

Detection of a relationship between Osteoporosis and *Helicobacter pylori* infection and Vitamin D3 deficiency

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium that has been implicated in influencing bone metabolism and potentially promoting the development and incidence of osteoporosis (OP). OP is characterized by low bone mineral density, which is associated with an increased risk of fractures. Emerging evidence suggests that *H. pylori* positivity rates appear to be higher among individuals with vitamin D deficiency. Vitamin D3 plays a crucial role in maintaining bone health, and its deficiency is often linked to compromised bone strength and density.

Objectives: This study aims to investigate the relationship between *H. pylori* infection and osteoporosis, the association between *H. pylori* infection and vitamin D3 levels, and the correlation between osteoporosis and vitamin D3 levels.

Methods: A total of 120 samples were collected and divided into two groups: 60 samples from osteoporosis patients (30 fecal samples and 30 blood samples) and 60 samples from healthy individuals (30 fecal samples and 30 blood samples). Fecal samples were used to detect *H. pylori* antigen (Ag), while blood samples were analyzed for *H. pylori* antibody (Ab) and vitamin D3 levels.

Results: Among osteoporosis patients, 75% tested positive for *H. pylori* Ag, while 25% were negative. In the healthy group, 20% were positive and 80% were negative for *H. pylori* Ag. Furthermore, 93.33% of osteoporosis patients who were positive for *H. pylori* Ag had vitamin D3 levels ≤ 29 ng/ml, and only 6.66% had vitamin D3 levels ≥ 30 ng/ml. Among the healthy individuals positive for *H. pylori* Ag, 100% had vitamin D3 levels ≤ 29 ng/ml. In the healthy group negative for *H. pylori* Ag, 53.33% had vitamin D3 levels ≤ 29 ng/ml, while 46.66% had levels ≥ 30 ng/ml. Additionally, 55% of osteoporosis patients had severe vitamin D3 deficiency (≤ 20 ng/ml), 35% had moderate deficiency (21–29 ng/ml), and 10% had sufficient levels (≥ 30 ng/ml). Among healthy individuals, 25% had severe deficiency (≤ 20 ng/ml), 40% had moderate deficiency (21–29 ng/ml), and 35% had sufficient levels (≥ 30 ng/ml).

Conclusion: The findings of this study indicate a strong association between *H. pylori* infection and osteoporosis, with a higher prevalence of *H. pylori* infection observed among osteoporosis patients. Moreover, both *H. pylori* infection and osteoporosis were associated with lower vitamin D3 levels, highlighting the potential role of *H. pylori* in contributing to vitamin D3 deficiency and impaired bone health. These results underscore the importance of screening for *H. pylori* infection and monitoring vitamin D3 levels in patients at risk for osteoporosis.

Keywords: *H. pylori* infection; OP; Vitamin D3 level; DEXA; fecal; blood

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Background

H. pylori is a gram-negative, spiral-shaped bacterium belonging to the *Helicobacteraceae* family. It is a transmissible and pathogenic microorganism, commonly spread through contaminated food (Bashir & Khan, 2023). Strains of *H. pylori* possess various virulence factors, with the most studied being genes such as CagA, VacA, DupA, OipA, and GGT. CagA is a bacterial protein that induces specific modifications in the morphology of epithelial cells, altering cell polarity and leading to a “hummingbird” phenotype. Additionally, changes in the cytoskeleton associated with the development of gastric adenocarcinoma can be triggered by this virulence factor (de Brito et al., 2019).

Furthermore, the vacuolating cytotoxin gene is induced upon contact with the epithelium, and other virulence factors include blood group antigen-binding adhesin, sialic acid-binding adhesin, and adherence-associated lipoproteins A and B (Šterbenc et al., 2019). The World Health Organization (WHO) designated *H. pylori* as a Class I carcinogen for gastric cancer in 1994. In 2014, the WHO recommended the global eradication of *H. pylori* as a critical preventive measure against gastric cancer (Mohamed et al., 2024).

