

# Helicobacter Pylori Seropositivity in Patients with Postmenopausal Osteoporosis

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**Abstract.** [Purpose] The results of studies investigating the role of *Helicobacter pylori* (HP) in osteoporosis are contradictory. In this study we investigated HP seropositivity in postmenopausal osteoporotic and non-osteoporotic females to elucidate the role of HP in postmenopausal osteoporosis. [Subjects and Methods] Serum was collected from fifty-eight osteoporotic patients and forty-seven nonosteoporotic subjects, whose status was diagnosed with dual-energy X-ray absorptiometry (DEXA). None of the subjects had received any prior treatment for osteoporosis. Subjects' sera were assessed for HP antibodies (Immunoglobulin A and G) by enzyme-linked immunoabsorbent assay (ELISA). Patients were interviewed about risk factors of osteoporosis. Prior fractures of patients and familial fracture history were also noted. [Results] Fifty-eight patients with osteoporosis and forty-seven nonosteoporotic patients, as determined by lumbar total or collum femoris in DEXA, were evaluated in this study. The familial fracture history was significantly higher in the osteoporotic patients than in the nonosteoporotic group. There was no significant difference in HP seropositivity between the osteoporotic and nonosteoporotic groups. [Conclusion] There was no difference in HP seropositivity between the groups, therefore HP infection seems not to be an important risk factor for postmenopausal osteoporosis.

**Key words:** Postmenopausal osteoporosis, *Helicobacter pylori* infection, *Helicobacter pylori* extragastric manifestations.

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## INTRODUCTION

Osteoporosis is characterized by low bone mineral density and increased predisposition to fracture. In postmenopausal females, one of the factors that determines the development of osteoporosis is estrogen deficiency<sup>1)</sup>. Estrogen loss in the postmenopausal period is associated with differentiation of cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha). TNF-alpha is a triggering factor for bone resorption and IL-6 increases the differentiation of osteoclast precursor cells into osteoclasts and decreases the maturation and function of osteoblasts<sup>2)</sup>. Other risk factors for osteoporosis are age, genetic and environmental factors, chronic diseases, and physical features of the bone<sup>3)</sup>.

Gastrointestinal diseases such as inflammatory bowel disease, celiac disease and gastrectomy have been proposed as being associated with osteoporosis<sup>4-8)</sup>. Since gastric acid

is thought to be necessary for the absorption of dietary calcium, osteoporosis in gastrointestinal diseases has been attributed to calcium deficiency<sup>9-12)</sup>. *Helicobacter pylori* (HP) infection also causes chronic gastritis and may cause inflammation by increasing local and systemic inflammatory markers such as TNF-alpha and IL-6<sup>13-15)</sup>. TNF-alpha and IL-6 were previously proposed as being part of the etiology of osteoporosis and the prevalence of HP infection increases with age<sup>2,16)</sup>. In this context, some studies exploring the role of HP in osteoporosis have been conducted<sup>3,17,18)</sup>. Since the results of these studies about the role of HP in postmenopausal osteoporosis are contradictory<sup>3,17)</sup>, we examined HP seropositivity in postmenopausal osteoporotic and nonosteoporotic patients.

## SUBJECTS AND METHODS

The study was conducted at the outpatient clinic of the

**Table 1.** Age, body mass index, age of menarche, menopause, duration of postmenopausal period, tea and coffee consumption and physical activity scores of osteoporotic and nonosteoporotic patients

	Osteoporotic patients (N=58)	Nonosteoporotic patients (N=47)
Age	65.3 ± 6.1	63.6 ± 6.5
BMI	28.1 ± 3.6	28.6 ± 3.7
Age of menarche	13.15 ± 1.18	12.76 ± 1.32
Age of menopause	45.27 ± 4.8	44.68 ± 3.5
Duration of postmenopausal period	20.01 ± 7.60	18.89 ± 7.33
Tea consumption (cup/day)	1.89 ± 1.05	2.29 ± 1.19
Coffee consumption (cup/day)	0.22 ± 0.46	0.25 ± 0.44
Physical activity score	12.5 ± 1.7*	13.7 ± 1.3*

Department of Physical Medicine and Rehabilitation of Denizli Government Hospital, Turkey. In this cross-sectional study, we examined 108 postmenopausal patients. The exclusion criteria were as follows: previous treatments for osteoporosis or osteopenia, or hormone replacement therapy, Paget's disease, gastrointestinal disorders, liver, kidney or thyroid diseases, malignancies, or treatments within the last 6 months with drugs affecting bone metabolism (anticonvulsants, cyclosporin, glucocorticoids) or with antibiotics for HP eradication, proton pump inhibitors or histamin H2 receptor antagonists. The bone mineral density of the lumbar and femur neck of patients who had osteoporosis risk factors and were at least five years into the postmenopausal period were measured by dual-energy X-ray absorptiometry (DEXA, Norland, XR 46). Osteoporosis was diagnosed according to WHO criteria<sup>19</sup>. According to WHO criteria<sup>19</sup>, osteoporosis is a bone mineral density that is more than 2.5 standard deviations (SD) below the young adult mean value and nonosteoporosis is a bone mineral density that is not more than 1 SD below the young adult mean value. We divided patients into osteoporotic and nonosteoporotic groups based on their DEXA bone mineral density values according to the WHO criteria. Three patients of the osteoporotic group were excluded from the study because their z scores of DEXA were below the level of -2 that can be a cause of secondary osteoporosis. The remaining 105 patients were evaluated for clinical findings and laboratory tests.

