

# The influence of vitamin D deficiency on eradication rates of *Helicobacter pylori*

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## Abstract

**Background.** *Helicobacter pylori* eradication therapy improves the healing of various gastro-duodenal diseases such as chronic gastritis and peptic ulcer, and also reduces gastric cancer incidence. Several studies have reported on risk factors other than antibiotic resistance related to *Helicobacter pylori* eradication failure.

**Objectives.** In this study, we aimed to investigate whether or not the serum levels of 25-hydroxy-vitamin D (25(OH)D) influence eradication rates of *H.pylori*.

**Material and methods.** 220 patients diagnosed with *H.pylori* gastritis using endoscopic biopsy had their 25-OH vitamin D levels measured via the electrochemiluminescence method before beginning eradication therapy of *H.pylori*. Gastric biopsies obtained at endoscopy were examined for *H.pylori* strains and histopathologic findings. All patients were treated with bismuth-containing quadruple therapy for 14 days. *H.pylori* eradication was determined via the 14C-urea breath test performed 4 weeks after the end of therapy. Based on the 25-OH vitamin D levels, the patients were divided into 2 groups: group 1 (deficient) had a vitamin D level of <10 ng/mL, while group 2 (sufficient) had a vitamin D level of ≥10 ng/mL.

**Results.** Eradication was successful in 170 (77.2%) patients and failed in 50 (22.7%) patients. The prevalence of 25(OH)D deficiency was 30.5%. Mean 25(OH)D levels were significantly lower in the eradication failure group compared to the successful treatment group ( $9.13 \pm 4.7$  vs  $19.03 \pm 8.13$ ;  $p = 0.001$ ). There were significantly more patients with deficient 25(OH)D levels in the failed treatment group compared to the successful treatment group ( $p = 0.001$ ).

**Conclusions.** Our findings suggest that 25-OH vitamin D deficiency may be considered a risk factor related to eradication failure of *H.pylori*, which may lead to a need for supplementation of vitamin D before eradication of *H.pylori*.

**Key words:** vitamin D, *Helicobacter pylori*, *Helicobacter pylori* eradication

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## Introduction

*Helicobacter pylori* (*H.pylori*) is a gram-negative bacterium that colonizes the human stomach; it is also increasingly prevalent, ranging from 25% in developed countries to 90% in developing countries.<sup>1</sup> *H.pylori* is a main causative factor in various gastrointestinal diseases such as chronic gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma (MALT) and gastric cancer.<sup>2</sup> *H.pylori* eradication significantly affects the treatment of both peptic ulcers and gastric lymphoma.<sup>3</sup> Therefore, successful eradication of *H.pylori* infection may prevent the development of gastric cancer. Clearly, it is important to eradicate this bacterium and its related risk factors.<sup>4</sup> Bacterial and host factors in *H.pylori* eradication therapy include antibiotic resistance, virulence factors and host-related genetic disorders (CYP2C19, IL-1B, multidrug-resistant transporter-1).<sup>5</sup> Host immunity also plays an important role against an infectious disease such as *H.pylori* infection. Meanwhile, vitamin D is responsible for regulating calcium and phosphorus metabolism, both of which are needed for bone formation. Beyond its well-known role in bone formation, vitamin D also has an immunomodulator role in targeting various immune cells, including monocytes, macrophages and dendritic cells, as well as T-lymphocytes and B-lymphocytes.<sup>6</sup> Hence, vitamin D deficiency may increase the incidence of immune system disorders and may be a risk factor for the progression of an infectious disease.<sup>7</sup>

Vitamin D deficiency might increase the risk of *H.pylori* infection, yet this association has yet to be evaluated. Therefore, in this study, we aimed to evaluate the association between vitamin D deficiency and the treatment of *H.pylori* infection.

## Material and methods

### Patients

Patients complaining of dyspeptic symptoms for at least 1 month underwent diagnostic esophago-gastro-duodenoscopy. All patients were non-ulcer dyspeptic patients. The study included 220 sequential patients diagnosed with *H.pylori* gastritis by endoscopic biopsy for prospective observation in a gastroenterology clinic between September 2014 and December 2015. The research excluded patients who had previously received *H.pylori* eradication treatment, vitamin D supplements, corticosteroids/immunosuppressive treatment, antibiotics, or anti-inflammatory or acid suppressive treatment in the prior 2 months. It also excluded those with a history of systemic inflammatory or autoimmune disorders, gastric surgery, renal failure, liver cirrhosis, and malignancies. The study was planned according to the ethics guidelines of the Helsinki Declaration, informed consent was obtained from all

the participants, and the research was approved by our hospital's Institutional Research Ethics Board.