Numerous studies have explored the association between *H. pylori* infection and osteoporosis. *H. pylori* can affect nutrient absorption in the digestive system, potentially reducing bone turnover. The infection also causes chronic gastritis and induces an inflammatory response, increasing pro-inflammatory factors such as IL-1 β , IL-6, IL-8, IL-17, and TNF- α . *H. pylori* influences bone metabolism through inflammatory reactions, oxidative stress, blood lipid metabolism, and insulin resistance—all of which contribute to the occurrence and progression of osteoporosis (Zhang et al., 2024).

Vitamin D3, also known as calciferol, is one of the four fat-soluble vitamins that can be stored in body tissues and synthesized by the human body. Vitamin D3 regulates multiple biological processes (Trivedi et al., 2023). The normal levels of vitamin D3 in the blood are classified by the Endocrine Society of Clinical Practice (ESCP) as follows: deficiency (less than 20 ng/mL), insufficiency (21-29 ng/mL), sufficiency (greater than 30 ng/mL), and toxicity (greater than 150 ng/mL) (Salim et al., 2023).

Shafir et al. (2021) observed that *H. pylori* positivity rates appear higher among populations with low serum vitamin D3 levels. This association led to the establishment of an inverse linear correlation between vitamin D3 sufficiency and the prevalence of *H. pylori* infection. This suggests that adequate vitamin D3 levels may offer protection against *H. pylori*. Moreover, increased vitamin D3 levels are associated with successful eradication of *H. pylori* in both wild-type and vitamin D receptor (VDR) knockdown (VDR-KD) mice, which were used to demonstrate that vitamin D3 inhibits *H. pylori* infection by enhancing the expression of the vitamin D receptor (VDR) and cathelicidin antimicrobial peptide (CAMP). VDR-KD mice with lower VDR expression were more susceptible to *H. pylori* infection. A meta-analysis by Yang et al. (2019) further confirmed the protective role of vitamin D3 in *H. pylori* infection. Additionally, El Shahway et al. (2018) found that low vitamin D3 levels were associated with treatment failure in *H. pylori* infection.

Research on cultured primary gastric epithelial cells in mice has demonstrated that the vitamin D/VDR complex binds to the CAMP promoter region, leading to increased gene expression. This mechanism provides a deeper understanding of the anti-*Helicobacter pylori* (*H. pylori*) activity of vitamin D3 at the molecular level. These findings suggest that vitamin D3 may serve as a novel approach in *H. pylori* eradication therapy, a crucial treatment for preventing chronic gastritis, peptic ulcers, and gastric cancer. By enhancing the immune response against *H. pylori*, vitamin D3 presents an opportunity for developing more effective therapies with reduced reliance on antibiotics. Given its potential clinical significance, further research is necessary to explore the relationship between vitamin D3 and *H. pylori* infection and to assess its viability as an adjunctive treatment (Zhou et al., 2020).

Diagnosing *H. pylori* is one of the most important steps in managing the infection. Several diagnostic methods are available, all with high sensitivity and specificity. These tests are classified into non-invasive and invasive procedures. Non-invasive diagnostic tests include the urea breath test, stool antigen test, serological tests, and molecular methods (Alfaro et al., 2023). The Stool Antigen Test (SAT) has several advantages: it is non-invasive, rapid, highly sensitive, specific, and dependable. It can be used to diagnose infections and assess the success of therapies. Its low cost, ease of implementation, and ability to gather samples have led to its increasing acceptance. Serological assays, such as enzyme immunoassay (EIA), detect antibodies against *H. pylori*, with immunoglobulin G (IgG) being the most commonly assessed. These tests have sensitivity and specificity values ranging from 60% to 100% (Khan et al., 2024).

Osteoporosis (OP) is a chronic metabolic bone disease characterized by a progressive decrease in bone mass and density, ultimately leading to increased bone fragility and susceptibility to fractures. This condition arises due to a complex interplay between genetic, hormonal, nutritional, and environmental factors, all of which contribute to the deterioration of bone microarchitecture and reduction in bone strength. The aging process, hormonal changes—particularly estrogen deficiency in postmenopausal women—nutritional deficiencies, and sedentary lifestyles are well-established risk factors for OP. Epidemiological data highlight the substantial global burden of this disease. According to the International Osteoporosis Foundation (IOF), more than 200 million people worldwide are affected by OP, with osteoporotic fractures occurring approximately every three seconds, imposing significant healthcare and socioeconomic burdens (Anish & Nair, 2024).