Patients were questioned about risk factors of osteoporosis (tea and coffee consumption, nutrition habits, age of menopause, duration of postmenopausal period, physical activity level). A 5-item, 5-point Likert-like scale instrument with higher scores indicating more physical activity was used to evaluate the activity level of patients<sup>20</sup>. The highest activity score was 20 and the lowest score was 0 on this Likert-like scale. Prior fractures of patients and familial fracture history were also noted.

Laboratory tests including calcium, phosphate, alkaline phosphatase levels were conducted and serum immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies to HP were measured by enzyme-linked immunoabsorbent assay (ELISA) in sera from all patients. Quantitative determination of IgA or IgG class antibodies to *Helicobacter pylori* in human serum was performed using a disposable device attached to a Chorus instrument

(Instrument: Chorus, method: microELISA, Diesse Diagnostica Senese, Siena, Italy). The Chorus method is based on the ELISA principle. The cutoff values HP IgG are positive: >12 UA/mL, negative: <12 UA/mL, and those of HP IgA are positive: when the index is > 1.2, negative: when the values are between 0.8–1.2. For HP IgG, the Chorus instrument expresses the result in AU/mL, calculated from a batch-dependent curve stored in the instrument. For HP IgA, the Chorus instrument gives the result as an INDEX (ratio of the O.D. value of the examined samples to the O.D. value of the cutoff) which can be used as a quantitative measurement since it is proportional to the quantity of specific IgA. Patients who had serum IgA or IgG levels which were over the laboratory cutoff level were considered HP seropositive for IgA or IgG.

The local Ethical Committee approved this study and all patients gave their permission for inclusion in this study in writing.

Statistical analyses were performed using SPSS 17.0 for Windows. All quantitative data are expressed as mean standard deviation (SD). Student's t-test was used to compare the variables between groups. Differences between prevalence rates were assessed by the Chi-square test. P values <0.05 were considered statistically significant.

## RESULTS

Fifty-eight osteoporotic and 47 nonosteoporotic patients were evaluated. The mean age of the osteoporotic patients was 65.29 ± 6.09 years and that of the nonosteoporotic patients was 63.57 ± 6.53 years. Between the groups there were no differences in age, education level, occupation, age of menarche or menopause, duration of postmenopausal period or daily consumption of tea, coffee, alcohol or diary products (p>0.05). The activity level of the osteoporotic group was significantly lower than that of the nonosteoporotic group (p<0.05) (Table 1).

There were 13 (22.4%) patients who had had prior fractures in the osteoporotic group and 7 patients (14.9%) in the nonosteoporotic group. The difference for prior fractures was not statistically significant between the groups (p=0.329). Eleven patients in the osteoporotic group (19%) and 2 patients in the nonosteoporotic group (4.3%) had a familial fracture history. The familial fracture history of the osteoporotic group was significantly higher than that of the

**Table 2.** Antecedent fracture and history of familial fracture of patients

	Osteoporotic (%) patients (n=58)	Nonosteoporotic patients (n=47) (%)
Antecedent fracture	13 (22.4)	7 (14.9)
History of familial fracture	11 (19)*	2 (15.4)*

\* p&lt;0.05

**Table 3.** Helicobacter pylori seropositivity of the osteoporotic and non-osteoporotic patients

	Osteoporotic patients (n=58) (%)	Nonosteoporotic patients (n=47) (%)
HP Ig A (+)	28 (48.3)**	22 (46.8)**
HP Ig G (+)	41 (70.7)**	35 (74.5)**

HP Ig A (+): Helicobacter pylori immunoglobuline A seropositivity.

HP Ig G (+): Helicobacter pylori immunoglobuline G seropositivity.

\*\* p&gt;0.05

nonosteoporotic group (p=0.023) (Table 2).

The calcium, phosphate, and alkaline phosphatase levels were within their normal ranges in both the osteoporotic and nonosteoporotic groups. Lumbar osteoporosis was detected in 7 patients (12.1%), femoral osteoporosis in 36 patients (62.1%), and lumbar and femoral osteoporosis in 15 patients (25.9%) in the osteoporotic group. The HP Ig A and Ig G seropositivities were not significantly different between osteoporotic and nonosteoporotic patients (p>0.05) (Table 3).

## DISCUSSION

This study was carried out to investigate the role of HP in postmenopausal osteoporosis. A possible role for HP in osteoporosis has been suggested due to the fact that HP infection may induce chronic low level systemic inflammatory status. Inflammatory markers such as TNF-alpha and IL-6 have roles in bone metabolism<sup>14,21</sup>. TNF-alpha increases bone resorption, and IL-6 raises the number of osteoclasts<sup>2</sup>. Estrogens have an inhibitory effect on the production of these cytokines<sup>2</sup>. Giannini et al.<sup>22</sup> reported that HP-infected patients showed decreased rates of conversion of steroids to estrogens.

