and without osteopenia/osteoporosis regarding only the grade of chronic inflammation according to Sydney classification (Table 3).

Table 3. The comparison of patients with osteopenia/osteoporosis and without osteopenia/osteoporosis according to Sydney classification .

	Patients with osteopenia/ osteoporosis (n=3)	Patients without osteopenia/ osteoporosis (n=3)	p
Chronic inflammation			
0 (normal)	-	27	0.56
1 (mild)	-	17	1.00
2 (moderate)	-	40	0.27
3 (severe)	3	10	0.001
Atrophy			
0 (normal)	3	97	1.00
1 (mild)	-	-	
2 (moderate)	-	-	
3 (severe)	-	-	
Neutrophil activity			
0 (normal)	-	-	
1 (mild)	-	31	0.07
2 (moderate)	-	30	0.55
3 (severe)	3	36	0.05
Intestinal metaplasia			
0 (normal)	3	97	1.00
1 (mild)	-	-	
2 (moderate)	-	-	
3 (severe)	-	-	
Density of H.pylori			
0 (normal)	-	28	0.55
1 (mild)	-	21	1.00
2 (moderate)	-	27	0.54
3 (severe)	3	21	0.01

P<0.05 is statistically significant

DISCUSSION

It has been proposed that gastrointestinal disorders, particularly those associated with malabsorption and maldigestion (celiac disease, postgastrectomy, peptic ulcers and atrophic gastritis, pancreatic insufficiency); inflammatory bowel diseases (Crohn's disease and ulcerative colitis) may have negative impact on bone metabolism leading to osteoporosis [3,7-9].

H. pylori causes chronic gastritis and induces both humoral and cellular complex and local (in the gastric mucosa) and systemic immune responses [8,10]). Systemic inflammatory cytokines related to H. pylori infection such as tumor necrosis factor- (TNF-) α and interleukin-1 stimulating osteoblasts to produce cytokines activating osteoclasts and interleukin-6 promoting osteoclast precursor cell differentiation are all associated with bone destruction [2,9,11]. The studies evaluating the local cytokine profile in children have shown that H. pylori infection induces production of proinflammatory cytokines and a Th1 response, similar to studies in adults [12]. In our study, all of three patients with osteopenia/osteoporosis had severe chronic inflammation as detected in histopathological examination.

It has been reported that inflammatory response also provokes reduced levels of osteocalcine, insulin-dependent growth factors (IGF-1) and their transportation proteins and these interleukins also exacerbate catabolism and induce anorexia, reducing the ingestion of nutrients such as calcium and vitamin D which play a crucial role in bone metabolism [2].

Another mechanism postulated is that impaired gastric acidification (gastric mucosal atrophy and hypo-, and achlorhydria) related to H. pylori infection might induce malabsorption of calcium and alter bone architecture [9,13-18]. Asaoka et al.[8] reported that endoscopic gastric mucosal atrophy tended to correlate with osteoporosis and suggested that the decrease of dissolution of calcium salts caused by the decrease in gastric acid secretion in atrophic gastritis may also result in the malabsorption of calcium.

Osteoporosis is defined by the World Health Organization as a systemic metabolic bone disease, characterized by reduced bone mass and deterioration of bone tissue microarchitecture with increased bone fragility and susceptibility to fractures. There is also reduced bone mass in osteopenia, but without involvement of microarchitecture [2,6]. While several studies

reported an association between H. pylori infection and osteoporosis [8,19,20], some of them suggested that H. pylori infection would not to be a risk factor for decreased BMD [10,18]. There are studies determining immune response and cytokines in children with gastritis [12], but the studies evaluating association between H.pylori infection and osteoporosis in children are limited [10]. Ozdem et al.[10] found that H.pylori infection was not accompanied by significant alterations in biochemical markers of bone metabolism in children.In our study, osteoporosis was observed in two of our patients with H. pylori, osteopenia in 19 and hypovitaminosis D in 48 (severe deficiency in 9) patients, but the differences were not significant when compared with H. pylori-negative patients.One of H. pylori-positive patients with osteoporosis, whereas 17 patients with osteopenia had low vitamin D levels.

H.pylori causes vitamin B 12 malabsorption and only a minimum concentration of vitamin B12 is needed for the proliferation of osteoblasts [10]. Although a significant reduction in serum vitamin B 12 levels in H.pylori-positive children compared to H.pylori-negative ones was observed in the study of Ozdem et al.[10], no changes were detected in markers of bone metabolism. They concluded that vitamin B12 levels in H.pylori-positive children may still be far above the minimum levels required for normal osteoblastic proliferation. Vitamin B12 levels were within normal limits in our patients.

Shih H-M et al.[16] reported that early eradication of H. pylori is associated with a relatively lower incidence of osteoporosis when compared with the late eradication group with chronic H. pylori infection, Although Asaoka et al.[20] stated that H. pylori infection is a risk factor for osteoporosis, they did not correlate the success of H.pylori eradication with the risk of osteoporosis [20]. Two of our patients with H. pylori who had osteoporosis and obesity and 19 who had osteopenia were reevaluated one month after eradication treatment, but no improvement was seen in their BMDs and they were referred to the department of pediatric endocrinology.

Limitation of this study is that the markers of bone formation include bone-specific alkaline phosphatase and osteocalcine could not be assessed due to inavailabilitiy of required test kits in our hospital.

In conclusion, considering the shorter duration of H. pylori infection in children, in this study any significant association was not found between H. pylori-positive and H. pylori-negative children in terms of markers of bone metabolism. Early eradication of H. pylori is important for preventing elevation of inflammatory cytokines due to chronic inflammation which causes osteoporosis. As delayed diagnosis may increase the risk of adult osteoporosis, further larger-scale studies are needed for determining whether routine screening of markers of bone metabolism and BMD is necessary in children with H. pylori.