The World Health Organization (WHO) recommends densitometric diagnosis of osteoporosis based on bone mineral density (BMD) measured by Dual-Energy X-ray Absorptiometry (DXA) (Nisar et al., 2023). DXA remains the gold standard for diagnosing OP, with the T-score being used for evaluation (Ünver et al., 2024). The T-score compares the patient's BMD to the mean value, and WHO diagnostic categories based on the T-score are as follows: Normal (T-score between +1 and -0.99), Osteopenia (T-score between -1 and -2.49), and Osteoporosis (T-score \leq -2.5) (Maschhoff et al., 2024).

Methods

Materials

The most materials and equipment used during the research are shown in table 1.

Table 1 Most material and equipment used in current study.

Name of the material or device	the manufacture company
VIDAS device	Biomérieux /France
VIDAS 25 OH Vitamin D Total Kit	Biomérieux /France
<i>H. pylori</i> Ag rapid test	Sure Bio-Tech/USA
<i>H. pylori</i> Ab rapid test	Sure Bio-Tech/USA
Stool collection container	China
Centrifuge	German
Gel tube	China
Syringe	Malaysian

Samples collection

The samples was collected from osteoporosis patients and healthy controls who underwent a DEXA examination at Ibn Sina General Hospital and Al-Salam Teaching Hospital in the city of Mosul/ Nineveh /Iraq. In addition the samples were taken after obtaining official approval from the Nineveh Health Department as well as with the patients' consent. The total number of samples was (120) divided as follows:

- a) The 30 fecal samples from osteoporosis patients (T-score ≤ -2.5) and (30) samples from healthy controls (T-score between +1 and -0.99).
- b) The 60 blood samples (about 5 – 10 ml from each person) were collected in disposable test tubes. Faecal samples were used for detecting the presence of *H. pylori* Ag, the samples were collected from patients after DEXA examination by giving each patient sterile stool collection container to place the sample in, with the patient being advised to put a sufficient amount of stool in the container, then the examination was performed in the hospital laboratory directly after collecting the sample.

The assay was performed according to the cassette manufacturer's instructions (PPA:99.69% and NPA:99.45%) which includes the following steps:

- 1) Allow the reagent to equilibrate to room temperature for 30 minute (10-30°C) prior to testing
- 2) For solid samples:
 - a. The cap of the stool collection tube containing the sample diluent was unscrew, then randomly stamp the stool collection applicator into the fecal sample in 6 different sites to collect approximately 50 mg of feces.
 - b. For liquid samples: Approximately 50 μ L of liquid fecal was adding into the stool collection tube containing the sample diluent.
- 3) The cap was tighten onto the stool collection tube, then the stool collection tube was shaking vigorously to mix thoroughly, then the tube was leaving alone for 2 minutes.
- 4) The outer packing was take off then the strip/cassette was putting onto the desk with the sample adding region of the strip/sample well of the cassette up.
- 5) The cap of the stool collection tube was break off, then drop 3 drops(80 μ L -100 μ L) of processed sample vertically into the sample adding region of the strip/sample well of the cassette
- 6) The test result was observed immediately within (10-20) minutes.
- 7) Interpretation of results :the appearance of both the test line (T) and control line (C) within the period-specific (15-20 minutes) designates that the collected sample tested positive for *H. pylori* infection. The appearance of only the control line (C) designates a negative test result, If the control line (C) does not appear the result is considered invalid and the procedure needs to be repeated.

As for the blood samples, the samples were collected from the same individuals whom stool samples were collected by drawing a blood sample and placing it in a gel tube then after separating the red blood cells and plasma from the serum by centrifuge, the serum samples was stored at (2-8°C) for less than 24 hours to perform the required tests, which included:

1. Detect *H. pylori* Ab by Rapid Test cassette (PPA:99.38% and NPA:99.17%) which includes following steps:
 - a) The reagent was allow to equilibrate to room temperature for 30 minutes(10-30°C) period to testing.
 - b) The test strip was removed from the sealed pouch and placed it on a clean and level surface with the sample adding area to up.