### Endoscopic evaluation

Endoscopy was conducted with Olympus Evis Exera 160 videoendoscopes (Olympus America Inc., Center Valley, USA). Two biopsy specimens were obtained from the antrum and 2 from the corpus for histological examination.

### Histopathologic examination

The biopsy samples were fixed in 10% formalin before being sliced into 4–6 mm pieces, dehydrated in ethanol, embedded in paraffin wax, sectioned (5 µm thick), and stained with hematoxylin and eosin (H&E) for histological examination and Giemsa stain for *H.pylori* identification. A blinded histopathologist examined all specimens and diagnosed cases as active or chronic gastritis. The updated Sydney system was used to grade the activity of gastritis, inflammation, atrophy and *H.pylori* density.<sup>8</sup> Thus, mucosal atrophy was defined by the loss of glandular tissue; inflammation of gastric mucosa was defined by the presence of an inflammatory infiltrate composed of lymphocytes and plasma cells; and activity of gastric mucosa was defined by the presence of neutrophil cells at superficial or deep layers. The degree of activity, inflammation, atrophy and *H.pylori* density were classified into 4 categories, scored on a scale of 0–3 (0 = none; 1 = mild; 2 = moderate; 3 = severe).

### Treatment protocol

All infected patients were treated for 14 days with bismuth-containing quadruple eradication therapy consisting of colloidal bismuth sub-citrate 300 mg q.i.d., pantaprazole 40 mg b.i.d., tetracycline 500 mg q.i.d., and metronidazole 500 mg t.i.d.

### Confirmation of *Helicobacter pylori* eradication (14C-urea breath test)

A 14C-urea breath test was performed at least 4 weeks after treatment completion. Following overnight fasting, patients used 25 mL of water to swallow 37 kBq (1 mCi) of an encapsulated form of 14C-urea/citric acid composition (Helicap, Noster System AB, Stockholm, Sweden). Breath samples of the patients were collected with a special dry cartridge system (Heliprobe BreathCard, Noster System AB) at 10 min. Patients exhaled gently into the cartridge mouthpiece until the indicator membrane changed color from orange to yellow. The breath-card was inserted into a special small desktop Geiger-Muller counter (Heliprobe analyzer, Noster System AB), and activity was counted for 250 s. The results were expressed both as counts per minute (HCPM) and as a grade

(0 – not infected, CPM < 25; 1 – equivocal, CPM 25–50; 2 – infected, CPM > 50). This procedure adhered to manufacturer guidelines regarding the counts obtained from the cartridges, and a negative test result was defined as *H.pylori* eradication.

## Laboratory measurements

Patients who had the histopathological diagnosis of *H.pylori* infection underwent the assessment of serum 25-hydroxyvitamin D3 levels and CagA seropositivity before *H.pylori* treatment. Serum 25(OH)D3 levels were measured using an electrochemiluminescence method (Roche Diagnostics GmbH, Mannheim, Germany), with inter-assay and intra-assay coefficients of variation (CVs) of 2.4% and 5.7%, respectively. Sera obtained by centrifugation were stored at -20°C and analyzed simultaneously by technicians who were blind to group allocation. Serologic assays for specific IgG antibodies against CagA protein were analyzed by enzyme immunoassays (DIA.PRO Diagnostic Bioprobes S.r.l, Milan, Italy). CagA antibody titers ( $\geq 8$  U/mL) were classified as positive, per manufacturer instructions. Vitamin D deficiency was defined as a condition in which the 25(OH)D serum level was lower than 10 ng/mL.<sup>9</sup> Before beginning *H.pylori* treatment patients were divided into 2 groups as follows: group 1 (vitamin D deficient) had a vitamin D level of <10 ng/mL, and group 2 (vitamin D sufficient) had a vitamin D level of  $\geq 10$  ng/mL.

## Statistical analysis

SPSS for Windows v. 17.0 was used for the statistical analyses of our study data. Mean standard deviations (SD) were used to identify the data related to the continuous variables, and categorical variables were provided as percentages. The Kolmogorov-Smirnov normalizing test was used to determine whether the continuous variable data fit a normal distribution. The comparison of the variables with normal distribution was tested with an unpaired t-test, and the comparison of the variables without normal distribution was tested with a Mann-Whitney U test. The categorical variables were compared with Pearson's  $\chi^2$  test, and a p-value <0.05 was considered statistically significant.

## Results

The study involved 220 patients. In 170 (77.2%) patients, *H.pylori* was eradicated successfully, while in 50 (22.7%) patients, eradication failed. At the end of therapy, all patients' compliance with the drug protocol was excellent. There were no significant differences between the eradication successful and eradication failure groups regarding age or sex ( $p = 0.54$  and  $p = 0.44$ , respectively) (Table 1). We evaluated the relationship of histopathologic findings

and eradication rates between the eradication successful and eradication failure groups. In the successful treatment group, the degree of activity and inflammation were significantly higher than that in the failed treatment group (both values of  $p = 0.001$ ). However, no significant differences were seen between the 2 eradication groups in terms of the degree of atrophy and *H.pylori* density (both had values of  $p > 0.05$ ) (Table 2). The *H.pylori* virulence marker CagA was positive in 108 (63.5%) patients in the successful treatment group, and it was positive in 8 (16%) patients in the failure treatment group. We found CagA-positive strains to have significantly higher eradication rates compared to negative ones ( $p = 0.001$ ) (Table 2). We divided patients into 2 groups according to vitamin 25(OH)D status; mean vitamin 25(OH)D levels were significantly lower in the eradication failure group compared to the successful treatment group ( $9.13 \pm 4.7$  vs  $19.03 \pm 8.13$ ;  $p = 0.00$ ) (Table 1). In addition, all patients had an overall 30.5% vitamin 25(OH)D deficiency. We found 42 (84%) patients in the failed treatment group and 25 (14.7%) patients in the successful treatment group to be vitamin 25(OH)D deficient. As shown in Fig. 1, vitamin 25(OH)D deficiency was significantly higher in the failed treatment group compared to the successful treatment group ( $p = 0.001$ ).

## Discussion

In this study, we found *H.pylori* eradication rates to be significantly lower in patients with low vitamin D levels. Bacterial and host-related risk factors such as antibiotic resistance, virulence factors and host-related genetic disorders are associated with *H.pylori* eradication failure.<sup>5</sup>

Table 1. Demographic and vitamin 25(OH)D level differences between the successful and failed eradication groups of *H.pylori*

Variable	Failure (n = 50)	Successful (n = 170)	p-value
Age (mean $\pm$ SD)	47.3 $\pm$ 14.3	47.5 $\pm$ 13.9	0.546
Male (n, %)	27 (54%)	102 (60%)	0.449
Vitamin 25(OH)D (mean $\pm$ SD)	19.0 $\pm$ 8.1	9.1 $\pm$ 4.7	0.001

Table 2. Histological scores and CagA seropositivity between the successful treatment group and the failed *H.pylori* eradication group (mean  $\pm$ SD)

Variable	Failure (n = 50)	Successful (n = 170)	p-value
Activity	1.5 $\pm$ 0.6	1.0 $\pm$ 0.2	0.001
Inflammation	1.7 $\pm$ 0.7	1.1 $\pm$ 0.2	0.001
Atrophy	1.3 $\pm$ 0.5	1.4 $\pm$ 0.5	0.360
<i>H.pylori</i> density	1.3 $\pm$ 0.5	1.5 $\pm$ 0.7	0.058
CagA seropositivity (n, %)	8 (16%)	108 (63.5%)	0.001

CagA – cytotoxin-associated antigen; *H.pylori* – *Helicobacter pylori*; SD – standard deviation.

