ORIGINAL ARTICLE

Is Vitamin D Deficiency a Risk Factor for Helicobacter Pylori Eradication Failure?

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SUMMARY

Background: Host factors related to failure of eradication of Helicobacter pylori (H. pylori) are increasingly studied. This work aimed to study the influence of 25-hydroxy-vitamin D [25(OH)-vitD] status on the rate of H. pylori eradication.

Methods: One hundred and fifty patients infected with H. pylori were tested for serum 25(OH)-vitD level prior to 14 days clarithromycin-based triple eradication therapy. Accordingly, patients were divided into: group I (eradication successful) and group II (eradication failure). Both groups were compared regarding mean level of serum 25(OH)-vitD and number and percentage of patients with deficient 25(OH)-vitD.

Results: Overall rate of eradication was 72%. Mean serum level of 25(OH)-vitD was higher in the eradication successful group compared to the group of eradication failure (28.12 ± 8.10 vs. 13.54 ± 6.37; p < 0.001). The percentage of patients with 25(OH)-vitD deficiency was higher in the group of eradication failure compared to the group of successful eradication [30 (71.5%) vs. 19 (17.5%); p < 0.001]. Patients with sufficient 25(OH)-vitD had a higher rate of eradication compared to patients with deficient 25(OH)-vitD (88% vs. 38.5%).

Conclusions: This study suggested that deficiency of 25(OH)-vitD could be a risk factor for H. pylori eradication failure, and it recommends to investigate the effect of vitamin D supplementation on H. pylori eradication.

KEY WORDS

vitamin D, deficiency, Helicobacter pylori, eradication, failure

INTRODUCTION

Infection with Helicobacter pylori (H. pylori) is considered the furthest spread infection all over the world, affecting on average half the world population with infection rates ranging from 25% in developed countries [1] to 90% in the developing countries [2]. It colonizes the human stomach and is an important contributing factor in the pathogenesis of many gastric diseases including gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma (MALT) and gastric
carcinoma [3,4], as well as colorectal polyps and cancer [5].

A recent meta-analysis concluded that *H. pylori* eradication could decrease gastric cancer risk, suggesting that persistence of *H. pylori* infection constitutes a relative risk of 65% for gastric cancers and an increased absolute risk from 1.1% to 1.7% [6]. Therefore, it is clearly important to successfully eradicate *H pylori infection* and its host contributing risk factors [7].

The standard triple therapy with clarithromycin, proton pump inhibitor (PPI), and amoxicillin or metronidazole is the first-line therapy for *H. pylori* eradication. A cure rate of 70 - 85% has been reported by the American College of Gastroenterology in 2007 [8]. A recent systematic review estimated a cure rate of 75.1% for the triple therapy, and 84.1% for the sequential therapy [9].

Apart from antibiotic resistance which has been suggested as the most important host factor affecting *H. pylori* eradication, the host immune system together with virulence factors and host-related genetic disorders (CYP2C19, IL-1B, and multidrug-resistant transporter-1) are important factors in *H. pylori* eradication [10].

Beyond the well-known role of vitamin D in calcium and phosphorous metabolism, both are needed for bone formation. Vitamin D also has an immune-modulator role in targeting different immune cells, including macrophages as well as T-lymphocytes and B-lymphocytes [11]. It enhances immunity by modulating the production of endogenous antimicrobial peptides (AMPs) inside immune cells including macrophages, [12] and through cytokine response, as well as it boosts the activity of macrophages and monocytes [13].

Therefore, vitamin D deficiency appears to be incorporated in immune system disorders; hence, it has been suggested as an important risk factor for *H. pylori* infection and failure of eradication [14].

**MATERIALS AND METHODS**

This study included patients presented to the family medicine and internal medicine clinic of Umm Alqura University Medical Center, Makkah, Saudi Arabia, during the period from March 2018 to October 2019. In this study, 150 patients of both genders, aged 18 to 65 years old, and complaining of symptoms of dyspepsia for two or more months were included. Full medical history was obtained, and complete physical examination was performed for every patient. A $^{14}$C-urea breath test (UBT) was performed, and those who tested positive were included in the study.

Exclusion criteria involves patients who received previous *H. Pylori* eradication treatment, are currently using vitamin D supplement, have used PPI or antibiotics any time in the prior two months, have known hypersensitivity to PPI or antibiotics, and/or have a history of autoimmune disorder, systemic inflammatory disease, gastric surgery, liver cirrhosis, renal failure, malignancies or are on oral corticosteroid therapy.