Ethics Committee Approval: İstanbul Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee approval was received (17/09/2019; 1349).
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REFERENCES

1. Radić M. Role of Helicobacter pylori infection in autoimmune systemic rheumatic diseases. World J Gastroenterol. 2014;20(36):12839-46. https://doi.org/10.3748/wjg.v20.i36.12839
2. Campos LM, Liphaus BL, Silva CA, Pereira RM. Osteoporosis in childhood and adolescence. J Pediatr (Rio J). 2003;79(6):481-8. https://doi.org/10.2223/JPED.1107
3. Turner J, Pellerin G, Mager D. Prevalence of metabolic bone disease in children with celiac disease is independent of symptoms at diagnosis. J Pediatr Gastroenterol Nutr. 2009;49(5):589-93. https://doi.org/10.1097/MPG.0b013e31819ca18e
4. Dixon MF, Genta RM, Yardley H, Correa P. Classification and grading of gastritis: the updated Sydney system. Am J Surg Pathol. 1996;20(10):1161-81. https://doi.org/10.1097/00000478-199610000-00001
5. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M; Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. 2008;122(2):398-417. https://doi.org/10.1542/peds.2007-1894
6. The WHO StudyGroup. Assessment of fracture risk and its application to screening for postmenapausal osteoporosis. Switzerland: World Health Organization; 1994.
7. Kim HW, Kim YH, Han K, Nam GE, Kim GS, Han BD, et al. Atrophic gastritis: a related factor for osteoporosis in elderly women. PLoS One. 2014;9(7):e101852. https://doi.org/10.1371/journal.pone.0101852
8. Asaoka D, Nagahara A, Hojo M, Sasaki H, Shimada Y, Yoshizawa T, et al. The relationship between H. pylori infection and osteoporosis in Japan. Gastroenterol Res Pract. 2014;2014:340765. https://doi.org/10.1155/2014/340765
9. Wu CH, Tung YC, Chai CY, Lu YY, Su YF, Tsai TH, Kuo KL, Lin CL. Increased risk of osteoporosis in patients with peptic ulcer disease: a nationwide population-based study. Medicine (Baltimore). 2016;95(16):e3309.

https://doi.org/10.1097/MD.0000000000003309
10. Ozdem S, Akcam M, Yilmaz A, Gultekin M, Artan R. Biochemical markers of bone metabolism in children with Helicobacter pylori infection. Dig Dis Sci. 2007;52(4):967-72. https://doi.org/10.1007/s10620-006-9292-0
11. Fotouk-Kiai M, Hoseini SR, Meftah N, Ghadimi R, Bijani A, Noreddini H, et al. Relationship between Helicobacter pylori infection (HP) and bone mineral density (BMD) in elderly people. Caspian J Intern Med. 2015;6(2):62-6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4478452/
12. Bontems P, Robert F, Van Gossum A, Cadranel S, Mascart F. Helicobacter pylori modulation of gastric and duodenal mucosal T cell cytokine secretions in children compared with adults. Helicobacter. 2003;8(3):216-26. https://doi.org/10.1046/j.1523-5378.2003.00147.x
13. Vitale G, Barbaro F, Ianiro G, Cesario V, Gasbarrini G, Franceschi F, et al. Nutritional aspects of Helicobacter pylori infection. Minerva Gastroenterol Dietol. 2011;57(4):369-77. Available from: https://www.minervamedica.it/en/journals/gastroenterologica-dietologica/article.php?cod=R08Y2011N04A0369
14. Franceschi F, Annalisa T, Teresa DR, Giovanna D, Ianiro G, Franco S, et al. Role of Helicobacter pylori infection on nutrition and metabolism. World J Gastroenterol. 2014;20(36):12809-17. https://doi.org/10.3748/wjg.v20.i36.12809
15. Schinke T, Schilling AF, Baranowsky A,Seitz S, Marshall RP, Linn T, et al. Impaired gastric acidification negatively affects calcium homeostasis and bone mass. Nat Med. 2009;15(6):674-81. https://doi.org/10.1038/nm.1963
16. Shih HM, Hsu TY, Chen CY, Lin CL, Kao CH, Chen CH, et al. Analysis of patients with helicobacter pylori ınfection and the subsequent risk of developing osteoporosis after eradication therapy: a nationwide population-based cohort study. PLoS One. 2016;11(9):e0162645. https://doi.org/10.1371/journal.pone.0162645
17. Sipponen P, Härkönen M. Hypochlorhydricstomach: a risk condi tion for calcium malabsorption and osteoporosis? Scand J Gastroenterol. 2010;45(2):133-8. https://doi.org/10.3109/00365520903434117
18. Chen LW, Chen FP, Hsieh CW, Kuo SF, Chien RN. Analysis of the associations among Helicobacter pylori infection, adiponectin, leptin, and 10-year fracture risk using the fracture risk assessment tool: A cross-sectional community-based study. PLoS One. 2017;12(4):e0175365. https://doi.org/10.1371/journal.pone.0175365
19. Lin SC, Koo M, Tsai KW. Association between Helicobacter pylori infection and risk of osteoporosis in elderly Taiwanese women with upper gastrointestinal diseases: a retrospective patient record review. Gastroenterol Res Pract. 2014;2014:814756. https://doi.org/10.1155/2014/814756
20. Asaoka D, Nagahara A, Shimada Y, Matsumoto K, Ueyama H, Matsumoto K, et al. Risk factors for osteoporosis in Japan: is it associated with Helicobacter pylori? Ther Clin Risk Manag. 2015;11:381-91. https://doi.org/10.2147/TCRM.S80647