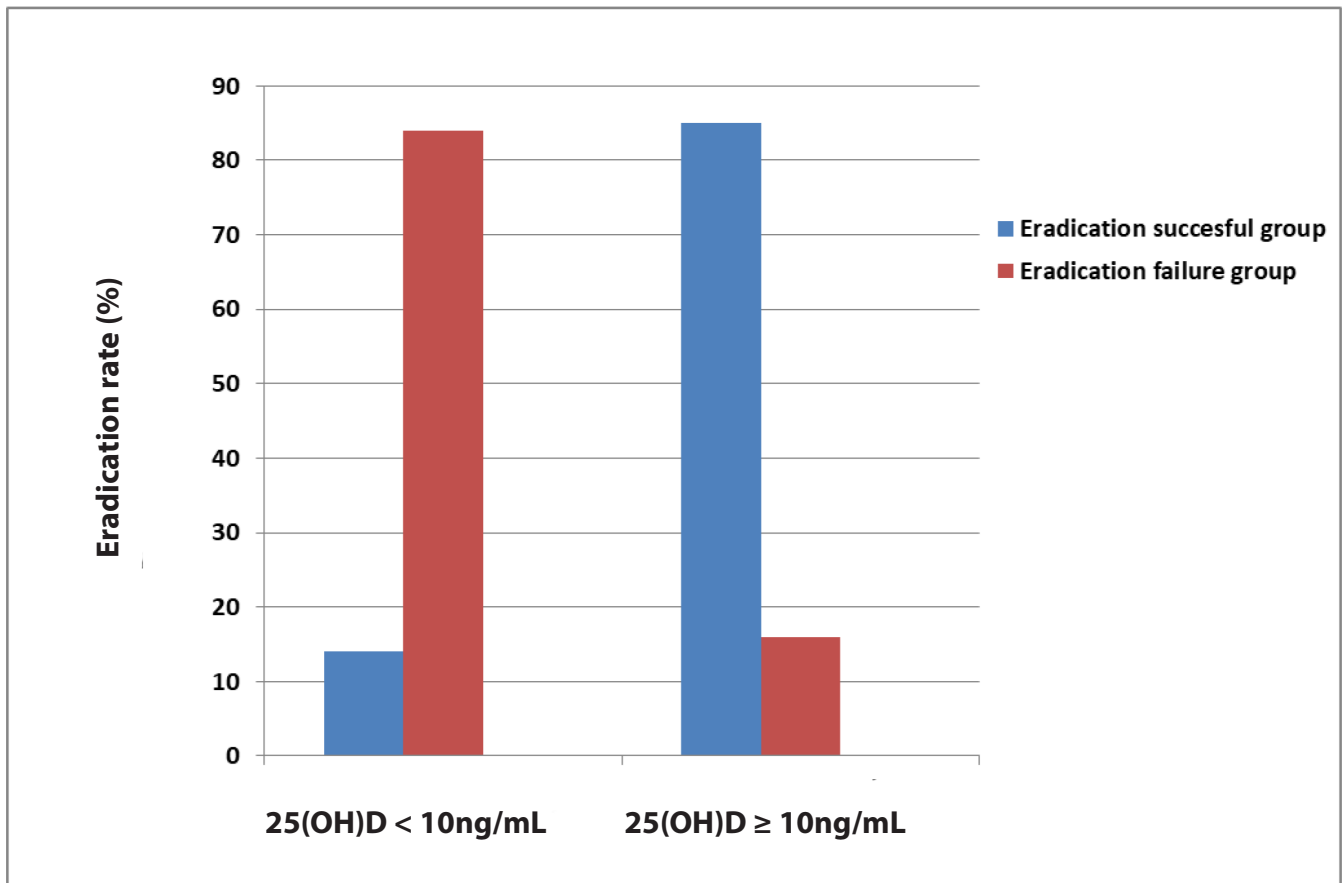


Fig. 1. Comparison of successful and failure rates of *H.pylori* eradication according to the vitamin 25(OH)D levels