Informed consent was obtained from patients after explanation of the objectives and the process of the study, and being assured of keeping their information confidential.

**Eradication therapy**

All patients were prescribed a 14-day course of clarithromycin-based triple therapy for *H. pylori* eradication (twice daily doses of clarithromycin 500 mg, amoxicillin 1,000 mg, and omeprazole 20 mg).

**Laboratory measurements**

Diagnosis of *H. pylori* infection via $^{14}$C-urea breath test (UBT)

Patients were asked to fast overnight and then ingested urea labeled with 37 kBq of $^{14}$C-urea/citric acid composition dissolved in 25 mL of water (1 mCi) (Helicap, Noster System AB, Stockholm, Sweden). Patient’s breath samples were obtained before ingestion and 15 minutes thereafter. The liquid $\beta$-scintillation counter was used to measure $^{14}$C content in breath samples in a Bq mode. A 3.33 Bq was set as a value below which the test result is negative for $^{14}$C in breath (successful eradication) and at or above which indicates the test result is positive (failure of eradication).

**Evaluation of vitamin D status by measurement of serum 25(OH)-vitD using a quantitative chemiluminescent immunoassay (CLIA) method**

The 25 OH Vitamin D TOTAL Assay (DiaSorin Inc. LIAISON 510(K), Italy; DiaSorin LIASON ® 25 OH Vitamin D TOTAL Assay) is an automated immunoassay that is a solid-phase competitive binding test. Sera were collected in separate tubes, mixed with denaturation buffer to extract the analyte as most circulating 25(OH)-vitD is bound to vitamin D-binding protein (VDBP) in vivo. Following neutralization, the enzyme conjugate biotinylated 25(OH)-vitD and the enzyme complex peroxidase-labeled streptavidin are mixed together and the solution is transmitted to the microtiter plate. Endogenous 25(OH)-vitD of the patient sample competes with a 25(OH)-vitD-biotin conjugate to bind to the VDBG. Binding of 25(OH)-vitD-biotin is identified by peroxidase-labeled streptavidin. Unbound components are eliminated by washing. In the next step, the reagents are added to initiate the chemiluminescent reaction. The light signal is detected by a photomultiplier as relative light units. An inverse relationship exists between the intensity of the light and the concentration of 25(OH)-vitD in the study sample [15].

This method measures total 25(OH)-vitD including 25(OH)-vitD2 and 25(OH)-vitD3 and other hydroxylated vitamin D metabolites in human serum [15]. A comparative study of the automated immunoassay DiaSorin LIASON (LSN) with liquid chromatography-tandem mass spectrometry (LC-MS/MS) demonstrated that LSN had comparable correlation coefficient [r] values to LC-MS/MS (0.936 and 0.933), and the LSN assay results were statistically equivalent to those given in the LC-MS/MS method.
Table 1. Demographics and laboratory findings of group I (eradication successful) and group II (eradication failure).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (n = 108)</th>
<th>Group II (n = 42)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>36.0 ± 10.30</td>
<td>35.16 ± 9.38</td>
<td>0.649</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male: 62 (57.5%)</td>
<td>male: 24 (57%)</td>
<td>0.977</td>
<td></td>
</tr>
<tr>
<td>female: 46 (42.5%)</td>
<td>female: 18 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>24.91 ± 2.037</td>
<td>25.35 ± 2.139</td>
<td>0.243</td>
</tr>
<tr>
<td>Smoking</td>
<td>36 (33.3%)</td>
<td>17 (40.4%)</td>
<td>0.411</td>
</tr>
<tr>
<td>DM</td>
<td>24 (22.2%)</td>
<td>8 (19%)</td>
<td>0.670</td>
</tr>
<tr>
<td>Hb</td>
<td>13.21 ± 1.059</td>
<td>12.89 ± 1.27</td>
<td>0.125</td>
</tr>
<tr>
<td>WBCs</td>
<td>6.13 ± 0.878</td>
<td>5.89 ± 0.75</td>
<td>0.126</td>
</tr>
<tr>
<td>ALT</td>
<td>22.14 ± 3.67</td>
<td>21.07 ± 4.27</td>
<td>0.126</td>
</tr>
<tr>
<td>AST</td>
<td>25.91 ± 5.71</td>
<td>27.50 ± 6.24</td>
<td>0.140</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.841 ± 0.12</td>
<td>0.81 ± 0.12</td>
<td>0.331</td>
</tr>
</tbody>
</table>

There were no significant statistical differences between both groups with regard to demographic characteristics and laboratory findings. BMI - body mass index, DM - diabetes mellitus, Hb - hemoglobin, WBCs - white blood cells, ALT - alanine aminotransferase, AST - aspartate aminotransferase.