- c) One drop(25 μ L)of serum was added onto the sample adding area of the strip with avoid formation of bubbles,then two drops(80 μ L -100 μ L) of the sample diluent was added onto the sample adding area of the strip.
 - d) The test result was observed immediately within 15-20 minutes.
 - e) Interpretation of results: The same instructions as in step number 7 when testing *H. pylori* Ag was followed.
2. Measurement level of vitamin D3 Which was performed by a VIDAS device which is an automated quantitative device use for the determination of 25-hydroxy Vitamin D Total in human serum using ELFA technique,this test was donig by inserting the SPR_s and strips into the instrument ,then 100 μ L of serum was added to first well of strep and selecting start,all the assay setps are performed automatically by the instrument,then the assay will be completed whithin approximately 40 minutes and finally the results were recorded. The stander T-tset was used to compared the results between the osteoporosis and control groups

Results

It was observed in this study that 75 % of osteoporosis patients had a positive result for the presence of *H. pylori* Ag in faeces as shown in Figure (1:A). The remaining percentage which includes 25% was negative for this test as shown in figure (1:B), while the percentage of presence of *H. pylori* Ag in the stool of healthy control was 20 % positive test while the negative tests result was 80%.

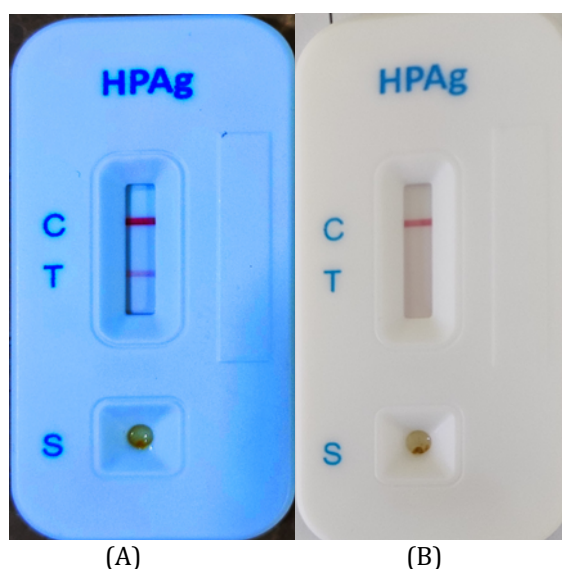


Figure 1 *H. pylori* rapid test. (A) Positive test. (B) Negative test

It was also shown through this study that the ratio of the presence of *H. pylori* Ab in the serum of osteoporosis patients was identical to the ratio of the presence of *H. pylori* Ag in stool which reached 75% positive test and 25% negative test. whereas examination of healthy people showed that 30% of them were positive test for *H. pylori* Ab and 70% were negative for this test.

The current study also included comparing the level of vitamin D3 with the presence of *H. pylori* Ag as shown in the table (2).

Table 2 levels of vitamin D3 and Positive samples for *H. pylori* test

Level of vitamin D3	<i>H. pylori</i> Ag test		
	<i>H. pylori</i> Ag (+) Osteoporosis Samples (%)	<i>H. pylori</i> Ag (+) Control Samples (%)	<i>H. pylori</i> Ag (-) Control Samples (%)
Vitamin D3 \leq (29) ng/ml	93.33	100	53.33
Vitamin D3 \geq (30) ng/ml	6.66	00	46.66

The samples from osteoporosis patients were positive for the presence of *H. pylori* Ag and whose vitamin D3 level \leq (29) ng/ml reached 93.33%, while the samples were positive for *H. pylori* Ag with a vitamin D3 level \geq (30) ng/ml was 6.66%. As for healthy people, the samples that were positive for *H. pylori* Ag and whose Vitamin D3 level was \leq (29) ng/ml reached 100%. As for measuring the level of Vitamin D3 in healthy people who were the result of examining their *H. pylori* Ag they were negative, It was found through this study that 53.33% of them have a level of vitamin D3 \leq (29)ng/ml and 46.66% of them have a level of vitamin D3 \geq (30)ng/ml,