Some studies have suggested that parietal cells of gastric mucosa of rats play an important endocrine role in secreting estrogens, and that this presumably is also the case in humans<sup>23</sup>. HP infection increases the risk of developing gastric mucosal atrophy<sup>24,25</sup>.

Some studies have determined the role of HP in osteoporosis. Nielsen et al.<sup>17</sup> suggested that HP seropositivity increased in postmenopausal osteoporosis. They evaluated 79 postmenopausal females having at least one low energy vertebral fracture and low BMD and 79 postmenopausal healthy females for antibodies to HP with the latex agglutination test. They found that HP seropositivity was 39.2% in osteoporotic patients and 22% in healthy controls. The difference was statistically

significant, so they proposed that HP infection is a risk factor for postmenopausal osteoporosis. In our study, we detected HP antibodies using the ELISA technique which is inexpensive, simple and readily available. We didn't find significant differences between HP antibodies, in contrast to Nielsen et al. This difference in study results may be due to the techniques used for detecting antibodies to HP. In addition, we didn't use X-rays of vertebra for defining the osteoporosis, rather we questioned osteoporotic and nonosteoporotic patients about previous fractures and familial fracture history.

Figura et al.<sup>18</sup> evaluated 80 osteoporotic males, and 160 healthy males for antibodies to HP, hormone levels and bone turnover markers and measured HP strains expressing Cag A antibodies in HP seropositive patients and controls. They found that HP seropositivity was not different but Cag A expressing HP seropositivity was significantly different between groups. Therefore, Figura et al.<sup>18</sup> proposed that HP strains expressing Cag A might increase the risk of osteoporosis in male patients by reducing the systemic levels of estrogens. They also proposed that bone resorption in patients infected with Cag A-positive HP strains, was higher than in those infected with Cag A-negative HP strains and HP negative cases. It is considered that detailed studies are needed to reveal the role of HP subgroups in postmenopausal osteoporosis.

Adriana et al.<sup>3</sup> evaluated 50 postmenopausal females with endoscopy and DEXA. They divided patients into two groups, those with and without osteoporosis. They found that seropositivity of HP was 75% in the osteoporotic group and 55% in the nonosteoporotic group but this difference was not statistically significant. It is known that HP infection increases the rate of development of gastric atrophy<sup>26,27</sup>. Gastric atrophy decreases the density of parietal cells which are important for gastric acid secretion and also for calcium absorption. In their study, Adriana et al. found that all patients infected with HP had atrophic gastritis but with a well preserved parietal cell density, contrary to expectation, in the osteoporotic group. They suggested that although the stomach had an important role for absorption of calcium, this role was not limited to its acid secretory function and that HP infection with or without atrophic gastritis seemed not to be an important risk factor for decreased bone mineral density in their postmenopausal patient group.

Previous studies about the possible role of HP infection in osteoporosis didn't explore the nutritional risk factors of osteoporosis or the physical activity level of patients. Although we asked about these factors, we didn't measure the bone turnover markers. This was one limitation of our study.

The presence of previous fracture was recorded on the basis of interviews with patients. This was the another limitation of our study. We asked about previous peripheral or vertebral fractures and familial fracture history, but we didn't explore the vertebral fracture of osteoporotic and nonosteoporotic patients with X-rays. X-ray examination might have been more useful and decisive for determining previous vertebral fracture and it might have helped to

determine presence of fracture more precisely.

The another limitation of our study was the detection method of HP infection. Several diagnostic tests are used to detect HP infection. In clinical practice biopsy-based methods remain the most reliable, but they are invasive, expensive, and not always appropriate. Therefore, several noninvasive tests have been developed over the last two decades which have become part of clinical practice. Noninvasive techniques, also have high sensitivity and specificity for the diagnosis of HP infection<sup>28</sup>). Therefore, serologic testing for antibodies to HP using ELISA has become a widely accepted diagnostic test. These tests are also suitable for epidemiologic studies<sup>29</sup>). We didn't use endoscopy which is a more decisive method for HP diagnosis and can also detect atrophic gastritis. We used the ELISA technique, which is the method available to our clinic, for this cross-sectional study.

While we found that the physical activity score of the osteoporotic group was significantly low and that familial fracture history of the osteoporotic group was significantly high compared to the nonosteoporotic group, we didn't find a difference in HP seropositivity between the two groups.

One study has suggested that HP infection is a risk factor for male osteoporosis. In the present study, we didn't find a difference in HP seropositivity between the postmenopausal osteoporotic and nonosteoporotic groups, therefore HP infection seems not to be a significant risk factor for postmenopausal female osteoporosis. Nonetheless studies with more decisive diagnostic methods for HP infection subgroups and osteoporosis are needed for determining whether HP infection is a risk factor of postmenopausal osteoporosis.

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