Table 2. Differences in vitamin 25(OH)-vitD levels in the eradication successful and eradication failure groups.

<table>
<thead>
<tr>
<th>25(OH)-vitD level (ng/mL) (mean ± SD)</th>
<th>Group I (eradication successful)</th>
<th>Group II (eradication failure)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with deficient 25(OH)-vitD [n (%)]</td>
<td>19 (17.5%)</td>
<td>30 (71.5%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Mean serum level of 25(OH)-vitD was higher in the eradication successful group compared to the group of eradication failure. Moreover, the percentage of patients with 25(OH)-vitD deficiency was higher in the group of eradication failure compared to the group of successful eradication.

Figure 1. 25(OH)-vitD status in relation to the rate of H. pylori eradication.

The rate of eradication in patients with sufficient vitamin D (25(OH)-vitD ≥ 20 ng/mL) was 88%, while the rate of eradication in patients with deficient vitamin D (25(OH)-vitD < 20 ng/mL) was 38.5%.
by LC-MS/MS (slope 0.93, intercept -2.5). For specificity, based on 25(OH)-vitD2 and 25(OH)-vitD3 levels assessed by LC-MS/MS, LSN showed minor differences in slope (0.9 in groups with detectable 25(OH)-vitD2 and 0.96 in groups with no detectable 25(OH)-vitD2. LSN demonstrated good precision (total coefficient of variation range 5.5 - 10.0%), and functional sensitivity was 2.15 ng/mL by LSN [16]. Similar findings were reported by the American College of Pathologists [17]. In general, the precision levels of LSN and LC-MS/MS are comparable; both have the required sensitivity to identify severe vitamin D deficiency [18].

The test was performed by a laboratory technician who was blind to the aim of the study. Vitamin-D level was considered sufficient if serum 25(OH)-vitD ≥ 20 ng/mL and deficient if serum 25(OH)-vitD < 20 ng/mL [15].

Confirmation of H. pylori eradication via 14C-UBT
This test was performed at least 4 weeks after treatment completion. Patients were advised not to use antibiotics nor PPIs in the 4 weeks prior to the test. According to the result of eradication, patients were divided into two groups as follows: group I (eradication successful) and group II (eradication failure).

Statistical analysis
Data collected through history, physical examination, and laboratory investigations were analyzed statistically by the SPSS software program, Version 20. Quantitative variables were presented as mean and standard deviation (SD), while qualitative variables were presented as numbers and percentages. To evaluate the variances on quantitative variables, unpaired t-test was used, while qualitative variables were compared with Pearson’s chi-square test. Statistical significance was set at p < 0.05.

RESULTS
The study included 150 patients diagnosed with H. pylori infection. Vitamin D status was determined for every patient before commencing eradication therapy. Overall, 101 (68.5%) of patients had serum 25(OH)-vitD ≥ 20 ng/mL (sufficient), while 49 (32.5%) had serum 25(OH)-vitD < 20 ng/mL (deficient). Overall rate of H. pylori eradication was 72%. There was no significant statistical difference between the eradication successful and eradication failure groups with regard to age, gender, BMI, smoking status, anemia, liver disease, kidney disease, and diabetes mellitus (Table 1, p > 0.05). The prevalence of 25(OH)-vitD deficiency was 32.6%. The group with eradication successful had a higher mean value of 25(OH)-vitD compared to the group with eradication failure (28.12 ± 8.1 vs. 13.54 ± 6.37; p < 0.001, Table 2). Furthermore, the percentage of patients with deficient 25(OH)-vitD was significantly higher in the group of eradication failure in comparison with the group of eradication successful [30 (71.5%) vs. 19 (17.5%); p < 0.001, Table 2]. Patients with sufficient 25(OH)-vitD had a higher rate of eradication compared to patients with deficient 25(OH)-vitD (88% vs. 38.5%, Figure 1).