Determining the antibiotic resistance of *H.pylori* strains is the most important factor for achieving effective eradication therapy. Worldwide, antibiotic resistance has been reported at an increasing rate (16.2–60.7%), particularly to clarithromycin and metronidazole.<sup>10</sup> Increasing the susceptibility of some antibiotics, another important factor is maintaining a high intragastric pH for 24 h. Although several studies have shown that standard triple therapies are effective in most patients as a first-line treatment, we treated the patients with a bismuth-containing quadruple eradication therapy, which is recommended as a first-line treatment in regions where clarithromycin resistance is >15%.<sup>11</sup> CagA expression in CagA-positive *H.pylori* strains is another bacterial factor in *H.pylori* eradication therapy. Research has shown it to be associated with the host inflammatory response and an increased risk for clinical outcomes.<sup>12</sup> Van Doorn et al. have shown an association between lower eradication efficacy and *H.pylori* strains missing the CagA gene, while Scholte et al. did not find this relation.<sup>13,14</sup> The present study indicated that *H.pylori* eradication rates were significantly higher in patients infected with CagA-positive strains compared to negative ones (CagA-positive, 63.5%; CagA-negative, 36.5%;  $p = 0.001$ ). It is possible that CagA-positive strains cause more intense gastric mucosal inflammation, which may play a role in eradication by increasing the blood flow, thus improv-

ing the flow of antibiotics.<sup>15</sup> Recent studies have shown higher *H.pylori* density by histopathology to be related with complications while indicating a negative correlation with *H.pylori* eradication rates.<sup>16,17</sup> Our study, however, found no significant difference. It is important to note that both of these studies used the triple therapy. Bacterial density does not seem to negatively affect differing treatment of bismuth + quadruple therapy adapted in our study. Previous studies have reported histopathological findings predicting *H.pylori* treatment failure.<sup>18,19</sup> Our study demonstrated that high histological scores of gastritis and activity are effective in determining eradication success. However, there was no significant difference in eradication rates of *H.pylori* according to the severity of atrophy in the antrum. The host immune system has been hypothesized to affect pharmacological treatment in *H.pylori* eradication. Some authors have found that impaired mucosal immune response may contribute to eradication failure in *H.pylori* infection.<sup>20,21</sup> Vitamin D has a long tradition of playing a role in regulating calcium and phosphorus metabolism, but it has also proven effective as a potent immune modulator of the adaptive immune system, stimulating the innate immune response upon infection.<sup>22</sup> Recent studies have demonstrated the relationship between vitamin D deficiency and infectious diseases.<sup>23,24</sup> Vitamin D regulates the innate immune system in macrophages against *Mycobacterium*

*bacterium tuberculosis* through the mechanisms of activated toll-like receptors (TLRs), leading to the induction of antimicrobial peptide cathelicidin that kills the organism.<sup>25</sup> A recent meta-analysis has found that low serum 25(OH)D levels are associated with a higher risk of active tuberculosis.<sup>26</sup> We have evaluated the possible association between vitamin D levels and *H.pylori* infection.

Our study primarily demonstrates the relationship between *H.pylori* eradication rates and low vitamin D levels. We found that *H.pylori* eradication rates were significantly lower in patients with vitamin D deficiency. A potential pathogenic mechanism explaining the observed association between vitamin D status and eradication rates is impairment of the vitamin D signal immune function, which may lead to inadequate immune response. There is limited data demonstrating the relationship between vitamin D and *H.pylori* infection. One in vitro study showed the selective antibacterial effect of vitamin D3 decomposition product (VDP1) against *H.pylori*.<sup>27</sup> Vitamin D is also known to regulate the expression of antimicrobial peptides – cathelicidin and  $\beta$ -defensin, which kill the bacteria. Although the effect of cathelicidin has been demonstrated only in macrophages infected with *M. tuberculosis*, antibacterial action against gram-negative and gram-positive bacteria has also been reported.<sup>28,29</sup> Another antimicrobial peptides  $\beta$ -defensin, which is secreted in the gastric mucosa after infection by *H.pylori*, constitutes immune defense against this bacterial pathogen at the mucosal surface.<sup>30</sup> In a vitamin D-deficient state, the infected macrophage is unable to produce sufficient 1,25-(OH)D2 to upregulate the production of cathelicidin and  $\beta$ -defensin, thus rendering them unable to kill the *H.pylori* strains.

In this paper, we have demonstrated low eradication success in infected patients with vitamin D deficiency. Vitamin D deficiency may be a risk factor associated with *H.pylori* infection treatment failure and may lead to a need for supplementation of vitamin D before *H.pylori* eradication therapy. More prospectively designed clinical trials considering pre-treatment vitamin D levels are needed to further evaluate the relationship between vitamin D status and *H.pylori* infection.

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