The current study also included a comparison between osteoporosis and levels of vitamin D3, the levels of vitamin D3 in osteoporosis patients as shown in Table (3) were low compared to healthy people, the results showed that 55% of osteoporosis patients had vitamin D3 levels less than (20) ng/ml, and 35% between (21–29) ng/ml, while the remaining 10% had a vitamin D3 level more than (30)ng/ml, As for healthy people, 35% of them had a vitamin D3 level of more than (30)ng/ml, and 40% between (21-29)ng/ml, while the remaining 25% had a vitamin D3 level of less than (20)ng/ml

Table 3 Level of Vitamin D3 in the current study

Individuals	Vitamin D3 level		
	<i>H. pylori</i> Ag (+) Osteoporosis Samples (%)	<i>H. pylori</i> Ag (+) Control Samples (%)	<i>H. pylori</i> Ag (-) Control Samples (%)
Osteoporosis	10	35	55
Healthy people (control)	35	40	25

Discussion

The results of the current study were consistent with previous research confirming the relationship between *H. pylori* and osteoporosis, such as Fisher et al. (2020). *H. pylori* infection may be a risk factor for osteoporosis through several mechanisms, including the production of systemic inflammation and the release of cytokines such as TNF- α , IL-1, and IL-6 (Zhang et al., 2024), which can indirectly cause bone turnover. Additionally, *H. pylori* infection may reduce calcium absorption by causing gastric mucosal atrophy and decreasing acid secretion (Tan et al., 2024). Furthermore, *H. pylori* infection may lead to decreased Vitamin B12 levels. When Vitamin B12 levels are reduced, folate becomes trapped as methyltetrahydrofolate, disrupting folate-related DNA synthesis, an essential process for bone regeneration. Consequently, the reduction in Vitamin B12 may contribute to decreased bone mineral density (BMD) and osteoporosis.

The presence of *H. pylori* antibodies (Ab) in the blood of osteoporosis patients, at the same proportion as *H. pylori* antigens (Ag), indicates active infection with the bacteria. In contrast, a higher rate of *H. pylori* antibodies in healthy controls compared to *H. pylori* antigens in the same individuals may result from prior treatment or from the patient's immune system successfully eliminating the bacteria. *H. pylori* antibodies may remain in the body for an extended period, with significant reduction in antibody titers occurring within one year after eradication treatment. However, it may take longer for complete negative conversion to be achieved. This finding aligns with previous studies, such as Tanaka et al. (2023), which noted that serological tests can remain positive for many months after eradication due to the persistence of antibodies in the blood.

Additionally, the results of the current study were consistent with previous research comparing vitamin D3 deficiency and the presence of *H. pylori* antigens (Săsăran et al., 2023; Habbash et al., 2022; Bahşi et al., 2022). Recent literature suggests that vitamin D3 has immunoregulatory functions that influence susceptibility to infections, including *H. pylori*. Vitamin D3 may reduce the risk of infection through various mechanisms, such as enhancing innate immunity by modulating the production of antimicrobial peptides and cytokine responses (Yang et al., 2019). Moreover, several clinical studies have shown that vitamin D analogues may have anti-*H. pylori* effects (Han et al., 2019; Wanibuchi et al., 2018), as *H. pylori* possesses virulence factors that make it resistant to antibiotics.

The current study revealed a significant decrease in vitamin D3 concentrations in patients with osteoporosis compared to healthy individuals. Since vitamin D3 is crucial for strengthening and building bones, any deficiency can lead to osteoporosis. These findings align with previous studies, such as Khadhim (2022), Obaid et al. (2021), and Salamanna et al. (2021).

Conclusion

Our study provides evidence for the relationship between *H. pylori* infection with OP and the level of Vitamin D3, The level of vitamin D3 in OP people and in patients with *H. pylori* is lower than the level of this Vitamin in control people, therefore, a periodic examination to detect the presence of *H. pylori* infection is recommended to prevent its complication.

Declaration Conflicting Interest

The authors have no conflicts of interest to declare.

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Author Contribution

All authors played an essential role in every phase of this article's development, including conceptualizing ideas, gathering and analyzing data, and drafting and refining the final manuscript. Their collaboration brought together diverse expertise and perspectives, ensuring the article's accuracy, depth, and overall quality.

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