DISCUSSION
In this study, the potential relationship between the rate of H. pylori eradication and levels of 25(OH)-vitD was examined. Diagnosis and post-treatment eradication of H. pylori was confirmed via the 14C-UBT. When compared with serology or stool antigen tests, this non-invasive and easy to perform test possesses the highest accuracy to detect H. pylori infection in patients without a recent use of antibiotics or PPIs [19]. In a recent systematic review, with specificity estimated at 90%, the sensitivity of these tests were 92% (95% CI; 89% to 94%) for 14C-UBT, 84% (95% CI; 74% to 91%) for serology, and 83% (95% CI; 73% to 90%) for stool antigen test [20]. Therefore, 14C-UBT is the most accurate test to diagnose and follow up eradication of H. pylori infection [19].

Overall, 72% eradication rate was achieved in the present study using the standard clarithromycin-based triple therapy. In comparison to the current study, a rate of 70 - 85% was reported by the American College of Gastroenterology in 2007 [8], and a rate of 75% was revealed by Feng et al. in their recent systematic review in 2016 with the same triple therapy [9].

A significantly lower rate of H. pylori eradication in patients with lower 25(OH)-vitD levels was demonstrated by the current study. Moreover, the eradication failure group had a higher percentage of patients with deficient 25(OH)-vitD. A likely explanation of the observed association between the status of 25(OH)-vitD and rates of H. pylori eradication is the inadequate immune response due to the impairment of 25(OH)-vitD immune function [21,22]. Vitamin D has been recognized as an effective immune modulator of the immune system, stimulating the immune response to infection [23].

Vitamin D deficiency status has been proposed as a potential factor for the susceptibility to various infections with the first observation of nutritional deficiency in children with rickets who experienced an increase risk for respiratory infections [24]. Recent epidemiologic studies have revealed an association between seasonal variations in vitamin D status and the increased rates of numerous infections including sepsis [25].

More recently, researchers have recognized the relationship between vitamin D deficiency and infectious diseases [26,27]. A large meta-analysis has demonstrated the relationship between low levels of serum 25(OH)-vitD and the increased risk to active tuberculosis [28]. Besides its long tradition on regulating calcium, phosphorus, and bone metabolism, vitamin D may decrease inflammatory markers such as C-reactive protein.
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(CRP), tumor necrosis factor (TNF-α), interleukine-6 (IL-6), and interleukine-8 (IL-18), while it may increase the level of anti-inflammatory cytokine (IL-10) [29]. A bactericidal effect of vitamin D has been demonstrated through increasing production of cathelicidin and β-defensin by macrophages which possess bactericidal effect against both gram positive and gram-negative bacteria [30]. There is shortage of data indicating a direct relationship between vitamin D and H. pylori infection. Hosoda et al. in their in vitro study have demonstrated a selective bactericidal effect of vitamin D3 decomposition product (VDP1) against H. pylori [31]. Interestingly, a recent study by Guo et al. demonstrated a very specific antimicrobial effect of vitamin D to protect against H. pylori infection through its important role in gastric mucosa homeostasis [33]. Moreover, the impaired gastric mucosal immune response may contribute to the eradication failure in H. pylori infection. The bactericidal effect of peptides cathelicidin and β-defensin, secreted in the gastric mucosa after infection by H. pylori, creates an immune defense against H. pylori at the mucosal surface. In the vitamin D deficient state, this action is hindered as the infected macrophages are incapable of producing adequate 25-(OH)-vitD to upregulate formation of both β-defensin and cathelicidin, thus leaving them incapable of eradication H. pylori [34].

CONCLUSION

This study has demonstrated a lower rate of H. pylori eradication in patients with vitamin D deficiency. Thus, vitamin D deficiency could serve as a risk factor for H. pylori eradication failure; thus, vitamin D supplementation to correct its status might be needed prior to H. pylori eradication. Clinical trials are needed to test the difference in success of H. pylori eradication in patients with vitamin D deficiency both before and after vitamin D supplementation.

Declaration of Interest:
The authors declare no conflict of interest for publication of this work.

Adherence to Ethical Recommendations:
The authors declare adherence to ethical recommendations. Patients’ information was kept confidential throughout the work and will not be breached.

References:


